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Environment Agency's Basic Policy on Environmental Endocrine Disruptors

Strategic Programs on Environmental Endocrine Disruptors SPEED '98

Approach and Achievement

Since the 1960s, concerns have been raised from the global observation of wildlife, that some chemicals in the environment may behave in the similar way to endogenous hormones and disrupt their endocrine systems. In 1996, a group of Dr. Theo Colborn, an American zoologist, published "Our Stolen Future" pointing out the serious effects of chemicals on wildlife, and sensationally arose the fears that there might be similar effects on humans as well.

In May 1998, Ministry of the Environment (MoE; the Environmental Agency formerly before January 6th, 2001) summarized its basic policy on Environmental Endocrine Disruptors in "the Strategic Programs on Environmental Endocrine Disruptors -SPEED '98-" (made revisions and additions based on more recent knowledge in November 2000) based on the examinations by the expert panel. According to the programs, MoE has promoted various measures including monitoring of chemicals suspected of having endocrine disrupting effects in the environment, pursuing a variety of effects possibly occurring in the organisms via the actions on the endocrine system, and participating internationally in the collaborative studies.

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What is the Endocrine Disrupting Effect?

What kind of mechanism is thought to be involved in the endocrine disrupting effect?

Some chemicals can attach to the so-called “receptors” that are the cellular binding proteins for the original hormones. When exogenous chemicals bind to the receptors, original hormonal actions can be inhibited, otherwise activated at undesirable growth stages. Some other chemicals can change the hormonal levels in the blood stream by affecting their biosynthesis or degradation. In SPEED ’98, these disrupting effects on the endogenous hormone functions were called as “endocrine disrupting effects.” MoE has decided to take a series of actions for this issue.

(1) Mechanism for estrogenic actions
(2) Estrogens
(3) Endocrine disruptors
(4) Cell
(5) Nucleus
(6) Transcription
(7) Protein synthesis
(8) Dimerization between an endocrine disruptor and an ER (estrogen receptor) can trigger estrogenic action.
(9) ERs (estrogen receptors): can bind to estrogens and activate the gene (DNA).
(10) Source: Strategic Programs on Environmental Endocrine Disruptors -SPEED ’98-

SPEED ’98 has taken the initiative for broad scientific research into how the endocrine disrupting effects occur.

Experimental study to reveal the localization of receptors in human cells and animals, or to investigate the effects of the receptor binding on the cellular gene expression is one of the approaches to clarify the mechanism of action. It was so far recognized that chemicals such as Nonylphenol and 4-t-Octylphenol could bind to the different receptors on the human cell nuclei. However, the practical effects caused biologically by such phenomena were not understood in detail, thus experimental studies are continuing further research.

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Concentration of Chemicals in the Environment and Situation of Wildlife

For 18 chemicals suspected of having endocrine disrupting effects, residual concentrations have being monitored in the water from approximately 100 sites of rivers, lakes and ponds in Japan. Similarly, 13 chemicals have being monitored in the air at approximately 20 sites. PCBs, Alkylphenols, Bisphenol A, and 17beta-Estradiol were detected in the water at more than half of the total sites in 2002, whereas 4-Nitrotoluene and trans-Nonachlor were detected in the air at more than 10 sites.

(1) Water sampling
(2) Kite
(3) Leopard frogs

Some Japanese wildlife such as kites and frogs have been investigated for their body burden of the chemicals suspected of having endocrine disrupting effects and their histopathological situation. No cause-effect relationship has been drawn so far between the detected body burden and anomaly in each species (such as ova-testis in frogs).

On the other hand, abnormal reproductive organs (male reproductive organs are found in female snails) of *Thais clavigera* (a snail species) have been observed over the broad coastal area in Japan. The same abnormality was identified when female animals of *T. clavigera* were exposed in an experimental condition to the same chemical concentration as found in the environment. [1)] Study on the mechanism is now in progress.

1) Experimental exposure of Tributyltin, Triphenyltin, Tripropyltin, and Tricyclohexyltin caused reproductive abnormality in *T. clavigera*. Among these chemicals, the detection of Tripropyltin and Tricyclohexyltin is relatively rare in the environment. Thus, the phenomena observed throughout Japan seem to have been caused by Tributyltin and Triphenyltin. Though these causal organic tin compounds had been used as the paints on the bottoms of ships (antifoulings), their domestic use has almost disappeared by approaches such as regulation by “the Law Concerning Examination and Regulation of Manufacture and Handling of Chemical Substances” and voluntary cancellation.

(1) Survey on imposex (defined as penis or vas deferens observed in female) in *T. clavigera* in Japan (from September 1996 to January 1999). The sample size was 20-30 at each site.
(2) Provided by Dr. Toshihiro Horiguchi, National Institute for Environmental Studies

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Studying the Endocrine Disrupting Effects on Various Biological Species

Avian, Amphibian and Invertebrate

The test methods with these biological species are now being developed.

