

UK-J Research Project for the Endocrine Disrupters in the Aquatic Environment

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My name is Koji Arizono, Faculty of Environmental and Symbiotic Sciences of Prefectural University of Kumamoto. I'd like to talk about joint research between Japan and the United Kingdom concerning endocrine disrupters in aquatic environment. Let's begin with the sequence of events. At the G8 conference of environment ministers held in March 1999, Kenji Manabe, the Director-General of the Environment Agency of Japan at that time, and Michael Meacher, the UK Minister of the Environment, agreed in principle for the two countries to conduct joint research on the problem of endocrine disrupters. The formal agreement was signed in Tokyo on December 7 this year. The terms of the agreement include 1) free exchange of technical information, 2) sharing of findings and development of joint research, 3) holding of technical discussions such as joint symposiums and 4) exchange of experts between the concerned parties. Along with conducting joint research for possible research themes, experts have been exchanged and we have begun collecting further scientific data on endocrine disrupters. It has been confirmed that this will contribute to future countermeasures against endocrine disrupters. Next I would like to talk about the situation of the joint research. Three joint symposiums/workshops have already been held, at which researchers enthusiastically exchanged information.

The first one was held at the Kobe International Conference Center in December 1999. Themes presented by researchers included studies of the freshwater environment, studies of the oceanic environment, TBT and imposex. A report concerning the first workshop has been published.

The second was held at Plymouth University in the UK in January 2001. Firstly, we presented the case of Japan and UK under the title of endocrine disrupters in the environments. Discussions were held following five sessions of presentations by researchers from the two countries [(1) fish, (2) invertebrates, (3) birds, amphibians and marine creatures, (4) environmental monitoring, (5) sewage treatment and disposal] (see table 1). A report of this conference has already been created.

Table 1: Topics

Session 1: Fish *Difference in susceptibility to endocrine disrupters according to stage and type of fish *Causes of intersex and fluctuation of numbers caused by intersex *Standardization of basic technologies among researchers and laboratories *Establishing new bioassays and biomarkers
Session 2: Invertebrates *Study of assessment techniques *Role of neuropeptides and steroids in invertebrates *Confirmation of endocrine disruption in invertebrates (by conducting field study)
Session 3: Birds, amphibians and predators at the top of the food chain *Improvement of frog metamorphosis assay *Appropriateness of using <i>Xenopus laevis</i> *Development of more sensitive biomarkers (vitellogenin antibodies, etc.)
Session 4: Environmental monitoring *Conducting UK-Japan joint environmental monitoring in Africa and South America *Standardization of measurement technology by measuring same sample and confirming data *Exchange of monitoring data
Session 5: Sewage treatment and disposal

*Study of cost effect of sewage treatment and disposal

*Metabolism of and measurement of metabolism of endocrine disrupters

The third was held at the Tsukuba International Conference Center yesterday. Data was exchanged concerning study of freshwater and oceanic environments, method of assessing the aquatic environment using molecular biological techniques, and endocrine disrupting effect on amphibians and invertebrates which are among themes thought to be for the future at the January conference.

I would now like to briefly describe the theme and findings of UK-J projects of endocrine disrupters conducted in 2000.

(1) Comparative analysis of study on the abnormality of marine organisms and freshwater organisms.

As for the endocrine disrupting effect on wildlife, the effect of endocrine disrupters on loaches and rainbow trout (freshwater) and flounder (marine) were studied in the UK, and the effect on carp (freshwater) and flounder (marine) were studied in Japan. The results of the studies were compared, and joint research was conducted to determine the index for evaluating the endocrine disrupting effect. Joint research to investigate the mechanism of spawning disorders by masculinization (imposex) of conches thought to be caused by organotin compounds was also conducted. The research enabled us to develop the technology to measure steroids in warty murex and ivory shell by high resolution gas chromatography and to determine that high concentrations of testosterone were detected in the ovaries of female shellfish suffering from imposex in sea areas that appear to be contaminated with organotins. We already know about ovotestis in lobster and intersex of Gammarus. We however conducted joint field studies of the effect on crustaceans such as Japanese freshwater crab, and succeeded in conducting the study in accordance with the manual shared by both countries. As a result of a study of the state of contamination by endocrine disrupters concerning higher sea dwelling creatures and fish/shellfish, we found that whereas contamination is reducing relatively quickly in land and coastal areas, it will take a longer time in the open seas. It was therefore concluded that it would be important to monitor long-term fluctuation of organochlorine compounds in the outer sea area. We are also conducting joint research on the toxic effect of these compounds, such as the mechanism by which ataxia of immunological and drug metabolism enzyme functions occurs.

