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Workshop 6.2

Hormonally active agents and plausible relationships to adverse effects on human health*

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Abstract: A hormonally active compound was first identified in the book *Silent Spring* by Rachel Carson in 1962, implicating the effect of pesticides such as DDT and the derivatives. Nearly four decades later, the book *Our Stolen Future* by Theo Colborn et al., and other pertinent publications have revisited and broadened the issue regarding a variety of possible chemicals and the area exposed. Translation and publication became available in Japan within the last four years. Since then, Japan joined the member countries involved in the global issue of endocrine disruptors, the "environmental hormone".

Although a significant number of chemicals possessing a hormone-like action have been recognized for many years, and the action of their biological plausibility related to the receptor-mediated effects strongly suggests possible human effects comparable to hormonal changes in wildlife, little is known about evidences or adversities in experimental animals and humans. The most essential key to resolving these dilemmas may be to understand the mechanism of actions (i.e., a possible low-dose issue). In other words, the mechanism at the low-dose effect may be resolved simultaneously by the mechanism of three major questions linked to the low-dose issue; namely, threshold, possible oscillation, and additive and/or synergistic action.

INTRODUCTION

The objective of this paper is to summarize all currently available information on hormonally active agents and plausible relationships to adverse effects on human health from the standpoints of the mechanisms of action of these chemicals.

It is not uncommon to come across agrochemicals and industrial chemicals that have hormone-mimic effects. These chemicals, the so-called "environmental hormones", often accumulate at detectable levels in the environment, and it has been feared that they may have adverse effects not only on wildlife but also on human beings. Following reports of feminization and decreased colony size of wild creatures, and reports suggesting a possible association of these chemicals with abnormalities of reproductive organs and oncogenesis in humans, attention has focused on the possibility that these occurrences may be associated with exposure to endocrine-disrupting chemicals (EDCs). In this connection, we would like to draw the attention of the reader to a Japanese translation of the book *Our Stolen Future*, written by Theo Colborn et al.

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This paper will review the subjects related to EDCs, the courses of arguments regarding the possible hazards of these chemicals, and current medical subjects pertaining to them.

CHEMICALS WITH HORMONE-MIMIC ACTIONS

Substances with hormone-mimic effects can be divided into four groups:

- hormones found in vivo;
- medicines with hormone-mimic actions manufactured for use in hormonal therapy, etc.;
- plant hormones known to exert phytoestrogen-like actions; and
- chemicals found in environments that can interact with hormone receptors.

In addition, substances that do not interact with hormone receptors but exert effects on gonads by their modifying effects on steroid metabolism may be deemed as hormone-mimics in the broader sense of the term. In this paper, however, emphasis shall be placed on the hormone-mimic actions mediated by receptors that play essential roles in the mechanism of actions of hormone-mimics.

CHARACTERISTICS OF THE RECEPTOR-MEDIATED ACTIONS OF HORMONE-MIMICS

The receptor-mediated actions of hormone-mimics are fundamentally characterized by the similarity in structures of the receptors involved, crossing the barrier of animal species. These characteristics allow us to speculate the possibility that the actions of these chemicals exerted in nature may also occur in humans.

Second, since similarities in the structure of various sex steroids and hormones are also known, it is possible that each individual hormone-mimic exerts diverse effects by acting on male hormone receptors, female hormone receptors, and nuclear receptors (including many orphan receptors), etc.

Third, many of these chemicals are excreted from the living body in the form of conjugated inactive substances instead of as degraded metabolites. They may also be eliminated in the unchanged form. Therefore, if feces and urine containing these substances are eliminated into river water, it is plausible to imagine that even inactivated hormones can sometimes become active and exert hormone-mimic actions in the environment. This is one of the characteristics unique to this class of chemicals.

Receptor-mediated responses involve many unresolved questions. Various undefined elements may be involved, including the relationship between receptor binding and signals, the relationship between receptor-ligand binding (ligand: substances that can bind to receptors) and the dissociation of ligands from receptors, signal cross-talks, involvement of unknown nuclear receptors, etc.

