International Symposium on Environmental Endocrine Disrupters 2001

Saturday, December 15 - Monday, December 17, 2001

パート 1
2001年12月15日（土）

Part 1
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我が国の取組の現状

Current Strategy in Japan

Current Strategies against Environmental Endocrine Disrupters by the Ministry of the Environment, Government of Japan

Kazuhiko Adachi

Ministry of the Environment, Government of Japan

1. Foreword

The Ministry of the Environment began holding the International Symposium on Environmental Endocrine Disrupters in 1998 for international exchange of opinion on the subject, and this year's session is consequently the fourth in the series.

In addition, March 2001 saw the completion of construction of the Environmental Hormone Research Building in the National Institute for Environmental Studies.

The Ministry is committed to continued contribution to the accumulation of scientific knowledge on the endocrine disrupter issues while exercising leadership in international approaches to it.

2. Approaches to Endocrine Disrupter Issues

Endocrine disrupters disrupt the action of endocrine system in the body and can cause impairment to the reproductive faculties as well as malignant growths. As such, they pose the risk of immense harm to human health and the ecosystem for generations to come, and have become a key issue of international concern.

In May 1998, the Ministry prepared the "Strategic Programs on Environmental Endocrine Disruptors '98" (SPEED '98) and presented them to the public. The Programs set forth the Ministry's basic perspectives on the problem and specific lines of action on it.

3. Outline of "Strategic Programs on Environmental Endocrine Disruptors '98"(SPEED '98)(last updated November 2001)

"The Strategic Programs on Environmental Endocrine Disruptors '98" (SPEED '98) advocated the promotion of investigative research toward the goal of ascertaining the presence or absence of a disrupting effect as well as (if present) determining the relative strength of the effect and elucidating its mechanism. More specifically, SPEED '98 called for work on the following four items.

(1) Promotion of a fact-finding study on detection in the environment and influence on wildlife

(2) Promotion of testing and research, and technology development

(3) Assessment and management of environmental risk, and provision of related information

(4) Efforts for reinforcement of international networking on endcrine disrupter issues

4. Assessment of environmental risk

In SPEED '98, a list was prepared of chemical substances suspected of having an endocrine disrupting effect. As a part of the national Millennium Project, the substances on this list have been undergoing study. This study identified 12 of them as endocrine disrupters in the year 2000 and another eight in the year 2001. These substances are now being assessed with respect to their environmental risk.

Among them, nonylphenol was subjected to an environmental impact test with medaka in August 2001. This test found that testes-ova appeared even at low concentrations like those in the environment. Through *in vitro* testing, it also provided the world's first confirmation of strong binding with the female hormone receptors.

5. Approaches to international joint research, etc.

The Ministry is participating in the development of screening test methods and other work being promoted and shared by the developed countries, mainly in the context of the Organization for Economic Cooperation and Development (OECD). Furthermore, it embarked on joint research with a U.K. counterpart on endocrine disrupting impact on the ecosystem in December 1999 and with a Korean counterpart in accordance with a bilateral agreement in the year 2001.

6. Action on POPs

Persistent organic pollutants (POPs) such as polychlorinated biphenyl (PCB) and dichloro-dipheyl-trichloro-ethane (DDT), which have a strong ability to remain in the environment, are reportedly polluting environments on a global scale and demand countermeasures within an international framework. This situation led to the adoption of the Stockholm Agreement on POPs (normally referred to as the "POPs Agreement") in May 2001.

Japan, too, intends to make the domestic arrangements necessary for the early ratification of this agreement.

note) POPS: Persistent Organic Pollutants

Outline of the Stockholm (POPs) Agreement

Background

Pollution of the global environment due to the 12 types of Persistent Organic Pollutants (POPs), including PCB, DDT, and other substances with a strong ability to remain in the environment as residue, cannot be prevented if only a few countries take action to this end. This situation heightened awareness of the need for international collaboration in prohibiting the production and use of POPs and reducing emissions of the same.

