International Symposium on Environmental Endocrine Disrupters 2000

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Panel Discussion

General Comment

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In 1962 Dr. Rachel Carson published a book "Silent Spring" dealing with serious influences of some agricultural chemicals including DDT on the wildlife, and gave us a warning that such chemicals possibly affect the human health, but her warning of this precious book was not earnestly heeded except some investigators and activist groups. In 1996, however, Dr. Theo Colborn et al. analyzed thousands of papers and reports, and then published a book "Our Stolen Future", which sounds a warning that anomalies in the reproductive system of wildlife are caused by some synthetic chemicals released to the environment, possibly resulting in the occurrence of similar anomalies in human beings sooner or later. This book has had a great impact on investigator, mass communication, administrative agency and industry, urging various groups of persons concerned to examine and investigate the toxicity of such chemicals, also causing a deep concern of citizen for the serious influence on the offspring. These hazardous synthetic chemicals released to the environment have a hormone-like action; most of them have the estrogenic property, and others have antiestrogenic, antiandrogenic and thyroid hormone-blocking actions; they are called endocrine-disrupting chemicals (EDCs).

Mature animals exposed to EDCs usually recover from the adverse influence after the withdrawal of the chemicals unless they have the exposure to a high dose of EDCs or the long-period exposure. On their way to development (fertilized egg - fetus - neonate - suckling infant), however, the organism shows an unrecoverable, irreversible response to EDCs as well as to estrogen despite a low - dose exposure for a short period. In addition, it has been reported that in mice and rats exposed perinatally to estrogen or antiestrogen, irreversible abnormalities occur not only in reproductive system but also in immune system, central nervous system (brain), bone tissue and behavior. These results suggest the possibility that EDCs produce diverse effects on various tissues and organs besides reproductive system when exposed in an undifferentiated state. Furthermore, the initial irreversible change is usually subtle at birth, appearing visible later as if a biological time - bomb.

The most important study of EDCs, therefore, appears to elucidate the toxicity for our coming offspring; accordingly, it seems necessary that a combined research organization of embryology, endocrinology, toxicology, immunology neurosciences and ethology is built up as soon as possible.

Contamination and Toxic Effects of Endocrine Disrupting Chemicals in Wildlife

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In recent years, several species of marine mammals and birds have been affected by uncommon diseases and unusual mortalities. While several factors have been attributed for these events, a prominent suspect is exposure to man-made toxic contaminants. Particularly, some of these man-made chemicals can disrupt normal endocrine physiology in animals and birds. Our study focuses on exposure and toxic effects of endocrine disrupting chemicals, particularly organochlorines, in wild higher trophic level animals. Endocrine disrupting chemicals, such as PCBs, PCDDs, PCDFs, *etc* are found in a wide variety of wildlife. Extremely high concentrations have been found in animals inflicted with disease and mass mortalities. Elevated contamination by organochlorines has been found in open sea animals such as cetaceans and albatrosses, which seemed to be attributable to their low capacity to metabolize persistent toxic contaminants. Significant correlations between biochemical parameters (serum hormone concentrations and cytochrome P450 enzyme activities) and residues of endocrine disrupting chemicals were found in some species of marine animals, which indicated that these chemicals may impose toxic effects in animals even at the current levels of exposure.

Concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs) were higher in marine mammals that suffered from mass mortalities, where adverse effects on immune systems by coplanar PCBs have been suggested. In general, water birds and marine mammals accumulated the dioxin-like compounds with much higher concentrations than humans, implying higher risk from these toxic contaminants in wildlife.

The future issues of endocrine disrupting chemicals in humans and wildlife will have to be focused on developing countries.

Environmental Signals: A New Way to Understand Endocrine Disruption

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Endocrine disrupting chemicals occur in an environment filled with biological signaling molecules. These signals include those occurring between plants and bacteria, plants and vertebrates, as well as internal signaling molecules like hormones and neurotransmitters, common to most vertebrates. By understanding the biologic basis of endocrine disrupting chemicals, we can place their potential deleterious effects in a more predictable context.

