MOE's Perspectives on Endocrine Disrupting Effects of Substances

ExTEND 2005

March 2005

Ministry of the Environment, Japan (MOE)

The ExTEND 2005 subtitle is an acronym for Enhanced Tack on Endocrine Disruption, followed by the year the document was created.

Introduction

The Ministry of the Environment, Japan (MOE) devised the "Strategic Programs on Environmental Endocrine Disruptors" in May 1998, made revisions and additions based on more recent knowledge in November 2000, and issued a November 2000 version (hereafter called SPEED '98). In accordance with this program, research on the mechanisms of endocrine disruption has progressed, along with environmental monitoring, test development and implementation through the Millennium Project, as well as annual international symposia and collaborative research between Japan and the United Kingdom of Great Britain and Northern Ireland(UK), or between Japan and the Republic of Korea.

International activities in which MOE has participated include the completion of the "Global Assessment of the State of the Science of Endocrine Disruptors" in August 2002 (hereafter "WHO Global Assessment", also available in Japanese translation), by the World Health Organization (WHO), International Labor Organization (ILO) and the United Nations Environmental Program (UNEP). During this time, at the Organization for Economic Cooperation and Development (OECD), MOE has made specific proposals and is proceeding with trials on test method development for endocrine disruptors, and new knowledge has continued to accumulate through a variety of efforts.

In May 2003, the government's definition of endocrine disruptors was agreed as follows "Injury and/or Hazardous Effects on Organisms Caused by Exogenous Substances Through Influence on the Endocrine System".

MOE established a SPEED '98 Revision Working Group in 2003, composed of specialists, experts in the field, and representatives from consumer groups etc. (hereafter called "Revision WG"). This Revision WG held meetings for two years and created a brochure on the achievements of the activities under SPEED '98 for the general public. It also compared the activities and results of SPEED '98 thus far to the issues that have been indicated internationally, and identified issues for the future. The Revision WG also held hearings from local governments on this problem.

Through this process, MOE has developed this document " MOE's Perspectives on Endocrine Disrupting Effects of Substances-ExTEND 2005-" (hereafter called "ExTEND 2005")

In addition to continuing to diligently conduct the necessary survey and research on endocrine disruptors as part of the continuing comprehensive measures to deal with chemicals based on ExTEND 2005, MOE will continue to make every effort to promote the information sharing and communication for the better understanding of general public.

March 2005

Environmental Health and Safety Division,

Environmental Health Department

Ministry of the Environment

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I. Activities to Date

1. Basic Framework of SPEED '98

With regard to the problem of endocrine disruptors, MOE stated in 1998 that "Environmental contamination by exogenous endocrine disruptors (so called environmental hormones) may be linked to the impairment of reproductive functions and the occurrence of malignant tumors through the disruption of the endocrine systems in humans and wildlife. At the present time, there are many questions still left unanswered by science, although these issues are related to the basic conditions for survival of living creatures, and may even have serious consequences for future generations, making this an important issue for environmental preservation."

The framework of SPEED '98 included (1) progress on detection in the environment and surveys of the wildlife, (2) promotion of test research and technology development, (3) proceeding with environmental risk assessments, environmental risk management and provision of information, (4) efforts to strengthen the international network. For the specific action planning, 67 chemicals were identified as chemicals having the highest priority in the survey and research in order to clarify the presence, the strength and the mechanisms of endocrine disrupting effects of each chemical. Subsequently, this list was revised to 65 chemicals in November 2000, and the various efforts have progressed.

2. Specific Measures of SPEED '98

(1) Environmental Surveys for Chemicals and Surveys of Effects on Wildlife

Since 1998, measurements of the concentrations of the chemicals listed in SPEED '98 have been conducted for wildlife and four media, water, sediment, soil and air (Table 1).

There has also been development of survey methods and partial surveys of the concentrations in indoor atmospheres, aquatic organisms, wild animals, as well as in food samples.

The results of the environmental surveys have been used as the basic data to select the chemicals for which tests will be implemented in order to assess the hazard and also to establish test concentrations (doses). In addition, the environmental concentration data itself can also be used effectively for general countermeasures to chemicals.

In the surveys of the effects on wildlife, it was shown that the females of the rock shell (*Thais clavigera*) had abnormal sexual organ development, with the formation of male type sex organs, over a wide range of the Japanese coastline. This was the evidence to be caused by organotin compounds, tributyltin and triphenyltin from antifouling paints in the marine environment.

On the other hand, surveys were conducted based on existing reports of elevated concentrations of vitellogenin, which is a protein that is a component of egg yolks, in male koi, but there was no causal relationship found between the detection of specific substances in the body of koi and abnormalities. There were also surveys conducted on frogs, covering the regions where the appearance of frogs with extra legs etc. are known to be obvious, but there was no causal relationship found between the detection of specific substances in the body and abnormalities.