Profile of the draft

1. Objective

The objective of the Agreement is to protect human health and preserve environments against the risk posed by POPs, with attention to the preventive approaches cited in Principle 15 of the Rio Declaration.

2. Major countermeasures

1) Prohibition of production and use as a general rule (aldrin, chtordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, and PCB)
2) Limitation of production and use (DDT, except for use against malaria)
3) Reduction of emissions of substances produced unintentionally (dioxin, dibenzofuran, hexachlorobenzene, PCB)
4) Proper disposal of waste and stockpiles containing POPs (preparation of an action plan by each country)

3. Other measures

* Instatement of measures for prevention of further production of POPs into existing regulations and schemes
* Sharing of information among signatory countries on the eradication of POP production and use, reduction of POP emissions, and POP alternatives
* Disclosure of information on POPs, provision of education about POPs, and determination and publication of POP emission and waste quantities through means such as the pollutant release and transfer register (PRTR)
* Promotion of investigative research of technology for assessment of POP impact on the environment and curtailment of emissions, and monitoring activities
* Provision of related technical and financial aid to developing countries

Future schedule

* Effectuation of the Agreement upon ratification by 50 countries

Efforts and Strategies to Deal with the Issue of Endocrine Disruptors in Japan Results of a Nationwide Survey by the Ministry of the Environment

Shinsuke Tanabe

Center for Marine Environmental Studies, Ehime University

Since the publication of the book "Our Stolen Future" by Theo Colborn, Dainne Dumanoski and John P. Myers, there has been a great concern regarding environmental pollution and ecological effects by endocrine disruptors. During the last few years, many research programs have been planned and conducted by Japanese research institutes and administrative organizations on endocrine disrupting chemicals. The Environment Agency (presently, the Ministry of the Environment) issued an interim report, "Exogenous Endocrine Disrupting Chemical Task Force" (chaired by Tsuguyoshi Suzuki, former Director General of the National Institute for Environmental Studies), and in May 1998, released a document titled "Strategic Programs on Environmental Endocrine Disrupters '98 (SPEED'98)", setting forth its basic position toward endocrine disrupters, as well as specific approaches to the problem. In SPEED'98, around 70 substances have been listed as chemicals suspected of causing endocrinological disruption, and the following future plans were presented.

1) Promotion of field investigations of the present status of environmental pollution and of the adverse effects on wildlife

2) Promotion of research, screening and testing method development

3) Promotion of environmental risk assessment, risk management, and information dissemination

4) Efforts to strengthen the international network

Based on the plan 1), the Ministry of the Environment has been conducting a nationwide survey since 1998 to elucidate the present status of environmental pollution (air, water, sediment, soil and aquatic biota) and the effects on wildlife by the endocrine disruptors. The results of this survey conducted during 1998 to 2000 have been discussed at research meetings and published as several reports in the homepage of the Ministry of the Environment (see below URLs).

In this presentation, the results of the nationwide survey conducted during 1998 to 2000 will be reviewed in terms of distribution and fate of endocrine disruptors in the Japanese ecosystem; the kinds of chemicals listed in SPEED '98, their occurrence in environmental media and their bioaccumulation and biomagnification in ecosystem including various trophic wildlife and humans will be discussed.

* Reports including the results of the nationwide survey by the Ministry of the Environment are available (most of them are written in Japanese) and can be downloaded from following URLs;
http://www.env.go.jp/chemi/index.html (Japanese page)
http://www.env.go.jp/chemi/end/index.html (Japanese page)
http://www.env.go.jp/chemi/dioxin/index.html (Japanese page)
http://www.env.go.jp/press/index.html (Japanese page)
http://www.env.go.jp/en/index.html (English page)

Present Status of Human Fetal Exposure to Several Endocrine Disruptors in Japan

Chisato Mori

Graduate School of Medicine, Chiba University

Introduction:

It has been recently clarified that there are many chemicals that disturb functions of natural hormones in the body. They are referred to as endocrine disruptors (EDs). EDs show some adverse effects on development and/or function of reproductive system and nervous system, particularly when exposure occurs in fetal or neonatal individuals.

