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TU016 New transgenic zebrafish models to study the expression of key steroidogenic enzymes and their perturbation by endocrine disrupting chemicals

n. hinfray, m. caulier, INERIS / Ecotoxicology Unit; y. guiguen, INRA / LPGP; O. Kah, IRSET; B. Piccini, INERIS / Ecotoxicology Unit; E. Chadili, J. Porcher, INERIS; F. Brion, INERIS / Ecotoxicology Unit. Concern about the effects of Endocrine Disrupting Chemicals (EDC) to fish reproductive health has stimulated the development and implementation of fish screening and testing procedures for EDCs which has become an important aim notably within the perspective of the EU regulatory framework for chemicals REACH (registration, evaluation, authorization and restriction of chemicals). In this context, small fish species such as the zebrafish had appear as relevant models to identify endocrine active substances, quantify their effects and explore their modes of action. In this context, transgenic zebrafish can provide suitable and practical biological models to study EDC while reducing costs and the number of animals. For instance, we recently demonstrated the usefulness and relevance of a vivo mechanism-based test (the EASZY test that uses transgenic cyp19a1b-GFP zebrafish embryos) for rapid and cost-effective screening of estrogenic activity of chemicals. In the present work, our aim was to develop a panel of new transgenic models to study the expression and the perturbation of several target genes involved in the endocrine system and known to be affected by exposure to EDC. These new transgenic zebrafish lines express Green Fluorescent Proteins (GFP) under the control of the zebrafish promoters of steroidogenic genes, cyp11c1 and cyp19a1a. These genes are known to play a critical role in the biosynthesis of androgens and estrogens respectively and are affected by exposure to EDC. We found that the transgenic cyp11c1-GFP and cyp19a1a-GFP lines are homozygous resulting in 50% of transgenic embryos when crossing transgenic animals with wild-type fish. Furthermore, the transgene is stably expressed across generation (>F3). Extensive immunohistochemistry experiments showed that there is a perfect co-expression of GFP with endogenous zebrafish Cyp11c1 and Cyp19a1a proteins in the gonads. In both transgenic lines, GFP was localized in the cytoplasm of oogonia, young oocytes and peri-follicular cells and in testes, GFP was localized in the cytoplasm of Leydig cells and germ cells. Monitoring the expression of these transgenes on the whole animal or on sections in control and exposed-fish will help to identify the interest of these models to study critical physiological process (e.g., sexual differentiation). Since the fish brain is characterized by a strong ability to synthesize neuro-steroids, their expression in the central nervous system will also be considered.