Table 1 Implementation of Environmental Surveys and Impact Surveys for SPEED '98

	Environmental survey				Indoor air survey	Aquatic organisms[1] (fish, shellfish)	Wildlife survey[2]	Food sample survey
	Water	Sediment	Soil	Air				
Number of measured chemicals	61	61	9	38	12	61	41	13

Materials for the Commission on Endocrine disruptors held during 1999 ~ 2004 and results of POPs monitoring surveys during 2002 ~ 2003

[1] **Fish**: greenling, ayu, butterfish, Japanese dace, rock greenling, common minnow, large-mouth bass, scorpion fish, dark chub, silver crucian carp, koi (carp), salmon, saury, white croaker, Japanese sea bass, Japanese sea bass (young), tilapia, skin carp, rainbow trout, goby, , Japanese dace (young), crucian carp, bluegill, striped mullet, common brakish goby, common crucian carp, far eastern dace, southern black sea bream, stone moroko, pond smelt; **Shellfish**: Japanese mussel, mussel, Japanese deck mussel, freshwater clam (Species measured differ in each year)

[2] **Mammals**: Japanese field mouse, Himalayan bear, raccoon dog, Japanese macaque, brown bear, harbor seal, ringed seal, stejneger's beaked whale, melon-headed whale, spectacled dolphin, blainville's beaked whale, Indian porpoise, fin whales, porpoise, hubbs' beaked whale, common dolphin, mink whale; **Birds**: brown hawk owl, golden eagle, black-tailed gull, ezo owl, Screech Owl, goshawk, Common Cormorant, Common Cormorant eggs, short-eared owl, white owl, hawk eagle, hawk eagle eggs, Eastern Marsh Harrier, kestrel, *Accipiter virgatus*, pigeon, kite, buzzard, vulture, shrike, falcon, falcon eggs, owl, owl eggs, osprey, gray starling; **Amphibians**: Tokyo potbellied frog, leopard frog, Japanese red frog, mountain red frog (Species measured differ in each year)

(2) Tests Using Fish to Assess the Effects on Ecosystems

Based on the list in SPEED '98, literature on the endocrine disrupting effects on wild animals and aquatic organisms as well as literature on *in vitro* testing were searched and collected for each chemical, and the reliability of them were assessed by experts. Based on the results of literature search, chemicals to be tested were selected and vitellogenin assay[3] and partial life cycle test[4] were conducted using medaka. As necessary, full life cycle test[5] was added. For the specification of the test concentrations (Figure 1), the concentrations in the environment, existing toxicity data and information on the nature of each chemical was considered.

The tests were conducted for 28 chemical and it was postulated that 4-Nonylphenol (branched form) and 4-t-Octylphenol have a strong endocrine disrupting effect on medaka at the concentrations believed to be present in the environment in Japan, and that Bisphenol A also has an endocrine disrupting effect on medaka. For the remaining 23 chemicals, it was determined that no clear endocrine disrupting effect could be recognized (as of December 2004, full life cycle tests were underway for o,p'-DDT and p,p'-DDE). The test results are summarized in Table 2.

Assessments of the endocrine disrupting effects were made based on the above-mentioned series of tests using medaka, but it has been pointed out that even for medaka, for which there has been significant accumulation of basic knowledge as a test animal, it is necessary to gather relevant information in order to further evaluate the results, and that there are issues relating to improving the efficiency, such as shortening the test period.

There have also been vitellogenin assay using *Xenopas laevis* , a sex-transformation test using Japanese quail, and an enhanced OECD test method using *Daphinia magna*. (Appendix (4))

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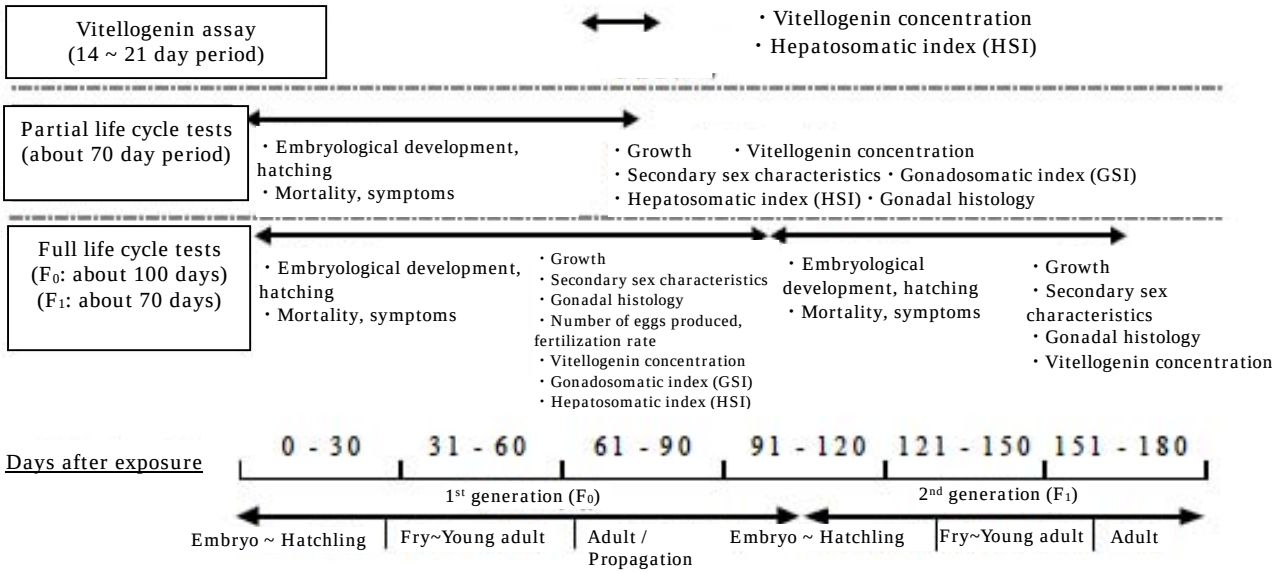
[3] Vitellogenin assay: Vitellogenin is a protein unique to the female that is a component of egg yolk, and absorbed by the oocytes during vitellogenesis. For this assay, the appearance of vitellogenin in the body of the males is observed. This is thought to be one indicator for endocrine disruption. Male medaka is exposed to a test substance for a 21-day period.