(1) Arthropoda
(2) Mollusca
(3) Annelida
(4) Nemertinea
(5) Platyhelminthes
(6) Porifera
(7) Aves
(8) Amphibia
(9) Aschelminthes
(10) Primal organisms
(11) Mammalia
(12) Reptilia
(13) Pisces
(13)' Vertebrates
(14) Protochordata
(15) Echinodermata
(16) Pogonophora
(17) Protozoa
(18) Evolutionary tree
(19) *Daphnia magna*
(20) Quail
(21) South African Clawed Frog
(22) Japanese Medaka in Laboratory

Fish

MoE has already developed the test methods with fish species such as the vitellogenin test [2)] and life cycle test. [3)] Among them, the life cycle test with Japanese Medaka was extensively performed and validated.

[2] The vitellogenin test is a method to detect female hormone-like effects by observing the phenomena in which male fish can produce a female specific substance (vitellogenin) when exposed to the test substances.

[3] The life cycle test is a method to observe the changes in vitellogenin production, fertility, and so on during the period from egg to adulthood in water containing a test chemical. Fertile eggs are exposed until 60 days after hatching in the partial life cycle test, while the exposure in the full life cycle test is prolonged as long as 100 days after hatching and the effects on the next generation is to be checked.

Results of life cycle and related tests with Japanese Medaka

Tests for the chemicals of higher priority in SPEED ’98 were carried out at the exposure levels relevant to those found in the environment. 24 chemicals already tested or evaluated so far (July 2004). [4] The endocrine disrupting effects of Nonylphenol, 4-t-Octylphenol and Bisphenol A were assumed though they were found less potent than that 17beta-Estradiol (the authentic hormone). The remaining 21 chemicals have shown no evident endocrine disrupting effects in the life cycle test with Japanese Medaka though some fluctuations in vitellogenin levels were observed depending on the exposure conditions (see Table 1).

[4] As of July 2004, 11 chemicals including Chlordane and DDE are now being tested (each isomer is the test chemicals for DDE and DDD). 4 chemicals including Dioxins and PCBs are independently regulated, thus excluded from the programs. Another 28 chemicals are being approached by ways such as reviewing available literature. Note that Nonylphenol and 4-t-Octylphenol are the members of “Alkylphenols”, and p,p’-DDT and o,p’-DDT belong to “DDT” on SPEED ’98.

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Table 1: Research Results

Test method / Test chemical	Result of life cycle test on Medaka *)	Result of 1-generation test on rats **)	Test method / Test chemical	Result of life cycle test on Medaka *)	Result of 1-generation test on rats **)
Hexachlorobenzene	D	-	Butyl benzyl phthalate	B	R
Pentachlorophenol	A	S	Di-n-butyl phthalate	E	Q
Amitrole	C	Q	Dicyclohexyl phthalate	D	S
Hexachlorocyclohexane	D	-	Diethyl phthalate	B	Q
p,p'-DDT	B	Q	2,4-Dichlorophenol	D	Q
o,p'-DDT	D	-	Di-(2-ethylhexyl) adipate	D	Q
p,p'-DDD	-	P	Benzophenone	D	S
Tributyltin chloride	D	S	4-Nitrotoluene	D	S
Triphenyltin chloride	B	S	Octachlorostyrene	A	S
4-Nonylphenol (branched type)	G	T	Dibenzyl phthalate	B	Q
4-t-Octylphenol	G	T	Dihexyl phthalate	B	S
Bisphenol A	F	P	Dipropyl phthalate	B	S
Di-(2-ethylhexyl) phthalate	C	Q			

*) Results from the life cycle test with Japanese Medaka and other related tests
http://www.env.go.jp./chemi/end/speed98/speed98-20.pdf

A: No finding as a result of the vitellogenin production test and the partial life cycle test.

B: Finding not relevant to the endocrine disrupting effects was observed as a result of the vitellogenin production test and the partial life cycle test.

C: Finding not evident but relevant to the endocrine disrupting effects was observed as a result of the vitellogenin production test and the partial life cycle test.

D: B and C above were observed as a result of the vitellogenin production test and the partial life cycle test.

E: Findings relevant or not relevant to the endocrine disrupting effects were observed as a result of the vitellogenin production test and the partial life cycle test. However, they were not considered the evident endocrine disrupting effects.

F: Finding relevant to the endocrine disrupting effects was observed as a result of the vitellogenin production test and the partial life cycle test. An endocrine disrupting effect was assumed. Finding not relevant to the endocrine disrupting effects was also observed.

G: Finding relevant to the endocrine disrupting effects was observed as a result of the vitellogenin production test and partial life cycle test. An endocrine disrupting effect was strongly assumed. Finding not relevant to the endocrine disrupting effects was also observed.

**) Result of 1-generation test on rats
http://www.env.go.jp/chemi/end/speed98/speed98-19.pdf

P: No significant response.