The actions of these chemicals add to the effects of intrinsic hormones. For this reason, these chemicals may exert their actions in a way different from that known for other chemicals that do not have structural or functional counterparts in vivo. For example, stimulation of hormone receptors by these extrinsic chemicals may modify homeostasis in vivo, leading to down-modulation of the physiological stimulation of these receptors by the intrinsic ligands. Therefore, the influence of the continued effects of environmental hormones needs special study.

PITFALL IN THE EFFECTS OF HORMONE-MIMICS

We must distinguish the interactions of endocrine hormone-mimics with hormone receptors from the hazards caused to endocrine tissue. Bearing this in mind, let us now summarize the problems related to the effects of hormone-mimics.

Antagonistic effects maintaining homeostasis

The endocrine system is regulated by homeostatic mechanisms. It is not uncommon for the effects of small amounts of hormone-mimics to interfere slightly with these mechanisms, often with no adverse influence; this is well known. However, this is not always the case. There seems to be a group of genes that act antagonistically to each other in the maintenance of homeostasis.

With the uterotrophic assay, which is used to check for estrogenic activity, the ovary is removed in advance and the blood level of the intrinsic female hormone is reduced to the minimum. Under the thus-created extremely shrinking state of the uterus, the test substance (a chemical or hormone) is administered to evaluate for its effects on the inflation of the uterus. This test (checking for growth of the uterus in ovariectomized animals) is designed to evaluate the hormone activity and effects of hormone-mimics under conditions of blockade of homeostasis.

This test method itself is valid. However, there is no sufficient rational evidence that indicates that the responses observed under such indirect control conditions of the living body can serve as an indicator of the health hazards of hormone-mimics. Although the ovo-testes seen in lower vertebrates may be used if the effects observed were to be valid as such an indicator, there is no consensus on what is valid as an indicator of the health hazards of EDCs when mammals are used as experimental animals.

Down-regulation of the expression of receptors

It is known that the expression of gene-encoding receptors is down-regulated by continuous stimuli, leading to reduced receptor activity. This can lead to a paradoxical outcome wherein the effects observed in the presence of low levels of a substance are not seen at high levels of the same substance. If this phenomenon occurred in individual organisms, the dose–response relationship will be nonlinear.

This means that extrapolation of results obtained at high levels of the chemicals, to conditions where low levels of the same substance are present, would be difficult. It is needed to test the validity of this hypothesis; analysis of the mechanisms underlying this phenomenon if the hypothesis were indeed valid, are thus important. Studies to resolve these questions are now under way.

Data gap concerning the effects of female hormones

In mature women, there are high levels of physiological hormones in vivo, and these are subject to cyclic control. It has been proposed that girls with inadequate physical growth begin menstruation at lower ages and undergo sexual maturation earlier than usual, and that hormone-mimics in these subjects can precipitate breast cancer.

The weak links in this hypothesis have been pointed out, and it has been shown experimentally that estrogen by itself may be teratogenic, although this tendency has been shown to be weak. It is known that organisms are programmed such that excessive exposure to estrogens during the intrauterine period or other developmental stages is avoided.

There are many open questions as to the mechanism by which mature females remain physiologically stable, even when exposed daily to high levels of estrogen (400 pM/l). Some additional dramatic effects may be needed to disturb this homeostatic physiology.

Multigeneration tests and effects on fetuses

It has been shown that exposure to hormones or hormone-mimics during intrauterine or early neonatal periods can lead to irreversible changes in the pattern of development. This susceptibility period is short, extending from the 13^{th} gestational day to about one week after birth. These effects are the so-called "intrauterine window effects."

In animal studies involving observation of experimental animals for two or more generations, no effects of EDCs have been demonstrated. The question therefore arises as to why window effects are observed during the short period mentioned above. It is unknown whether or not these effects really do occur, and if they do, how they are produced.

Delayed growth of the thalamic nucleus specific to males (called sexual dimorphic nucleus) is seen in male rats treated with female hormones. We may say that under conditions of homeostasis of the physiological hormones in mature individuals, exposure to dose levels that usually cause only reversible changes can lead to irreversible changes, if the exposure occurs during genesis, morphogenesis, or functional development. However, there are no ample data endorsing this view in humans.

Considering the biological plausibility inferred from the experimental data accumulated to date*, we may say that there are no sufficient data that clearly rule out this view. Close attention has therefore been paid to these effects in children.