Environmental Hormones and Reproductive Medicine

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Introduction

The tremendous expansion of science and technology occurred in the 20th century, especially the latter half of the century, has brought not only prosperity for mankind but also a significant problem known as environmental pollution of the earth. Some man-made chemicals including dioxins alter reproductive functions in wildlife, causing some species to become endangered as they are introduced in "Our Stolen Future". Also there are growing concerns on reduced reproductive functions in humans evidenced by decreasing sperm volume and increasing number of patients with infertility or endometriosis, resulting in attention to the association between these human health effects and environmental pollution. However, studies of altered human reproductive functions have just started and there still are many unexplained details. This report presents the actual conditions of contamination with environmental hormones and the approaches to evaluations from the viewpoint of a practitioner in reproductive medicine.

1. The current status of reproductive medicine

Although it used to be said that infertility ratio was one in every 10 couples, now it is one in every seven couples, indicating that the increasing number of couples requires therapy. Causes of infertility are roughly grouped into three, ovulation/ovarium factors, oviduct factors, and male factors. Treatment methodologies for these factors are improved and now known as the Assisted Reproductive Technologies (ART). One of the modality most widely known is *in vitro* fertilization (IVF). Although IVF has a history of mere 20 years, it has becomes the essential general treatment that treats almost 100,000 cases annually. But, the success rate is only 10%, requiring further improvement. Determining the association between infertility and environmental hormones may contribute improvement of the treatment results.

2. Environmental hormones contamination of human reproductive organs and its evaluation

1) Environmental hormone contamination of follicular fluid

Human exposure to environmental hormones is expected to affect human reproductive organs. Indeed, environmental hormones are detected in follicular fluid (fluid which compose the environment for an ovum to grow and mature) and sperm sampled from the IVF patients who agreed with informed consent. 1pg/ml (0.01 pg. TEQ/ml) of a dioxin and 1ng/ml bisphenol A (a chemical commonly used for materials of polycarbonate and epoxy resins) were determined in the follicular fluid samples. Detectable levels of dioxin and bisphenol A in follicular fluid indicate that the contamination with these environmental hormones reaches a reproductive organ. It is necessary to evaluate the risk of this contamination. The early stage embryo development model that we are using, and the results obtained with the model are shown below.

1) Evaluation of contamination with environmental hormones using the early stage embryo development model

We incubate fertilized ova (embryos in the pre-implantation period) as a part of the reproductive medical treatment. Although the incubation is done under *in vitro* environment, the incubated embryo goes back to the *in vivo* environment after implantation. The *in vitro* culture has a meaning of reproduction in the *in vivo*

environment using *in vitro* method. Embryo development is generally susceptible to exogenous compounds [8]. We are trying to evaluate the effects of environmental hormones using embryo development as an indicator.

As the results, influence on the embryo development rate was detected when 1 - 5 pM of dioxin (TCDD) was added. Addition of bisphenol A showed an enhancement effect at 1 - 3 nM. In contrast, it showed an inhibitory effect at 100 μM. The effect at the high concentration of bisphenol A may be considered as a traditional dose-response effect seen with the administration of toxicity dose. On the other hand, the low concentration of bisphenol A at 1 - 3 nM presented no dose-response effect and showed reversible effect of toxicity inconsistent with toxicity dose (low dose effect). Moreover, this low concentration of bisphenol A is actually seen in the environment, and is not significantly different from the concentration detected in human blood or follicular fluid. It was reported that the effects of parental bisphenol A exposure on offspring included enhanced growth and sexual maturity after birth. We also observed enhanced development of offspring born to a surrogate mother in the experimental study using exposed embryos, indicating that in utero exposure during the early embryo influences the next generation.

3. Endometriosis and dioxin

1) Increased incidence of endometriosis

Endometriosis is a disease in which endometrium proliferates and grows outside uterus, causing dysmenorrhea, persistent pelvic pain, and infertility, and remarkably impairing QOL of females at the reproductive age. It is said that incidence of endometriosis has increased significantly during the last 20 to 30 years with the change of female life style such as early menarche, decreased number of children, and childbirth at the higher age. According to the recent report of a Welfare Ministry research group, it is confirmed that 130,000 females are receiving the medical treatment for endometriosis, and the estimated number of patients with endometriosis totaled to over one million females.

There are concerns on the effects of environmental hormones that are increasing in the environment as a cause of increased incidence of endometriosis. The beginning of these concerns was traced back to the experimental study using monkeys conducted by Rier et al. The result showing that a trace amount of Dioxin, as small as 126 pg, increased the incidence and severity of endometriosis indicated that one of the possible human health effects caused by dioxin is endometriosis.