(2) Cross check of fish vitellogenin antibodies

As was stated in (1), research of the effect of endocrine disrupters on marine organisms and freshwater organisms is being conducted in both Japan and the UK. The antibodies of the enzyme immunity antibody method used for evaluation are unique to each researcher, so there were problems with universality and comparison of study results. Vitellogenin antibodies of fish such as carp, grey mullet, medaka, and dace were mutually exchanged, the antibodies were cross checked, and the methods of creating and evaluating antibodies were standardized by the two countries to conform to international standards for biomarkers in order to grasp endocrine disrupting effect. In 2000, researchers from the Hara laboratory were sent to Brunel University and CEFAS of the UK to conduct a comparative study of antibodies. Some antibodies that could be used by both were confirmed.

(3) Development of the estimate method of endocrine disrupting action by invertebrate animal

The effect of endocrine disrupters on invertebrates such as crustaceans and Nematoda has gained a lot of notice. Last year, researchers from Japan and the UK began working on development of a new method of evaluating endocrine disruption by molecular biological techniques using invertebrates.

Exchange researchers from the UK were sent to Japan to study a method of evaluation that utilizes endocrine disruption using Nematoda, which are thought to have genes that are extremely homologous to those of human beings. This research continues to be conducted this year. The two countries plan to propose the jointly developed technique to the OECD.

(4) Standardization of medaka experiment conditions, etc.

A symposium on medaka was held by the Environment Agency in Nagoya in March 2000, where it was confirmed that medaka was an excellent specimen for research of endocrine disruption. By establishing the Web site (see fig.1) desired by the foreign researchers participating in preparation of means of arranging and publishing basic findings concerning the endocrine disruption of medaka, we were able to convey that information both in Japan and overseas, and to exchange information with basic data of UK concerning zebrafish and fathead minnows. We also supplied the UK with the medaka (d-rR strain) developed primarily at Ozato laboratory of Nagoya University in Japan and exchanged data for the purpose of jointly standardizing the medaka experiment method concerning breeding conditions, sex ratio, and exposure conditions. The Web site created by Professor Oshima of Kyushu University also enabled us to collect information on experiments using medaka and to promote the use of medaka. The Web site contains a database of literature, test methods and news. It also functions to exchange opinions by means of a mailing list and condensed questionnaires.



Fig. 1: Medaka Web site home page

I would now like to talk about the exchange researchers involved in the joint research project on endocrine disrupters for the year 2000. Prof. Toru Takahashi visited AstraZeneca to conduct a field study of the effect of endocrine disrupters on crustaceans such as Japanese freshwater crab. Prof. Haruhisa Fukada and Prof. Toshiaki Fujita went to CEFAS to conduct a cross check of fish vitellogenin antibodies and to conduct research for evaluation of endocrine disruption of fish living in coastal areas and rivers. Prof. Mark Hurst of CEFAS came from the UK to Japan to learn the technique of evaluating endocrine disruption using Nematoda and to study to establish the validation of the endocrine disruption technique using yeast. A report on UK-J joint research on endocrine disrupters for 2000, the first year, was created with the support of the Overseas Environmental Cooperation Center.

Joint research by representative researchers of the following themes was also conducted in 2001 (see table 2).

Table 2: Research themes and representative researchers

Comparative analysis of research concerning abnormalities in marine and freshwater organisms.	Akihiko Hara, Hokkaido University
Molecular biological approach to sex differentiation of fish	Taisen Iguchi, Okazaki National Research Institutes
Current status of and mechanism by which endocrine disrupters act on crustaceans	Toru Takahashi, Minamata Environment Technocenter
Development of method of evaluating endocrine disruption using invertebrates	Koji Arizono, Prefectural University of Kumamoto
Standardization of test food for fish, etc.	Kenjiro Ozato, Nagoya University
Study of current status of endocrine disruption in marine organisms of developing countries	Shinsuke Tanabe, Ehime University

Finally I would like to express our appreciation to everybody at the Environmental Health and Safety Division of the Environmental Health Department of the Ministry of the Environment and all of our joint researchers. This brings my presentation to a close. Thank you very much.