New theories of methodology, focusing on effects in fetuses and children, are now being developed, primarily in the United States, or the World Health Organization, within the framework of children's program, etc.

HEALTH HAZARDS AT LOW LEVELS OF EXPOSURE

Chemicals used for agriculture or industrial purposes are marketed, in general, only after their effects on living beings have been investigated. We may therefore understand that they are used on the premise that the possibility of these chemicals exerting hazardous effects on health at relatively high-dose levels has been almost ruled out. Nevertheless, problems with EDCs have begun to be highlighted. These problems may not be confined to those related to the accumulation of these substances through food chains in the ecosystem, but also to the additional possibility that these chemicals may exert effects at low-dose levels even if they have been declared safe at high-dose levels. The latter possibility may apply, however, only to some cases and not to others.

We may say that a major issue pertaining to EDCs that must be resolved urgently is whether or not they pose health hazards at low-dose levels. This issue can be summarized into the following three questions:

- presence/absence of threshold level;
- presence/absence of synergistic or additive effects; and
- possibility of extrapolation of high-dose effects to low-dose levels (i.e., presence/absence of a linear dose–response relationship).

No clear-cut answers have as yet emerged to these questions. Considering the above-mentioned characteristics of the effects of hormones, it is plausible to imagine how difficult it may be to resolve these questions.

To determine if these chemicals exerted hazardous effects on health at low-dose levels, the following basic questions may need to be considered; their biological plausibility is hardly denied.

- Regarding the presence or absence of threshold levels, it seems likely that many chemicals suspected of being EDCs can easily permeate across the cell membrane, which is composed of phospholipids. Therefore, assuming that one receptor molecule reacts with one chemical molecule, the lower limit of the dose level exerting the chemical's effects would be extremely low.

  Of course, since the probability of the binding of a ligand to the receptor will be low if the dose level is low, we cannot say that there is no threshold level for the effects seen in the low-

*Biological plausibility: Likelihood of a phenomenon as judged by considering the difference or similarity of elements of reactions in individual organisms, on the basis of the results of a series of related biological experiments. (Cf. probability)

dose-level range. In fact, for bisphenol A (which has been attracting close attention because of its hazardous effects on health at low-dose levels), the presence/absence of a threshold level has not yet been reported. It seems rational, therefore, to assume that these health hazards occur in a very low-dose-level range.

- If we consider not only the affinity of each substance for the receptor, but also the nonlinearity of responses (e.g., waveform responses as a result of reduced receptor expression following an increase in dose level), it is possible to assume that there are U-shaped or reverse U-shaped reactions, or oscillational dose–response curves. Interim data endorsing such a view are being accumulated.
- Regarding the possibility of synergistic or additive effects, the observation of additive effects among different nuclear receptors has been reported. Data yielded by analysis of interactions between receptor signals also suggest such a possibility. In fact, the dose–response curves for some composite materials were reported to be additive, but not synergistic.

Thus, the questions on health hazards at low-dose levels have several aspects:

- type of receptor-mediated actions of the hormone mimics;
- diverse reactive characteristics on the part of the receptors;
- diverse modification during expression of intracellular signals; and
- factors involved in irreversible changes related to morphogenesis and functional development.

Resolution of all these aspects of the question will lead to clarification of the mechanism of actions of the substances from each of the aforementioned standpoints. While these questions are among the hottest research themes at present, they are certainly unlikely to be resolved easily.

At a workshop held in North Carolina, USA, in October 2000, health hazards of chemicals at low-dose levels were discussed. Investigators for and against the possibility of these substances posing health hazards at low-dose levels gave detailed accounts of their studies, and no definitive conclusions could be reached, as the arguments of both sides appeared to be tenable.

This means that reports affirming the plausibility of these substances posing health hazards at low-dose levels in animal experiments cannot be immediately rejected. The workshop concluded by pointing out the necessity of paying attention to the possible hazards on fetuses and neonates.

HEALTH HAZARDS OF HORMONE-MIMICS TO HUMANS

The possibility of health hazards of hormone-mimics to human beings have not been supported by adequate epidemiological data, and the number of cases for which the data clearly endorse such effects is quite small. The U.S. National Research Council (NRC) emphasizes the necessity of conducting further epidemiological studies on this topic (NRC, 1999).