Q: Though a significant response was observed at a dose lower than the one already confirmed to have an effect, it was considered within the range of physiological fluctuation.

R: A significant response was observed at a dose lower than the one already confirmed to have an effect. Its significance is the issue for future examination.

S: P and Q above were observed.

T: P and Q above were observed (Ethinyl estradiol, a synthetic female hormone, was used as a positive substance).

-: Tests are in progress.

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Approach to the Effects on Humans

Tests on Rats

The 1-generation test method is carried out to apply the result obtained on rats to the evaluations of the effects on humans. According to this method, a test chemical is gavaged to mother animals during pregnancy until weaning. The changes in the mother and offspring are to be observed.

Results of the 1-generation test on rats

Tests were implemented for 22 chemicals at the doses relevant to the levels found in the environment. Effects such as increase or decrease in testis weight were observed for 17 chemicals at higher doses than environmental levels. However, none of them was confirmed to have the evident endocrine disrupting effects as far as observed in the 1-generation test on rats. (See Table 1)

Epidemiological Study on the Situation

The endocrine disrupting effects in fetuses are suggested to be more severe than the one found in adults. For this concern, the maternal transfer of various chemicals to fetus was monitored in the blood from umbilical cords, resulting in the detection of many lipophilic Organochlorines (such as DDT related pesticides and its metabolites, PCBs, Dioxins and Dibenzofurans). The high volume but shortly degradable material products for plastic, and/or the chemicals mainly found in soybean and related plants (Phytoestrogens) [5] that work similarly to the female hormones are also identified. However, it is still difficult to evaluate the potential effects caused by the transferred chemicals.

On the other hand, it has been concerned and suggested that the substances may trigger some congenital abnormalities such as cryptorchidism[6] and hypospadia[7]. Therefore, epidemiological studies [8] were employed to clarify the cause-effect relationship between exposure to Bisphenol A and the congenital abnormalities. The results obtained so far were rather ambiguous.

(1) Fetus

5) Phytoestrogens: A chemical group of the natural products found in plants and biologically active similarly to female hormones. This time, Genistein, Daidzein, and Equol have been monitored and their transfer was recognized.

6) Cryptorchidism: A symptom in which the testis should fail to descend from its intra-abdominal location down into the scrotum. High incidence is suggested among premature babies.

7) Hypospadias: A male congenital abnormality in which the urethra is opened not at the tip of the glans but on the midline from the glans to the perineum.

8) Epidemiological study: A research methodology to study the cause-effect relationship between the incidence of health-related effects such as congenital abnormalities and some suspected causal factors in statistical manners on special groups exposed to certain substances. Precise planning and a certain magnitude of sample size and period will be further necessary to identify another suspected factors such as smoking and multiple pregnancies.

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International Cooperation

There are many problems and issues to be solved regarding the endocrine disrupting effects including its mechanism, its relationship with the chemicals, and the development of simple test methods. Thus, international coordination to share the study is an important as the cooperation between relevant authorities and agencies within Japan.

Since 1998, MoE has organized “the International Symposium on Environmental Endocrine Disruptors” every year in Japan. In the symposium, not only the information exchange among eminent specialists from the world, but events for the public audience such as special lectures and panel discussions are held. About 10,000 people in total including 500 participants from overseas have attended.
http://www.env.go.jp/chemi/end/index3.html

(1) International Symposium
(2) OECD Conference

Furthermore, Japan has actively participated in the task force for the Endocrine Disruptor Testing and Assessment (EDTA) promoted by the Organization of Economic Cooperation and Development (OECD) and in the works on “Global Assessment of the State-of-the-Science of Endocrine Disruptors” published by the World Health Organization (WHO).

MoE also implements the collaboration research with the Republic of Korea and the UK.

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Future Problems

MoE has promoted studies on endocrine disrupting effects according to “the Strategic Programs on Environmental Endocrine Disruptors SPEED ’98” since 1998.

Through MoE’s approach and achievement, MoE has gradually recognized the importance of efforts to accumulate scientific knowledge via broader basic research and constant wildlife observation, focusing on a variety of ecological effects (not only human effects) as well as the effects on the immune and nervous systems via the endocrine system (not only the effects on the reproductive endocrine system). In addition, MoE hopes more active international partnership to develop the practical test methods and evaluate the suspected substances.

Responding to growing concern about the chemical safety, another important issue is the approach toward the risk communication. MoE needs to make efforts to explain the state of the science knowledge to the public at large in order to enlighten them on correct understanding concerning the endocrine disrupting effects.

MoE is now seeking for the better ways to summarize the issues already clarified and problems not yet clarified, to address the needs of people in Japan, and to play the international roles.

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[Related website]
Approach to the Problem of Endocrine Disruptors by Ministry of the Environment
http://www.env.go.jp/chemi/end/index.html