Our group has investigated fetal exposure to EDs in Japan by analyzing umbilical cords and cord blood. Human umbilical cords, a part of the fetal tissue, were collected from normal newborns. Our studies have been approved by the “Congress of Medical Bioethics” in Chiba University, Yamanashi Medical College and Kyoto University. We also obtained their mothers' permission to analyze their babies' umbilical cords.

Results and Discussion:

1) We detected dioxins (PCDDs + PCDFs + co-PCBs), PCBs, DDTs, DDEs, aldrin, hexachlorocyclohexane (HCH), chlordanes, HCB, heavy metals (Cd, Pb), bisphenol A and phytoestrogens in both human umbilical cords and cord blood.
2) The concentration of dioxin, PCB and DDE (persistent chemicals) in the first babies seems to be higher than that in the second or the third babies.
3) There is a correlation between concentration of PCBs and DDEs/HCB/HCH in human umbilical cords.
4) Exposure level of persistent chemicals to the fetus is correlated with the maternal age. As persistent compound chemicals accumulate in the human body, previously stored chemicals will be transferred from the mother to the fetus even if avoided during pregnancy.
5) Fetal hormonal condition is affected by phytoestrogen highly transferred from mothers.

By studying umbilical cord tissue and blood, we found that fetuses in Japan were exposed to several chemicals. It became clear that fetuses in Japan are contaminated by chemical cocktail.

It is very important to assess the risk of the chemical exposure to solve the EDs problem. In this presentation, I will mention the necessity to establish the new risk assessment for human fetal exposure to chemical, including EDs.

Testing System for Hazard Assessment of Endocrine Disruptors in Fish

Hirofumi Yokota, Masanori Seki, Toshiki Nozaka, Makoto Nakai, Masanobu Maeda, Yoshikuni Yakabe, Hiroshi Tadokoro

Chemicals Evaluation and Research Institute, Japan (CERI)

Ministry of the Environment has begun assessing the hazard of suspected endocrine disruptors according to priorities based on the results of environmental monitoring and scientific surveys of endocrine-disrupting effects. The testing system we use for assessing the hazard of endocrine disruptors in fish consists of the following screening and testing methods using medaka *(Oryzias latipes)* to evaluate mainly estrogenic and androgenic effects. In this symposium, we present the basic outlines of the screening and testing assays we have developed.

< Screening >

1. Vitellogenin (VTG) induction assay

The assay investigates the effects of test substances on the synthesis of VTG in the liver of medaka. Mature medaka are exposed to test substances for 21 days under flow-through conditions. At the end of exposure, hepatic VTG concentrations in exposed fish are measured. VTG is an estrogen-dependent glycoprotein that is usually synthesized only in the liver of mature females. Therefore, VTG synthesis in male fish can be used as a specific biomarker for estrogenic exposure.

2. Partial life-cycle test

This test is intended to assess the endocrine-disrupting effects of test substances on sex differentiation of medaka. The fish are exposed to test substances from the fertilized egg stage to about 60-day posthatch under flow-through conditions. Embryological development, hatching, posthatch mortality, growth, secondary sex characteristics, gonadal histology, and VTG concentration are examined. Medaka is an ideal test species for evaluating endocrine-disrupting effects because it typically develops an intersex condition (i.e. testis-ova) in the gonadal tissues when exposed to estrogens or androgens.

<Testing>

1. Full life-cycle test

This test is conducted to elucidate the chronic effects of test substances on the life cycle of medaka over two generations in continuous exposure. We examine embryological development, hatching success, posthatch survival, growth, secondary sex characteristics, gonadal histology, fecundity, and fertility of the F_0 generation; for the F_1 generation, we examine all these endpoints except fecundity and fertility.

< *In vitro* assay >

1. Competitive binding assay of medaka estrogen receptor

This assay uses medaka estrogen receptors expressed in *E. coll.,* and measures the binding affinities of test substances to the receptors. The purpose of this *in vitro* assay is to supplement *in vivo* screening and test results, and to identify the mode of action of the test substance.