In conclusion, this paper summarizes the current knowledge concerning the health hazards of hormone-mimics to humans. Reports dealing with the effects of these substances on humans are confined to those pertaining to the effects of dioxins and polychlorinated biphenyls (PCBs); the validity and usefulness of these results have not yet been established.

The following information is based on case studies conducted to date.

Health hazards of dioxins

Regarding health hazards of dioxins, two-year dosing studies revealed weight loss and liver damage, and three-generation reproductive studies in rats disclosed intrauterine death and a decrease in litter size. Onset of endometriosis in rhesus monkeys has also been reported.

A causal relationship of EDCs to the following episodes in humans has been suggested: biased male-to-female ratio in children born in the dioxin-exposed Seveso area of Italy, and increased inci-

dence of cleft palate in the Diemerzeedijk district of the Netherlands, probably due to steroids. In both of these cases, the U.S. Environmental Protection Agency (USEPA) did not affirm a causal relationship, and classified them as cases requiring special attention.

No consensus has been reached concerning the relationship of hypothyroidism observed in the inhabitants along Lake Michigan to the ingestion of PBB- (polybrominated biphenyls-) contaminated fish.

Effects on mature females (e.g., increased incidence of breast cancer)

No reports affirm the effects of dioxins on mature human females (e.g., effects on breast cancer or endometriosis as discussed below). There are many unresolved questions on this topic. However, none of the studies conducted in mature experimental animals revealed data endorsing the plausibility of occurrence of such effects. On the other hand, it is known that the age at menarche is lower and the incidence of breast cancer higher in females exposed to dioxins. Some investigators cite these data when discussing the health hazards of dioxins. It is also known that females exposed to dioxins are often taller.

In European countries, a height increase of about 3.5 mm per year and an approximately one-year decrease in the age at menarche have been reported during the past 30 years. It is difficult to identify the influence of extrinsic endocrine factors on these changes, and no studies addressing this issue have been reported to date. Although several studies have been published concerning the effects of female hormone preparations, including pills used for contraception and hormone replacement therapy in postmenopausal women, no studies have provided data that establish the effects of EDCs.

Endometriosis

Endometriosis is a disease of unexplained origin that is seen in primates with sexual cycles. It has been pointed out that this disease tends to be more severe in individuals exposed to dioxins (2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD] and to PCBs). Data yielded from experiments in rhesus monkeys are used as evidence to corroborate the causal relationship between dioxins and endometriosis. Thus, we cannot rule out the biological plausibility of these effects. However, no reports affirming the causal relationship in humans have been published.

Possibility of other effects on humans

Biological plausibility has also been considered for the following effects of hormone-mimics on humans: qualitative dysfunction of human sperm, effects on neurobehavior of neonates, and immune functions. The effects on immune functions have been suggested by reports of cases with Yu-sho (PCB intoxication).

CONCLUSION

The International Program of Chemical Safety (IPCS), a section of the World Health Organization, has released a Web site publication "Global Assessment of the State-of-the Science of Endocrine Disruptors" (GAED), June 2002 (URL: <http://ehp.niehs.hih.gov/who/>). WHO/IPCS started the GAED program in March 1998 after the publication of *Our Stolen Future* (Theo Colbone et al., 1996). The publication took three years to edit; covering a policy to document all the published pertinent literatures, to summarize them as descriptive manner solely based on those published literatures. Twenty-seven expert scientists and 20 independent peer-reviewers participated in editing the GAED.

Other reports on nonylphenol and octylphenol, released by the Japanese Ministry of Environment (MoE), revealed an "ovotestes" formation that was observed in the assay of the laboratory experimen-

tal fish (*Medaka*) exposed to doses close to those recorded in the monitoring fields in the MoE surveillance. Further, phthalates, such as di-(2-ethylhexyl)phthalate, di-cyclohexylphthalate, and butylbenzylphthalate, as selected and prioritized chemicals by the MoE, showed some unique data in different endpoints, including mRNA expression, in dose ranges lower than those no observed effect levels (NOELs) and/or no observed adverse effect levels (NOAELs) reported previously.