[4] Partial life cycle test: Observation of the effects on gonadal histology etc. by exposing medaka to a test substance throughout the growth period starting from the fertilization stage.

[5] Full life cycle test: Observations to grasp the effects throughout the entire lifespan by exposing Medaka to a test substance for at least two generations.



The exposure to the test substance is 21 days to male medaka for the vitellogenin assay, about 70 days throughout the growth period starting from fertilization for the partial life cycle test, and at least two generations (about 180 days) for the full life cycle tests. For medaka, the period from hatching to spawning is about 90 to 120 days.

Figure 1 Medaka test outline

Table 2 Results of tests using Medaka

name of chemicals	Test results
Di-2-Ethylhexyl adipate	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
Amitrole	Clear endocrine disrupting effects were not recognized.
Triphenyltin chloride	Clear endocrine disrupting effects were not recognized.
Tributyltin chloride	Clear endocrine disrupting effects were not recognized.
Octachlorostyrene	Clear endocrine disrupting effects were not recognized.
4-t-Octylphenol	(1) strong binding to the fish female hormone receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the liver, (3) emergence of testis-ova, (4) the number of eggs produced and the fertilization rate were both low. It is postulated that there is a strong endocrine disrupting effect on fish.
cis-Chlordane	Clear endocrine disrupting effects were not recognized.
2,4-Dichlorophenol	Clear endocrine disrupting effects were not recognized.
4-Nitrotoluene	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
trans-Nonachlor	Clear endocrine disrupting effects were not recognized.
4-Nonylphenol (branched)	(1) strong binding to the fish female hormone receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the liver, (3) emergence of testis-ova, (4) the fertilization rate is low. It is postulated that there is a strong endocrine disrupting effect on fish.
Bisphenol A	(1) binding to the fish female hormone receptors, although weak, was recognized (2) elevated vitellogenin (egg yolk protein precursor) concentration in the liver, (3) emergence of testis-ova, (4) higher number of days of incubation (delayed hatching). It is postulated that there is an endocrine disrupting effect on fish.
Diethyl phthalate	Clear endocrine disrupting effects were not recognized.
Di-2-ethylhexyl phthalate	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
Dicyclohexyl phthalate	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
Di-n-butyl phthalate	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
Dipropyl phthalate	Clear endocrine disrupting effects were not recognized.
Dihexyl phthalate	Clear endocrine disrupting effects were not recognized.
Dipentyl phthalate	Clear endocrine disrupting effects were not recognized.
Butylbenzyl phthalate	Clear endocrine disrupting effects were not recognized.
β-Hexachlorocyclohexane	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
Hexachlorobenzene	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
Benzophenone	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized for low concentrations (comparatively low concentration considered for the fish estimated exposure dose obtained from literature).
Pentachlorophenol	Clear endocrine disrupting effects were not recognized.
p,p'-DDD	Frequency is low, but the appearance of testis-ova was confirmed. There did not appear to be a negative effect on fertilization rates. Clear endocrine disrupting effects were not recognized.
p,p'-DDE	Since elevated vitellogenin (egg yolk protein precursor) concentration in the liver and a concentration-dependent emergence of testis-ova were recognized, the assessment is planned after the completion of full life cycle testing
o,p'-DDT	Since elevated vitellogenin (egg yolk protein precursor) concentration in the liver and a concentration-dependent emergence of testis-ova were recognized, the assessment is planned after the completion of full life cycle testing
p,p'-DDT	Clear endocrine disrupting effects were not recognized.

