

# An ecotoxicological study on early life stages of Japanese medaka(*Oryzias latipes*) using transcriptome analysis

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(トランスクリプトーム解析を用いたメダカの初期発生における生態毒性学的研究)

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### 論 文 内 容 の 要 旨

From last century, organic compounds have been widely used in industry and other areas of human daily life. Without appropriate treatments, discharge of them usually cause pollutions and negative effects on ecosystem. Many of these compounds are bioaccumulative and have extended toxicity to human and other living organisms in the ecosystem, especially in the aquatic environment when the pollutants are flowing into waters. Some of pollutants can cause various damage to fish, especially in the early life stages from embryonic to juveniles stages when fish are developing and more sensitive to chemicals. These chemicals not only affect the present organisms, but also cause reproduction problems and further influence the population and structure of ecosystem. In order to use these chemicals in an appropriate and environmental-friendly way, ecotoxicological studies of these pollutants on early life stages of fish are important and necessary. In the present study, three typical environmental pollutants, 3-hydroxybenzo[*c*]phenanthrene (3-OHBcP), perfluorooctanesulfonate (PFOS) and tributyltin (TBT), are used to exposing embryo or juvenile of Japanese medaka (*Oryzias latipes*). The effects of these chemicals on transcriptomes level were analyzed using mRNA sequencing analysis (mRNA-Seq), which is a powerful and accurate genomic method of quantifying gene expression for understanding the disease states and biological processes.

In the first study, 3-OHBcP, higher toxicity metabolite of benzo[*c*]phenanthrene *in vivo* of fish, was used on medaka embryo exposure test. BcP is known as one of the many polycyclic aromatic hydrocarbons in the environment. The *in ovo*-nanoinjection, which can inject accurate amount of chemicals directly into fertilized eggs, was applied in the present experiment to simulate the maternal transfer of 3-OHBcP to embryos. At some stages, development of the 1-nM 3-OHBcP exposed medaka embryos was significantly accelerated. The significant expression change of genes related to eye development, muscle development, energy supply, and stress-response had been found significantly changed between control and exposure groups. The heart rate of medaka embryos was significantly higher than that of the control groups, on the 5<sup>th</sup> dpf. This study indicates that the 3-OHBcP have adverse effects on the development of medaka embryos, even at low exposure level (1-nM).

In the second experiment, PFOS, which has been widely used in our daily life and industry products, was

exposed to medaka embryo. It has been reported to have adipogenesis and endocrine disruption effects on animals. In this study, 0, 0.05, 0.5, and 5 ng PFOS had been in ovo-nano-injected into fertilized medaka embryos. Hatching time was significantly delayed in the 0.05 ng exposure group, and accelerated in the 5 ng group than that of the control groups. Larvae adipocyte areas of the 0.05 and 0.5 ng PFOS exposure group were significantly enlarged. In the mRNA-Seq result (0.5 ng), most of the genes, which had significantly changed expressions, were involved in metabolism, genetic information processing, environmental information processing, *et al.* However, no genes with significant expression changes were found to be involved in the PPAR signaling pathway, which is the key regulation pathway of adipogenesis. The obesogenic effect of PFOS may come from its disruption with other lipid metabolism processes. This study indicates the potential of PFOS on being an obesogen, and proved one-time low dose exposure of PFOS on medaka embryo has further effect on the gene expression of larvae.

In the third experiment, TBT was used to expose the medaka juvenile. Although it has been banned in most counties due to its high toxicity, TBT still remains in the environment. TBT was exposed to 4-week old medaka juveniles (0, 1, and 10 ng/g body weight/day) through dietary exposure. Nile red dye staining of whole fish body showed that the adipose tissue area was significantly increased in the 1 ng/g bw/d TBT exposure groups after 2-week and 4-week exposure. The mRNA-Seq result indicated that 2215 genes had significantly changed expressions in the 2-week 1 ng/g bw/d TBT exposure group compared with those in the control groups. Expressions of 26 genes, including *pparg* and *rxr*, in the PPAR signaling pathway have significantly changed after exposure. Over 100 genes, which have significantly changed expressions, are related to obesogenic effect. This experiment shows not only the obesogenic activity of TBT on medaka juvenile development, but also explanations of its toxic effect on biological pathways from gene expression level.

In summary, 3-OHBCP, PFOS and TBT have effects on early stage development of Japanese medaka, even at low exposure level. Their effects have been seen through abnormal physiological development, like heart rate and adipose tissue growth. The *in ovo*-nanoinjection on medaka embryos can successfully simulate maternal bioaccumulation and biotransportation of chemicals. Meanwhile, the result of mRNA-Seq analysis supported the physiological effects and cleared the toxicity mechanisms of the organic pollutants at gene expression level. The mRNA-Seq is a powerful and accurate method on profiling potential toxicity of chemical compounds at molecular level, and to help clarify the biological pathways and processes affected by these pollutants. The combination method of *in ovo*-nanoinjection, physiological endpoints and mRNA-Seq analysis can be a useful and efficient way to identify biological processes and clarify the mode of actions of organic pollutants in fish and other organisms.