An Approach for Evaluating Low-dose Effects of Suspected Endocrine Disruptors

Hiroaki Aoyama

Institute of Environmental Toxicology

Effects of chemicals on living organisms have generally been recognized to become intense as an exposure level increases. However, not a few studies have recently suggested that certain estrogenic chemicals may not exhibit such a simple dose-response relationship. In these studies, suspected endocrine disruptors, many of which are known to manifest estrogenic effects, are suggested to exert some effects at a very low-dose level that is fur below the no-observed-adverse-effect-level (NOAEL) obtained in traditional toxicology studies. The purpose of our project is to develop a new bioassay for precisely predicting low-dose effects of suspected endocrine disruptors with estrogenic activities, such as nonylphenol and octylphenol. We are currently conducting improved one-generation reproductive studies in rats (trans-generation assay) by using ethynylestradiol, a synthesized reference estrogen, to confirm the availability of new bioassay for detecting low-dose effects.

In common reproductive studies with rodents (rats and mice), the number of pups per litter is adjusted to 8 or 10 on a certain day during the lactation period and only a small number of offspring (usually 1/sex/litter) is examined closely for the sexual development and reproductive abilities after weaning. The advantages of this methodology are (1) nutritional condition of pups is kept constant among litters so that scientists can precisely evaluate the effect of test compound on pup growth, and (2) a small number of test animals enables scientists to conduct detailed examination by limited numbers of persons and/or limited spaces. At the same time, however, such a methodology (only selected animals are examined for certain endpoints) may cause missing of low-incidence abnormalities among offspring. Another disadvantage may be the suspected overestimation of chemical effects due to unexpectedly deviated data from an “odd fellow” that is selected for the examination by chance in a treated group. In our trans-generation assay, the number of pups per litter is not adjusted during the entire period of lactation and all weanlings are examined for the same endpoints at the same time to outweigh the shortage in common reproductive studies.

Our food as well as animal feed is rich in estrogenic compounds derived from plants (phytoestrogens). It also contains low levels of several environmental contaminants. For examining the endocrine disrupting effects of test chemicals at very low doses, basal diets should be free from unexpected “noise” of estrogenic contaminants. Animals in our experiments were supplied for phytoestrogen-free diets. The diet was also analyzed for any other contaminants before use to ensure the exact evaluation of chemical effects on animals at very low doses.

Although our project is still ongoing at present, obtained results have satisfactory demonstrated the low-dose effects of ethynylestradiol. Interim results will be explained for the audience who are not familiar with toxicological sciences as plainly as possible.

Development of a Test Method for Risk Assessment of Endocrine Disrupting Chemicals Using DNA Microarray Technology

Akihiro Kondo

Biotechnology Research Laboratory, Takara Shuzo Co., Ltd.

General concern among the public over adverse effects of endocrine disrupting chemicals (EDCs) on wildlife and humans, especially the effects of exposure during fetal development, has been increasing in recent years. The effects, or "toxicity", caused by EDCs are not traditionally examined thoroughly in conventional toxicological studies. This is because the effects of EDCs are mainly mediated through endocrine receptors that are known to involve new paradigms of toxicity, such as low dose effects, unusual dose-response relationships, and possible direct and indirect effects on fetuses. "Low dose effects" refers to biological changes that occur at doses lower than those typically used in standard toxicological testing for the evaluation of reproductive and developmental toxicity. Some recent observations regarding induced endocrine disruption in animals has stemmed from investigation of the possibility that exposure to low levels of EDCs can result in adverse effects. However, other researchers have reported failure to repeat these observation. When observations are found to be difficult to repeat, the original observation of low dose effects is not invalidated. To clarify uncertainties and better characterize low dose effects, we recommended using genetic approaches to determine mechanisms of action and to characterize the dose-response relationship. We have worked to establish a new test method for risk assessment of EDCs based on systematic monitoring of receptor signaling and gene expression profiling. In this symposium, I will present a brief overview of some of our progress.