The effects of EDCs on human health are unknown at this moment. However, due to the biologically plausible data currently accumulated, the existence of endocrine disruptions under certain circumstances seems to be a reality. Thus, by the time of the SCOPE/IUPAC symposium, the EDC research for the next stage may shift from plausibility to possibility, and put forward further mechanistic research.

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Implications of Endocrine Active Substances for Humans and Wildlife—a SCOPE/IUPAC Project

- Molecular Mode of Action of Nuclear Receptors: Fundamentals for Understanding the Action of Endocrine Active Substances
- Environmental Fate and Metabolism of Endocrine Active Substances
- Effects of Endocrine Active Chemicals in Rodents and Humans, and Risk Assessments for Humans
- Effects of Endocrine Active Substances in Wildlife Species
- Effectiveness of OSAR for Prescreening of Endocrine Disruptor Hazard
- Toxicogenomics as a Rational Approach to Endocrine Disruptor Research
- The Need for Establishing Integrated Monitoring Programs
- Simple, Rapid Assay for Conventional Definitive Testings of Endocrine Disruptor Hazard
- Precautionary Principle Approach and Weight of Evidence in Endocrine Disruptor Issues
- Risk Management Options for Endocrine Disruptors in National and International Programs

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Special Topic Issue

Health Hazards of Endocrine-Disrupting Chemicals on Humans as Examined from the Standpoints of Their Mechanisms of Action

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Abstract: Hormonally active compounds were first recognized in "*Silent Spring*" by Rachel Carson in 1962, which implicated pesticides, such as DDT and derivatives. Nearly four decades later, the book "*Our Stolen Figure*," by Theo Colborn *et al.*, and other pertinent publications have revisited and broadened the issue to a variety of chemicals and areas exposed. Translations of these books have just become available in Japan in the past three or four years, and since then Japan has started to join the debate and/or discussion of the global issue of endocrine disruptors—"Environmental Hormones." Although significant numbers of chemicals possessing a hormone-mimicking action have been recognized for many years and based on biological plausibility their receptor-mediating effects strongly suggest effects in humans similar to those seen in wildlife, little is known about the experimental evidence related to human adverse effects. The key issue in resolving the dilemmas posed by the biological plausibility and poor experimental evidence may be to clarify their mechanism of actions at low levels. In other words, the mechanisms of the possible low-dose effects may be resolved simultaneously by defining three major properties threshold, oscillation, and additive-synergism.

Key words: Receptor; Hormone mimics; Homeostasis; Effects at low dosage; Human hazards

Introduction

The objective of this paper is to summarize all the currently available information on the possible hazards of endocrine-disrupting chemicals (EDs) on human health from the stand-

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points of the mechanisms of actions of these chemicals.

It is not uncommon to come across agrochemicals and industrial chemicals that have hormone-mimicking effects. These chemicals, the so-called "environmental hormones," often accumulate at detectable levels in the environment, and it has been feared that they may have adverse effects on living beings. Following reports of feminization and decreased colony size of wild creatures, and reports suggesting a possible association of these chemicals with abnormalities of reproductive organs and oncogenesis in human, attention has been focused on the possibility that these occurrences may be associated with exposure to EDs. In this connection, a Japanese translation of the book entitled "*Our Stolen Future*," written by Theo Colborn *et al.*, was published some time ago.

This paper will review the problems related to EDs, the courses of arguments regarding the harmful effects of these chemicals, and current medical topics pertaining to them.

Chemicals with Hormone-Mimicking Actions

Substances with hormone-mimicking effects can be divided into four groups: (1) hormones found *in vivo*, (2) medicines with hormone-mimic actions manufactured for use in hormonal therapy, etc., (3) plant hormones known to exert phytoestrogen-like actions, and (4) chemicals found in environments that can interact with hormone receptors.

In addition, substances which do not interact with hormone receptors but exert effects on gonads by their modifying effects on steroid metabolism may be deemed as hormone-mimics in the broader sense of the term. In this paper, however, emphasis shall be placed on the hormone-mimicking actions mediated by receptors which play essential roles in the mechanism of actions of hormone-mimics.