http://www.env.go.jp/chemi/end/speed98/speed98-20.pdf

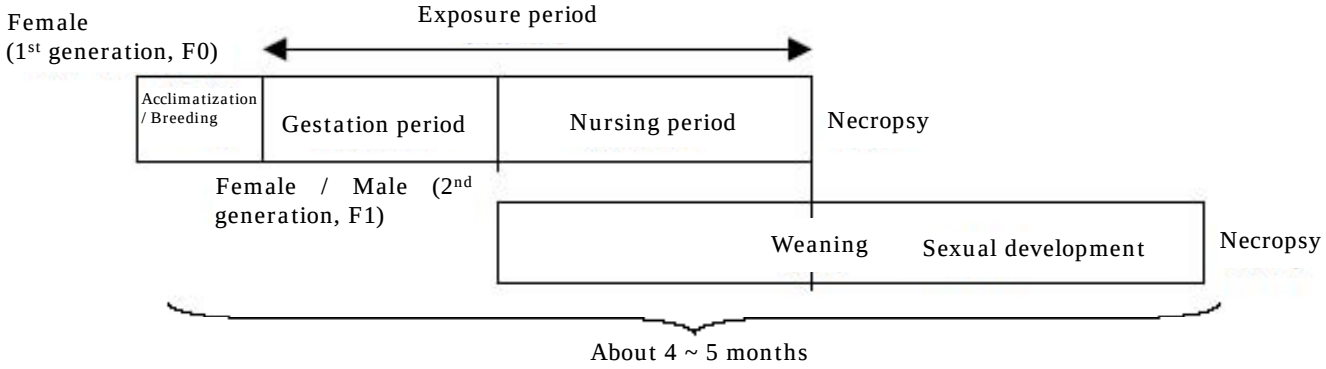
(3)Tests Using Mammals and Epidemiological Surveys to Assess the Effects on Human Health

① Tests Using Mammals

An enhanced one-generation test using rats (Figure 2) was developed, and based on the list in SPEED '98, literature on the endocrine disrupting effects on mammals as well as literature on *in vitro* testing were searched and collected for each chemical, and the reliability of them was assessed[6] by experts. Tests were conducted using the test chemicals selected based on the results of literature-search. The test doses and endpoint were selected based on consideration of the environmental concentrations of the test chemicals, the estimated exposure doses to humans, existing toxicity data and information on the nature of each chemical. For the specification of the test doses, the goal was set to detect any reactions from a dose to which a human was likely to be exposed and also non-dose correlated reactions. The purpose was not that of conventional risk assessments. In principle, there were four groups receiving doses considered to be the estimated human exposure doses, and one group receiving a dose already reported to be associated with some kind of hazardous effects. The results were assessed with reference to test results from the uterotrophic test[7], Hershberger assays[8], and the enhanced 28-day repeated dose toxicity test conducted mainly by the Ministry of Economy, Trade and Industry [9].

Tests were conducted for 28 chemicals using rats. It was determined that none of these chemicals showed clear endocrine disrupting effect at the doses considered to be the estimated human exposure doses. The test results are summarized in Table 3.

Since these were tests using animals, the tests where done in the presence of that could not be completely eliminated from the diet, nor could be quantitatively controlled (for example, phytoestrogen(plant-derived substances with estrogen like effects) and phthalate esters). Since it is not practically possible to establish a control group with a zero exposure dose of endocrine disruptors, it was clear that there is a limit for understanding precise variation over the dose levels considered to be the estimated human exposure doses. In the future, it is necessary to investigate not only at the possible human exposure doses, but also at dose levels that consider the various types of toxicity assessments and methods.



The gestation period of rats is about 22 days, and about 21 days from birth until weaning. The time from weaning until pregnancy is possible is about 30 ~ 35 days. For males, it takes about 40 days for separation of the prepuce. Exposure to the test substance is made throughout the gestation period until weaning, about 43 days.

Figure 2 Enhanced one-generation rat test outline

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[6] Materials for the Commission on Endocrine Disruptors held during 2000 ~ 2004 (in Japanese).
[7] Uterotrophic test: Immature female rats or female rats with the ovaries removed are dosed with the test substance, and the estrogen-like effects are assessed from changes in the weight of the uteri.
[8] Hershberger assay: Immature male rats or male rats with the testicles removed are dosed with the test substance, and the androgen effects are assessed from prostate weight and examinations of secondary sex glands.
[9] Enhanced 28-day repeated dose toxicity test: The subject is dosed with the test substance for a 28 day period, and indicators related to endocrine disruption, including genital morphology, sperm formation, and serum hormone concentrations, are assessed.