2) Endometriosis in human and dioxin

Some studies compare dioxin exposure levels between endometriosis patients and non-endometriosis controls to determine the relationship between endometriosis and dioxin. According to these studies, dioxin exposure levels were higher in some results but lower or equal in others. We are also conducting a joint research with National Institute for Environmental Studies. There are genetic level studies as another approach. In endometrium or endometriosis site, the dioxin receptor (the Ah receptor) and expression of the related gene are detected in human. We hope that the studies of dioxin will break through the identification of endometriosis of which even the cause is currently unknown.

3) Breast milk and endometriosis

Although the tolerable daily intake of dioxin is 4 pg/kg, the dioxin level in human breast milk is high and the intake amount for breast-fed infants totals to 100 pg/kg/day. The infants' intake of dioxin from mother's milk lasts only a short period of time, but there is an apprehensive matter on the post-lactational effects. Therefore, we

investigated if breast-feeding can be a possible risk factor to induce endometriosis in the later life of breast-fed female infants. The results showed that the breast-feeding rate was lower in endometriosis group than in non-endometriosis group, indicating that breast-feeding does not constitute a risk factor to induce endometriosis although breast-feeding causes a relatively higher level of infant exposure to dioxin. Conversely, breast-feeding may have an endometriosis inhibitory effect. These findings support the facts that breast milk is the superior source of nutrients and did not have any safety problem in the past.

Conclusion

Many of the details of the relationship between environmental hormones and human diseases or reproductive functions remain unclear. Progress and accumulation of pollution would adversely affect the future of mankind. We must make greater efforts in exploring solutions for the problem from the standpoint as medical practitioner. We also would like to expand the studies to develop the methods to excrete environmental hormones including dioxin from human body.

Industry Perspective: "To What Extent Have We Known About Endocrine Disruption Chemicals?"

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Scientific research is the foundation of environmental and public health stewardship and regulatory policy. Industry has responded to public concern about the endocrine disruption issues by sponsoring independent research and working closely with the NGO, scientific and government communities. We have supported, and continue to support, a thorough investigation of the endocrine disruption based on sound scientific principles.

Research has indicated that the endocrine-mediated processes for synthetic and naturally occurring substances are more complex than originally thought. The challenge in resolving the endocrine disruption issues is to substantiate whether chemical substances at low levels result in adverse - that is, harmful - effects to the endocrine systems of wildlife and humans. Overall, the following criteria need to be satisfied:

(1) The body of scientific knowledge must support that adverse endocrine effects are clearly demonstrated in animal models from validated screens and tests.

(2) The relevance and implications of these effects in humans are clear.

Scientific evaluation of the endocrine disruption continues to advance. In February 1997, the United States Environmental Protection Agency (US EPA) published the "Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis." This report represented a review of more than 300 scientific papers published over 25 years. The US EPA found that a link between chemical exposures and adverse endocrine effects in wildlife only at high environmental doses, that is, after accidental spills or severe environmental pollution. Additionally, the causal relationship between exposure to a specific environmental agent and an adverse health effect in humans operating via endocrine disruption has not been established, and more research is needed.

Since 1997, similar conclusions have been reached by representatives of environmental authorities in Germany and Sweden and by scientific forums organized by the International Unions of Pure and Applied Chemistry, Pharmacology and Toxicology, the U.S. National Academy of Sciences, the Scientific Committee for Toxicity, Ecotoxicity and the Environment of the European Commission and the International Life Sciences Institute.

Some NGOs have stated that thus far no compound has been tested for endocrine disruption. This is not accurate. Already much is known about the ability of all currently registered pesticides and many industrial chemicals to affect both mammalian and wildlife endocrine systems. Existing regulations in the US and OECD countries have required pesticides to undergo safety evaluations before entering the marketplace. These safety evaluations include acute, developmental, multi generation reproduction, sub-chronic and chronic toxicology tests that have a long history of use and include endocrine sensitive endpoints including the determination of reproductive cancers and effects on sperm. Furthermore, during the next several years, as part of the High Production Volume (HPV) Initiative, the majority of industrial chemicals used in the US and international commerce will also be voluntarily tested for reproductive and developmental effects using the OECD SIDS battery.

To summarize, we support the refinement and use of tests to evaluate the safety of high production volume chemicals, the validation of appropriate screens and tests that measure endocrine effects, and the continued evaluation and regulation of pesticides and industrial chemicals by science-based safety assessment. Through science and the cooperation of the industry, NGO, scientific and government communities, we can continue to advance global public health for all.