Characteristics of the Receptor-Mediated Actions of Hormone-Mimics

The receptor-mediated actions of hormone-mimics are fundamentally characterized by the similarity in the structures of the receptors involved, crossing the barrier of species. This characteristic allows us to estimate the possibility of the actions of these chemicals exerted in nature also occurring in humans.

Secondly, since similarities in the structure to various sex steroids and hormones are also known, it is possible that each individual hormone-mimic exerts diverse effects by acting on male hormone receptors, female hormone receptors, receptors in the nuclei (including some unknown receptors), etc.

Thirdly, many of these chemicals are eliminated from the living body in the form of conjugated inactive substances instead of as degraded metabolites. They may also be eliminated in the unchanged form. Therefore, if feces and urine containing these substances are eliminated into river water, it is plausible to imagine that even inactivated hormones can sometimes become active and exert hormone-mimic actions in the environment. This is one of the characteristics unique to this class of chemicals.

Receptor-mediated responses involve many unresolved questions. Various undefined elements may be involved, including the relationship between receptor binding and signals, the relationship between receptor-ligand binding (ligand: substances that can bind to receptors) and the dissociation of ligands from receptors, signal cross-talks, involvement of unknown nuclear receptors, etc.

The actions of these chemicals add to the effects of intrinsic hormones. For this reason, these chemicals may exert their actions in a way different from that known for other chemicals which do not have structural or functional counterparts *in vivo*. For example, stimulation of hormone receptors by these extrinsic chemicals may modify homeostasis *in vivo*, leading

to weakening of the physiological stimulation of these receptors by the intrinsic substances. Therefore, the influence of the continued effects of environmental hormones needs special study.

Pitfall in the Effects of Hormone-Mimics

We must distinguish the interactions of endocrine hormone-mimics with hormone receptors from the hazards caused to endocrine tissue. Bearing this in mind, let us now summarize the problems related to the effects of hormone-mimics.

1. Antagonistic effects on the maintenance of homeostasis

The endocrine system is regulated by homeostatic mechanisms. It is not uncommon for the effects of small amounts of hormone-mimics to interfere slightly with these mechanisms, often with no adverse influence; this is well-known. However, this is not always the case. There seems to be a group of genes that act antagonistically to each other in the maintenance of homeostasis.

With the uterus growth test, which is used to check for estrogenic activity, the ovary is removed in advance and the blood level of the intrinsic female hormone is reduced to the minimum. Under the thus-created extremely undeveloped state of the uterus, the test substance (a chemical or hormone) is administered to check for its effects on the growth of the uterus. This test (checking for growth of the uterus in ovariectomized animals) is designed to evaluate the hormone activity and effects of hormone-mimics under conditions of blockade of homeostasis.

This test method itself is valid. However, there is no sufficient rational evidence that indicates that the responses observed under such indirect control conditions of the living body can serve as an indicator of the health hazards of hormone mimics. Although the ovotestes seen in lower vertebrates may be used if the effects observed were to be valid as such an indicator, there is no consensus on what is valid as an indicator of the health hazards of ED's when mammals are used as experimental animals.

2. Down-regulation of the expression of receptors

It is known that the expression of genes encoding receptors is down-regulated by stimuli, leading to reduced receptor sensitivity. This can lead to a paradoxical outcome wherein the effects observed in the presence of low levels of a substance are not seen at high levels of the same substance. If this phenomenon occurred in individual organisms, the dose-response relationship will be non-linear.

This means that extrapolation of results obtained at high levels of the chemicals to conditions where low levels of the same substance are present would be difficult. It is needed to test the validity of this hypothesis, and analysis of the mechanisms underlying this phenomenon if the hypothesis were indeed valid, are thus important. Studies to resolve these questions are now under way.

3. Data gap concerning the effects of female hormones

In mature women, there are high levels of physiological hormones *in vivo*, and these are subject to cyclic control. It has been proposed that girls with inadequate physical growth begin menstruation at lower ages and undergo sexual maturation earlier than usual, and that hormone-mimics in these subjects can precipitate breast cancer.

The weak links in this hypothesis have been pointed out, and it has been shown experimentally that estrogen by itself may be teratogenic, although this tendency has been shown to be weak. It is known that organisms are programmed such that excessive exposure to estrogens during the intrauterine period or other developmental stages is avoided.