Using recent developments of DNA microarray technology, the expression and/or suppression of tens of thousands of genes can be measured simultaneously. This will have a big impact on EDC research, with the potential to increase the specificity of individual EDC classification. Gene expression profiles can be used for screening and testing risk assessment. Developed and validated methods for risk assessment of EDCs using DNA microarray technology could provide alternatives to traditional animal bioassays that would be much faster, more sensitive, and more informative.

The EDCs that we have tested were listed in the SPEED '98 report, and were selected for priority risk assessment in 2000 by the Exogenous Endocrine Disrupting Chemicals Task Force. We have examined gene expression regulation by the test chemicals on steroid-hormone-sensitive human cell lines and mouse fetuses. In the case of human cell lines, a microarray comprising about 8,400 human genes was used for the test. A statistically significant change in expression was observed for about 1,000 genes in the microarray. In the case of mice, about 1,500 of approximately 9,000 genes were identified. These selected genes can be called "expression regulated genes associated with EDC effect". We are currently preparing microarrays (EDC chip) including selected expression regulated genes by EDCs, both for humans and mice. In the future, to demonstrate the utility of the EDC chip for risk assessment of EDCs, we will characterize the sensitivity and reproducibility of gene expression profiling tests using pharmacological approaches, e.g. uterotrophic assay. The goal includes establishment of test methods, classification of expression profiles, and construction of databases on individual EDCs using the EDC chip.

I gratefully acknowledge the collaborative work of Prof. Ken Takeda and his colleagues (Tokyo University of Science) and Prof. Taisen Iguchi and his colleagues (Okazaki National Research Institutes). Our research was funded in part by a grant from the Ministry of the Environment, Japan.

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

Koji Arizono

Prefectural University of Kumamoto

Research Cooperation on Endocrine Disrupters in the Aquatic Environment was initiated in April 1999 by Michael Meacher, Minister of State for the Environment and Kenji Manabe, the then Japanese Environment Minister. At that time research groups in the UK and Japan were actively investigating different aspects of endocrine disruption. It was agreed that by cooperating we would be able to address more rapidly some of the questions vital to improving our understanding of the impacts of endocrine disrupters in the aquatic environment and be able to move faster towards regulatory action where necessary.

The Arrangement for implementing the research cooperation was formally signed on 7 December 1999 in Tokyo by senior officials in the Department of the Environment, Transport and the Regions and the Japan Environment Agency. It was followed by a one-day technical workshop of Japanese and UK scientists on 8 December and attendance at the International Symposium on Environmental Endocrine Disrupters on 9-11 December, 1999. The second workshop was held on on Monday 29th January 2001 at Plymouth University.

The following items were executed as UK-J projects at current year. 1) The comparative study on the abnormality of marine animals and fresh water animals 2) the cross check of vitellogenin antibodies, 3) the development of the estimate method of endocrine disrupting action by invertebrate animal, 4) open web site for medaka home page.

Endocrine Disrupters Research in National Institute for Environmental Studies

Masatoshi Morita

National Institute for Environmental Studies (NIES)

National Institute for Environmental Studies has initiated research activity for endocrine disrupters EDs mainly focusing on dioxin and organotin compounds. Dioxin research has advanced after completion of dioxin facility in 1995. In 2001, a new building for endocrine disrupters research is completed and is open to research scientists for collaboration.

The endocrine disrupters research laboratories building is, we expect, to function as a place for experimental research of interdiscipline. In the laboratories, we are performing research on the effect of EDs to terrestrial and marine organism, chemical and biochemical method development for analysis, toxicological study of mammals and birds, and informational analysis of environmental fate.

Major equipment includes high-resolution nuclear netic spectrometry, liquid-chromatography-mass spectrometry, magnetic resonance imaging (MRI: 4.7T) and several types of clean rooms.

The laboratory is also planned to function as a focus for information. Development of EDs database is under preparation, and information service on chemicals and their effect to living organism will be available near future.