Table 3 Results of enhanced one-generation test using rat

name of chemicals	Test Results
Di-2-Ethylhexyl adipate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Amitrole	Clear endocrine disrupting effects were not recognized for doses (3 dose groups) believed to be likely human exposure doses obtained from literature.
Triphenyltin chloride	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Tributyltin chloride	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Octachlorostyrene	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
4-t-Octylphenol	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
cis-Chlordane	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
2,4-Dichlorophenol	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
4-Nitrotoluene	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
trans-Nonachlor	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
4-Nonylphenol (branched)	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Bisphenol A	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Diethyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Di-2-ethylhexyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Dicyclohexyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Di-n-butyl phthalate	Clear endocrine disrupting effects were not recognized for doses (5 dose groups) believed to be likely human exposure doses obtained from literature.
Dipropyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Dihexyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Dipentyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Butylbenzyl phthalate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
β-Hexachlorocyclohexane	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Hexachlorobenzene	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
Benzophenone	Clear endocrine disrupting effects were not recognized for doses (3 dose groups) believed to be likely human exposure doses obtained from literature.
Pentachlorophenol	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
p,p'-DDD	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
p,p'-DDE	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
o,p'-DDT	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.
p,p'-DDT	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) believed to be likely human exposure doses obtained from literature.

http://www.env.go.jp/chemi/end/speed98/speed98-19.pdf

② Epidemiological Survey

For the investigation of effects on humans, there are epidemiological methods in addition to the tests using mammals.

Surveys and studies on exposure to chemicals such as Dioxins using human umbilical cords and surveys on the prevalence of congenital abnormalities have been conducted, so that some understanding of the exposure situation has been obtained.

There have also been studies on sex ratios at birth, the effects on urogenital organs, as well as research on human testis weights and sperm formation, but these have not extended to an assessment of phenomena which raise concerns about effects on human health.

It is difficult to implement epidemiological surveys that enable assessment of the relationships between exposure in the environment and suspected health effects. (Tables 4-1 and 4-2)

Table 4-1 Outline of epidemiological surveys (1)

	Outline	Results
Survey of human congenital abnormalities	It has been pointed out that endocrine disruptors may contribute to the occurrence of congenital abnormalities in humans through the disruption of the endocrine system. Therefore concentrations of Bisphenol A and Nonylphenol in the umbilical cord blood and in the blood of human females, both during pregnancy and while not pregnant, were measured in order to understand the relationship between Bisphenol A, etc. and the occurrence of congenital abnormalities. There have also been measurements of the serum concentrations of the two mentioned substances of infants with hypospadia and their mothers (while not pregnant).	It was not possible to form a conclusion about a causal relationship between the concentration of chemicals in the blood or umbilical cord blood and the occurrence of hypospadia.
Surveys and studies on exposure to chemicals, such as Dioxins using human umbilical cords	There is concern that fetuses are highly sensitive to chemicals, but detailed investigations on fetal exposure to chemicals have not been conducted. There have been surveys on detection rates and concentrations in the umbilical cord, umbilical cord blood, and the mother's blood for dioxins, PCBs, organic chlorides, estrogens, and phytoestrogens.	Dioxins, PCBs, DDTs, hexachlorobenzene (HCB), hexachlorohexane (HCH), Endosulfan, Chlordane, and phytoestrogens (Genistein, Daidzein, Equol) were detected in more than 80% of the survey subjects. Aldrin and Endrin were not detected. Results of preliminary tests show that Bisphenol A, phthalate esters and heavy metals were also detected. There was shown to be a correlation between the total PCB concentration in the mother's blood and the concentrations in the umbilical cord and the umbilical cord blood. However, there was no apparent correlation with PCB congeners/isomers. The concentrations of phytoestrogens, Genistein and Daidzein tended to be higher in the umbilical cord blood than in the mother's blood. It is necessary to investigate the relationship between chemical concentrations and the age of the mother (or age at delivery).

Materials for the Commission on Endocrine Disruptors held in 2004(First and Second)

Table 4-2 Outline of epidemiological surveys (2)

	Outline	Results
Survey of sex ratios at birth	As there was a report of a decline in the percentage of males among infants born in the Kasumigaura region[10], a survey was conducted on sex ratios nationally and around Kasumigaura.	There was no clear change in the sex ratios observed in the area around Kasumigaura.
Survey of effects on urogenital organs.	Since it was pointed out that endocrine disruptors may contribute to reproductive function abnormalities in humans, a survey of reproductive function in young males was conducted on groups of college students. A national survey was conducted on cryptorchidism in male children under the age of 3 years. A survey on the frequency of testicular cancer was also conducted.	For the survey of reproductive function, there was no clear result obtained. For the cryptorchidism, there were no apparent peculiarities in patients or parents regarding pregnancy history, birth measurements, parent's diet, medication or occupation. It was thought that the probability of effects from environmental factors, including exposure to endocrine disruptors, is extremely low. With regard to the frequency of testicular cancer, although there is a gradual increase in the number of cases, it does not constitute evidence of a connection to chemicals.
Research on human testicular weight and spermatogenesis	There is concern about reduced sperm counts and reduced testicle size in men. Investigation of testicle weight and testicular tissue was performed by analyzing autopsy records of abnormal corpses at the Tokyo Medical Examiner's Office	Sorted by age at the time of death (from 20s to 60s), height increased in each age group for more recent birth dates. With regard to testicular weight, however, there was no linear increase, even for more recent birth dates. The results of the investigation of testicle weight by age at the time of death in 5-year groupings indicated a tendency for testicle weight to reach the maximum value at a younger age as the subject group becomes of more recent birth. A relationship between testicle weight and sperm production was observed. With regard to a relationship between testicle weight and cause of death, there was a tendency for low testicle weight for cases of death due to malnutrition, and higher testicle weight associated with instances of sudden death. Data on normal, healthy adults were not obtained.