A Discussion on Risk of Endocrine Disruptors

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1 Introduction

Endocrine disruptors have attracted much concern of people recently and the media reported news on this subject almost everyday, a couple of year ago. This phenomena significantly raised people's wish to understand about potential risk from chemical exposure, and also urged people in charge of regulatory sciences to review existing safety evaluation scheme.

However, despite of significant expertise in various fields in this country, the deficiency both in scheme and in ideas in integrating knowledge of various disciplines to make science-based judgement on which risk assessment is based, renders appropriate understanding of risk and its management lagged in Japan until now. The author, having been engaged in work on risk assessment in international co-operative effort, truly hope to nourish understanding and interest in risk assessment and appropriate measures taken based on it, spurred by these concerns.

2 Quantitative estimation of effect and exposure, weighing limitation of the methods

It is most important to know that how high concentrations are detected under which conditions, or how strong are activities of the compounds in what test system, as compared to know simply that some compound was detected in a certain environment/in a eluate from food package, or a certain activity was detected in a in-vitro system. One can get some suggestions on the possibility of some activities or on mode of action from in-vitro studies, but can not tell the risk from exposure to that chemical. For example, more than a score before, it was claimed that carcinogens can be screened out by using mutagenicity tests, and amino-acid pyrolysates could be a human carcinogen, because some of them showed strong mutagenicity in in-vitro assays. Thereafter, it was shown that intake of several kilograms per day of pyrolysates only, may be able to cause tumours in humans, however the mutagenicity studies contributed much to understanding the mechanism of cancer[1)].

3 Elucidation of mode of action through integration of knowledge in chemistry and biology

Some phenomena observed among wild animals can suggest possibility of health effects to humans. PCB and organotins which contaminate some coastal fish or clams can cause immune suppression at fairly low levels. It was speculated that massive deaths of marine mammals living in coastal waters could be caused by these compounds accumulated high in their bodies. Japanese people eat also much sea foods and accumulate quite high these compounds in their bodies that one needs to examine carefully potential health effects on immune system and others such as effects on drug metabolising systems by these compounds[2)].

It is also important to understand about critical period of exposure to chemicals, because embryo and foetus can be highly sensitive to insults since they are in the process of development and any adverse effects to them can be irreversible one. From this point of view, authors stated through co-operation of interdisciplinary team.

Organisms are responding exquisitely to stimulation or effects from both inside their bodies and outside of it by attenuation or by counter-action to keep their homeostasis. They also have capacities to protect them against attack from xenobiotics including bacteria, virus and foreign molecules. It is imperative to have deeper and probably wider understanding of these mechanisms to answer the question of so-called "U-shaped response phenomena" suggested to occur at low doses.

4 Examining possibility of effects to humans

Which compounds are most probable to exert effects to Japanese populations among various putative endocrine disruptors? I started to examine quantitative effects observed in humans related to these compounds, and assumed soybean estrogens can be a good candidate for their effects to humans taking into consideration of high exposure from these compounds among Japanese populations, levels of activities, and mode of actions of these estrogens.

Quantitative estimation of intake, blood and urine excretion levels, combined with effect levels found in various clinical and epidemiological studies together with their mode actions, resulted that the effects are most likely at the levels observed among Japanese populations to explain various beneficial effects[3]. This evidence is very important in examining allegedly claimed effects to humans for compounds such as bisphenol A which has activities similar to or lower than soybean estrogens but have about one-thousandth chance of exposure compared to soybean estrogens. Authors are currently examining potential effects of soybean estrogens to Japanese women through surveys on food intake pattern and their physiology, and also from a clinical epidemiology study.

5 Prediction of risk and estimation of uncertainty in risk assessment[4]

Appropriate measures against risk will never be taken without pertinent estimation of risk. Recently many voices in this country shout for crisis management, however analytical and integrated assessment of risk and judgement based on it plus preparedness before something happens, are indispensable before one talks about crisis management. To this goal, we need to assume two situations, one is ordinary case or average case, and other is worst case. In many cases, the assumption of ordinary case can apply, but in some cases, unexpected bad conditions can cumulatively cause worst outbreaks. Therefore, risk assessment and pertinent measures always necessitate examination of existence of uncertainty factors and their levels.

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