There are many open questions as to the

mechanism by which mature females remain physiologically stable, even when exposed daily to high levels of estrogen (400pM/*l*). Some dramatic effects are probably needed to disturb this physiology.

4. Multi-generation tests and effects on fetuses

It has been shown that exposure to hormones or hormone-mimics during intrauterine or early neonatal periods can lead to irreversible changes in the pattern of development. This susceptibility period is short, extending from the 13th gestational day to about one week after birth. These effects are the so-called "intrauterine window effects."

In animal studies involving observation of experimental animals for two or more generations, no effects of EDs have been demonstrated. The question therefore arises as to why window effects are observed during the short period mentioned above. It is unknown whether or not these effects really do occur, and if they do, how are they produced.

Delayed growth of the thalamic nucleus specific to males (called sexual type II nucleus) is seen in male rats treated with female hormones. We may say that under conditions of homeostasis of the physiological hormones in mature individuals, exposure to dose levels that usually cause only reversible changes can lead to irreversible changes, if the exposure occurs during genesis, morphogenesis or functional development. However, there are no ample data endorsing this view in humans.

Considering the biological plausibility inferred from the experimental data accumulated to date,[*1] we may say that there are no sufficient data that clearly rule out this view. Close attention has therefore been paid to these effects in children.

New theories of methodology, focusing on the effects in fetuses and children, are now being developed, primarily in the United States, within the framework of children's program, etc.

Health Hazards at Low Levels of Exposure

Chemicals used for agriculture or industrial purposes are marketed, in general, only after their effects on living beings have been investigated. We may therefore understand that they are used on the premise that the possibility of these chemicals exerting hazardous effects on health at relatively high dose levels has been almost ruled out. Nevertheless, problems with EDs have begun to be highlighted. These problems may be not confined to those related to the accumulation of these substances through food chains in the ecosystem, but also to the possibility additionally that these chemicals may exert effects at low dose levels even if they have been declared safe at high dose levels. The latter possibility may apply, however, only to some cases and not to others.

We may say that a major issue pertaining to EDs that must be resolved urgently is whether or not they pose health hazards at low dose levels. This issue can be summarized into the following three questions: (1) presence/absence of threshold level, (2) presence/absence of synergistic or additive effects, and (3) possibility of extrapolation of high-dose effects to low-dose levels (i.e., presence/absence of a linear dose-response relationship). No clear-cut answers have as yet emerged to these questions. Considering the above-mentioned characteristics of the effects of hormones, it is plausible to imagine how difficult it may be to resolve these questions.

To determine if these chemicals exerted hazardous effects on health at low dose levels, the following basic questions may need to be considered; their biological plausibility is hardly denied.

[*1] Biological plausibility: Likelihood of a phenomenon as judged by considering the difference or similarity of elements of reactions in individual organisms, on the basis of the results of a series of a related biological experiments. (cf. probability)

(1) Regarding the presence or absence of threshold levels, it seems likely that many chemicals suspected of being EDs can easily permeate across the cell membrane, which is composed of phospholipids. Therefore, assuming that one receptor molecule reacts with one chemical molecule, the lower limit of the dose level exerting the chemical's effects would be very low.

Of course, since the probability of the binding of a ligand to the receptor will be low if the dose level is low, we cannot say that there is no threshold level for the effects seen in the low dose level range. In fact, for bisfenol A, which has been attracting close attention because of its hazardous effects on health at low dose levels, the presence/absence of a threshold level has not yet been reported. It seems rational, therefore, to assume that these health hazards occur in a very low dose level range.

(2) If we consider not only the affinity of each substance for the receptor, but also the non-linearity of responses (e.g., waveform responses as a result of reduced receptor expression following an increase in dose level), it is possible to assume that there are U-shaped or reverse U-shaped reactions or oscillational dose-response curves. *Interim* data endorsing such a view are being accumulated.

(3) Regarding the possibility of synergistic or additive effects, the observation of additive effects among different nuclear receptors has been reported. Data yielded by analysis of interactions between receptor signals also suggest such a possibility. In fact, the dose-response curves for some composite materials were reported to be additive, but not synergistic.

