Abstract

[Project Information]

Project Title :	Development of an Avian in Ovo Method for Evaluating Abnormalities in Sexual Differentiation Induced by Chemicals and Proposal to Establish a Test Guideline
Project Number :	JPMEERF20225001
Project Period (FY) :	2022-2024
Principal Investigator :	Kawashima Takaharu
(PI ORCID) :	
Principal Institution :	National Institute for Environmental Studies 16-2 Onogawa Tsukuba, Ibaraki 305-8506, JAPAN Tel: +81-29-850-2174 E-mail: kawataka@nies.go.jp
Cooperated by :	Hokkaido University, Saitama University
Keywords :	Chemicals, Reproductive toxicity, Sex differentiation, Birds, OECD test guidelines

[Abstract]

In recent years, the biodiversity of wild avian species has been rapidly declining both in Japan and overseas. Although it has been suggested that chemical substances dispersed in the environment may be one of the adverse factors, the effects of the environmental chemicals on the survival and reproduction of birds remain unclear. In this study, we developed a method to evaluate the abnormalities in the avian sexual differentiation caused by administration of test substances *in ovo*. We explored endpoints for detecting abnormalities in sexual differentiation in early embryos in 1) reproductive organs (testes, ovaries, and oviducts), 2) germ cells (sperm, oocytes, and primordial germ cells) and 3) the brain. Based on the results obtained, we concluded that the optimal endpoints for convenient, rapid and accurate detection of estrogenic effects were morphological abnormalities of the Müllerian duct (and in the future, oviducts) and changes in the size ratio of the left and right testes, and the endpoints for the detection of anti-estrogenic effects was an increase in the size of the right ovary (which should naturally regress). In each of the three subthemes, we also succeeded in presenting molecular markers for detecting toxicity.

Furthermore, in each of the three sub-themes, a correlation was shown between abnormalities during embryogenesis and adverse effects after sexual maturation, suggesting the endpoints found in each of the three subthemes can be used to assess postmaturity reproductive effects.

In addition, we have also succeeded in developing a multiple, shell-less culture technique for quail embryos that enables easier and more efficient observation of embryonic development and

evaluation of the effects of test substances.

To establish the *in ovo* assay constructed based on the results of this study as a new test guideline, Japan, jointly with France, submitted a proposal entitled "Avian *In Ovo* Assay for Sex Steroid Hormone Disrupting Properties" to the OECD, which was adopted as an international work plan. The *in ovo* assay is simple, cost-effective, timesaving, and considers animal welfare, and is expected to contribute to the efficiency and sophistication of chemical substance screening and domestic and international management.

This study was supported by the Environment Research and Technology Development Fund of the ERCA (JPMEERF20225001) funded by the Ministry of the Environment.