Materials for the Commission on Endocrine Disruptors held in 2004(First and Second)

[10] Mizuno, Reiko (2000) Decline in birth rates of male infants in the Kasumigaura basin and Tonegawa estuary. Kagaku, 70:113-118 (in Japanese)

(4) International Activities

There are many issues to be solved concerning endocrine disruptors, including clarification of the mechanisms and the causal relationship with exposure, and development of test methods. It is important to advance research and study under international collaboration and cooperation.

MOE has been conducting an annual International Symposium on Endocrine Disruptors since 1998. In addition, joint research projects are being conducted with UK and the Republic of Korea. There is also active cooperation with international agencies such as OECD and WHO. (Table 5)

Table 5 Outline of international activities

International symposium on Endocrine Disruptors	These were held in Tokyo 1998, Kobe 1999, Yokohama 2000, Tsukuba 2001, Hiroshima 2002, Sendai 2003 and Nagoya 2004. Each symposium runs for 3 days. The first day is a presentation of information on the status of domestic and international activity as a program for the general public. The second and third days are programs for experts, with presentations of the latest research by foreign researchers and discussions of future directions. So far about 10,000 people have participated, including 500 participants from abroad.(http://www.env.go.jp/chemi/end/index3.html)
Bilateral research projects	Bilateral collaborative workshops have been held in order to exchange technical data, conduct joint research and provide interaction among experts. Specific themes cover a broad range, from basic research on genetic cloning and identification of hormone receptors to monitoring of effect of endocrine disruption on the native species of each country. Bilateral research has been underway since 1999 with UK, and since 2001 with the Republic of Korea. In 2004 a bilateral collaboration with the USA was started to share information on test method development. (Materials for the Commission on Endocrine Disruptors held in 2004)
Cooperation with the Organization for Economic Cooperation and Development (OECD)	Some of the test methods developed in Japan have been proposed to the OECD as international standard test methods (an invertebrate test method 'enhanced TG211', a bird test method 'enhanced TG206'). In addition, Japan actively participates in test method validation work on fish and frogs screening methods being advanced by the OECD (http://www.oecd.org/home)
Cooperation with the World Health Organization (WHO)	WHO/ILO/UNEP issued the WHO Global Assessment in 2002 summarizing the latest scientific information on endocrine disruptors. MOE participated in preparing this summary, and translated the WHO Global Assessment into Japanese. This Japanese translation (MOE version) is accessible on MOE website.(http://www.env.go.jp/chemi/end/index4.html)

II. Future Direction

1. Basic Concepts

The origin of the focus on endocrine disrupting effects was the suggestion of a connection between reproductive abnormalities in wildlife and an exposure to substances with hormone-like effects. However, the understanding of abnormalities in wildlife is not the only issue for endocrine disruptors, it is also the starting point for countermeasures for chemicals in view of the protection of the ecosystem.

Conventionally, the effect of a chemical on the ecosystem, which is related to many factors, is difficult to prove directly through laboratory experiments. For this reason, it is necessary to first continuously observe wildlife in the nation, determine whether the observed phenomena are normal or abnormal through scientific surveys, and understand the variations of individual organisms (groups). Next, there should be an estimate of the effects on the ecosystem based on these results. It is also necessary to collect basic biological knowledge and learn more about the interrelationships between species in order to assess whether observed phenomena are normal or abnormal. Also for the estimation of the effects on the ecosystem, essential is basic biological knowledge.

When investigating the effects of endocrine disruptors on the ecosystem, there is also a need for continuous observation and accumulation of basic biological knowledge. In particular, it is necessary to make progress on collecting the basic knowledge on the endocrine systems of each species and on the basic research on the various mechanism of endocrine disruption. In order to understand the effects of substances in the environment on the ecosystem and the human health, it is necessary to understand concentrations of substances in the environment and whether there is exposure. As is already known, there are natural hormone-like substances (phytoestrogens and hormones derived from humans and animals). In order to grasp the situation in the environment, it is necessary to also consider the exposure to these substances.

In addition to the effects via hormone receptors, it has been pointed out that endocrine disruption can also occur through metabolic processes without affecting receptor-binding processes. It has also been pointed out that in addition to changes that are apparent during the development of an individual organism, there is also possibility that latent effects received during the development process will become apparent later in the adult stage. Endocrine disruption is an important topic of research, but for measures against chemicals in general it is important to gain a comprehensive understanding of the various hazardous effects of chemicals on living organisms.