Thus, the questions on health hazards at low dose levels have several aspects: (1) the type of receptor-mediated actions of the hormone mimics, (2) diverse reactive characteristics on the part of the receptors, (3) diverse modification during expression of intracellular signals, and (4) factors involved in irreversible changes related to morphogenesis and functional development. Resolution of all these aspects of the question will lead to clarification of the mechanism of actions of the substances from each of the aforementioned standpoints. While these questions are among the hottest research themes at present, they are certainly unlikely to be resolved easily.

At a workshop held in North Carolina, USA, in October 2000, health hazards of chemicals at low dose levels were discussed. Investigators for and against the possibility of these substances posing health hazards at low dose levels gave detailed accounts of their studies, and no definitive conclusions could be reached, as the arguments of both sides appeared to be tenable.

This means that reports affirming the plausibility of these substances posing health hazards at low dose levels in animal experiments cannot be immediately rejected. The workshop concluded by pointing out the necessity of paying attention to the possible hazards on fetuses and neonates.

Health Hazards of Hormone-Mimics on Humans

The possibility of health hazards of hormone-mimics on humans have not been supported by adequate epidemiological data, and the number of cases for which the data clearly endorse such effects is quite small. The US National Research Council emphasizes the necessity of conducting further epidemiological studies on this topic (National Research Council, 1999).

In conclusion, this paper summarizes the current knowledge concerning the health hazards of hormone-mimics on humans. Reports dealing with the effects of these substances on humans are confined to those pertaining to the effects of dioxins and PCB, and the validity and usefulness of these results have not yet been established.

The following are based on case studies conducted to date.

1. Health hazards of dioxins

Regarding health hazards of dioxins, two-year dosing studies revealed weight loss and liver damage, and three-generation reproductive studies in rats disclosed intrauterine death and a decrease in litter size. Onset of endometriosis in rhesus monkeys has also been reported.

A causal relationship of EDs to the following episodes in humans has been suggested: biased male-to-female ratio in children born in the dioxin-exposed Seveso area of Italy, and increased incidence of cleft palate in the Diemerzeedijk district of the Netherlands, probably due to steroids. In both of these cases, the Environmental Protection Agency (EPA) of the United States did not affirm a causal relationship, and treated classified them as cases requiring special attention.

No consensus has been reached concerning the relationship of hypothyroidism observed in the inhabitants along Lake Michigan to the ingestion of PBB (polybrominated biphenyls)-contaminated fish.

2. Effects on mature females, e.g., increased incidence of breast cancer

No reports affirming the effects of dioxins on mature human females (e.g., effects on breast cancer or endometriosis as discussed below). There are many unresolved questions on this topic. However, none of the studies conducted in mature experimental animals revealed data endorsing the plausibility of occurrence of such effects. On the other hand, it is known that the age at menarche is lower and the incidence of breast cancer higher in females exposed to dioxins. Some investigators cite these data when discussing the health hazards of dioxins. It is also known that females exposed to dioxins are often taller.

In European countries, a height increase of about 3.5 mm per year and an approximately one-year decrease in the age at menarche have been reported during the past 30 years. It is difficult to identify the influence of extrinsic endocrine factors on these changes, and no studies addressing this issue have been reported to date. Although a number of studies have been published concerning the effects of female hormone preparations, including pills used for contraception and hormone replacement therapy in postmenopausal women, no studies have provided data that establish the effects of EDs.

3. Endometriosis

Endometriosis is a disease of unexplained origin that is seen in primates with sexual cycles. It has been pointed out that this disease tends to be more severe in individuals exposed to dioxins (TCDD/PCBs). Data yielded from experiments in rhesus monkeys are used as evidence to corroborate the causal relationship between dioxins and endometriosis. We cannot thus rule out the biological plausibility of these effects. However, no reports affirming the causal relationship in humans have been published.

4. Possibility of other effects on humans

Biological plausibility has been pointed out also on the following effects of hormone-mimics on humans: qualitative dysfunction of human sperm, effects on neurobehavior of neonates, and immune functions. The effects on immune functions have been suggested by reports of cases with Yu-sho (PCB intoxication).