In addition to the basic and fundamental research mentioned above, it is also important to establish a variety of test assessment methods in order to estimate the effects on the human health and on the ecosystem using the knowledge MOE has at the moment. In the studies so far, there have been no chemicals found to have endocrine disrupting effects on rats at doses corresponding to concentrations in the environment. For medaka, however, some chemicals have been found to

possess endocrine disrupting effects at concentrations in the environment. According to the agreement on the inter-ministerial cooperation on endocrine disruption, the main focus of MOE is environmental protection. To accomplish this, it is considered important to establish test methods for the assessment of effects on the ecosystem, and to focus on the implementation of tests. MOE will continue to actively cooperate in the establishment of test methods being advanced by the OECD, etc., and work to make positive contributions internationally.

As measures to handle chemicals in general, it is necessary to perform comprehensive risk assessments based on the exposure situations, and to link this to risk management, based on the data obtained from the perspective of assessing the hazards from a variety of viewpoints, not only the data on endocrine disruption.

Much of the information related to chemicals requires effort to fully understand, including scientific details. Furthermore, since there are still many uncertainties regarding endocrine disrupting effects, in order to avoid irrational anxiety, it is extremely important to provide accurate information and promote risk communication based on accurate understanding and shared knowledge.

From this perspective, the following will be promoted as the foundations of the activities to deal with the endocrine disruptor problem: (1) Observation of wildlife, (2) Evaluation of the environmental concentrations and exposure level, (3) Promotion of basic research, (4) Effects assessment, (5) Risk assessment, (6) Risk management, and (7) Promotion of information sharing and risk communication.

The issues indicated internationally have been compared with the items already handled by MOE to identify and classify specific issues for the future. References used for the comparison include the WHO Global Assessment[11], WHO workshop reports[12], IUPAC reports[13], and EC strategies on endocrine disruptors[14].

[11] WHO (2002) Global assessment of the state-of-the-science of endocrine disruptors, WHO/IPCS/EDC/02.2.
[12] WHO/UNEP/ILO (2004) Report of the joint IPCS-Japan workshop on “Endocrine disruptors: Research needs and future directions”, WHO/IPCS/EDC/01/04.
[13] J. Miyamoto and J. Burger (2003) Implication of Endocrine Active Substances for Human and Wildlife, Scope/IUPAC.
[14] EC (2001) Communication from the Commission to the Council and the European Parliament on the implementation of the Community Strategy for Endocrine Disruptors- A range of substances suspected of interfering with the hormone systems of humans and wildlife (COM(1999)706) , COM(2001)262 final.

2. Specific Objectives

(1) Observation of Wildlife

Reproductive abnormalities in wildlife in various regions throughout the world had been observed, and it had been suggested that there would be a connection to exposure to chemicals. Especially endocrine disruption was pointed out as the causative mechanism. (Page 16, Note 11) As a result, surveys were being made on the effects of chemical exposure on wildlife in various regions all over the world. Examples in Japan of possible causal relationships between abnormalities in wildlife and exposure and/or accumulation of substances (including natural and synthetic hormones) in organisms, include reproductive organ abnormalities observed in female rock shell (*Thais clavigera*), and elevated vitellogenin concentrations in the blood of male koi (Page 6), but there have not been many reports on wildlife abnormalities.

Finding abnormalities in wildlife is the starting point for measures against chemicals for the ecosystem, not just for the endocrine disruptor problem.

It is important to contribute to the preservation of the environments, which is irreplaceable, and efforts are required to grasp changes and precursory phenomena along with time-course in wildlife. It is necessary to continue to build a scientific monitoring system of the ecosystem with experts, but there is a limit to the activities of them. Observation at the local level by children and ordinary citizens can enable them to gain knowledge to determine whether changes in the ecosystem are one of the nature variations there and can be developed into the more detailed studies by a limited number of specialists.

Specific examples that can be started at the present time are presented below.

① Continuous observation of wildlife at the local level

For the continuous monitoring of wildlife in the community, it is desirable to make use of the observation activities of local citizens and the nature observation training conducted at schools in various regions. This kind of activity is steady and continuous monitoring rooted in the local region. Although there are limits to the precision and identification of species due to lack of involvements of specialists, it is believed to be invaluable for obtaining an understanding of the current status of the ecosystem, including the various types of wildlife. By creating networks of local schools and existing activities, and collecting a standardized level of information, such as the species to be observed, the items to be reported and the location of the surveys, it will be possible to contribute to selection of fields, etc. for the studies by experts.

In addition, the participation of children and ordinary citizens in the observation of wildlife is expected to nurture interest and concern for the ecosystem, and enable people to learn the diversity of the ecosystem.

As the first species for common observation, aquatic organisms have the advantage of having easily-identifiable habitats. It is currently planned to start with medaka, for which test methods on endocrine disrupting effects have been developed, and later add target species to achieve

comprehensive monitoring.

② Survey and evaluation by experts

Starting with observation data from the local level, a limited number of experts can utilize those data and extend their fields nationwide. In the case that experts suspect significant changes from field surveys, more detailed studies should be conducted through examination of specimens captured for pathological examination, if possible, or based on the observation details, such as measurements of the concentrations of various substances in the habitats, studies using biomarkers to investigate sources of variation and teratogenicity, hybridization experiments between abnormal organisms or between abnormal and normal organisms, or studies of possible causes other than chemicals, such as radiation. (Figure 3)

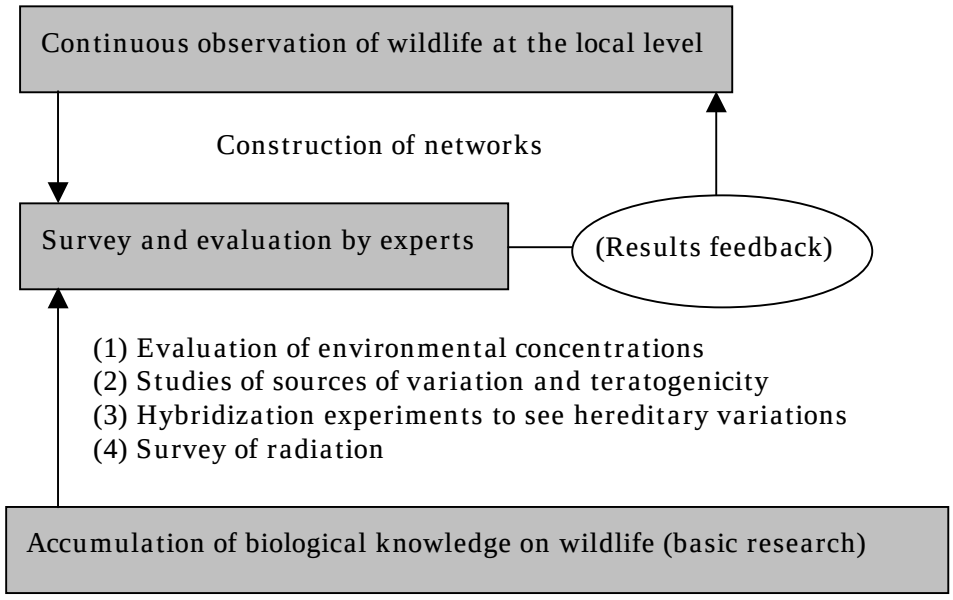

Figure 3 Conceptual diagram of observation of wildlife and comprehensive surveys

(2) Evaluation of the Environmental Concentrations and Exposure Level

In order to properly assess the environmental risk of chemicals, it is crucial to understand the existing concentrations in the environment, as well as data related to the hazard. Therefore, since 1998, environmental surveys have been underway to determine the levels of the chemicals listed in SPEED '98. Environmental survey and wildlife monitoring of chemicals (called "black book reports", because the reports have black covers) have been conducted since 1973, presenting the presence of chemicals in the environment using samples from a variety of media, including water, soil and air etc.. However, there are tens of thousands of chemicals present in the environment, and there is a limit to the survey and analysis capabilities.

In the future, the selection of substances for the black book reports will include consideration of endocrine disrupting effects. In addition, natural substances, such as phytoestrogens (plant-derived estrogens) will be also considered in the selection process. Using the limited survey and analysis capabilities to the utmost, it is desirable to maintain a constant and nationwide understanding of the environmental situation and to make broad, effective use of the results obtained for various countermeasures for chemicals, including measures against endocrine disruptors. (Figure 4)

① Environmental survey and wildlife monitoring of substances

i) Preliminary environmental survey

For substances for which the presence in the environment is not clear, conduct surveys with the primary goal of verifying the presence.

ii) Detailed environmental survey

For substances confirmed to be present in the environment, make quantitative measurements using advanced analysis methods for each environmental medium, water, soil and air.

iii) Monitoring survey

For chemicals which require knowledge about residual amounts in the environment over time, because of accumulation in organisms or persistence in the environment, conduct regularly scheduled surveys using advanced analysis methods. If there is a switch to a higher-sensitivity analysis method, conduct the high-sensitivity analysis on samples from past surveys in order to maintain the data comparability over time.

iv) Exposure level survey

Gain an understanding of the exposure levels through exposure routes via media like foods and indoor air. Verify the accumulated quantities within the bodies of wildlife.

v) Human sample survey

As part of the effort to understand exposure levels, measure substance concentrations in body samples, specifically, human blood and human umbilical cord blood etc..

② Estimation of concentration levels of substances in the environment

Starting with specific rivers and regions, create and verify estimation models using basic data

obtained through actual measurement in order to simplify the process to detect detailed changes in the concentrations of substances, and to estimate changes in concentration levels in specific areas.

③ Environmental sample preservation

Since it is likely that the substances that will be the target of study will change, and that analysis methods will advance, a portion of the samples used in the surveys will be placed in freezing storage to enable re-analysis in the future.

④ Development of more sensitive analysis methods

For the selection of analysis methods, in addition to reproducibility, convenience, and cost considerations, it is also necessary that the methods satisfy the required sensitivity. For substances for which the existing analysis methods do not satisfy the sensitivity required for risk assessment, promote the development of high-sensitivity analysis methods to enable analysis of the concentrations present in the environment and contribute to the accurate understanding and assessment of exposure levels.

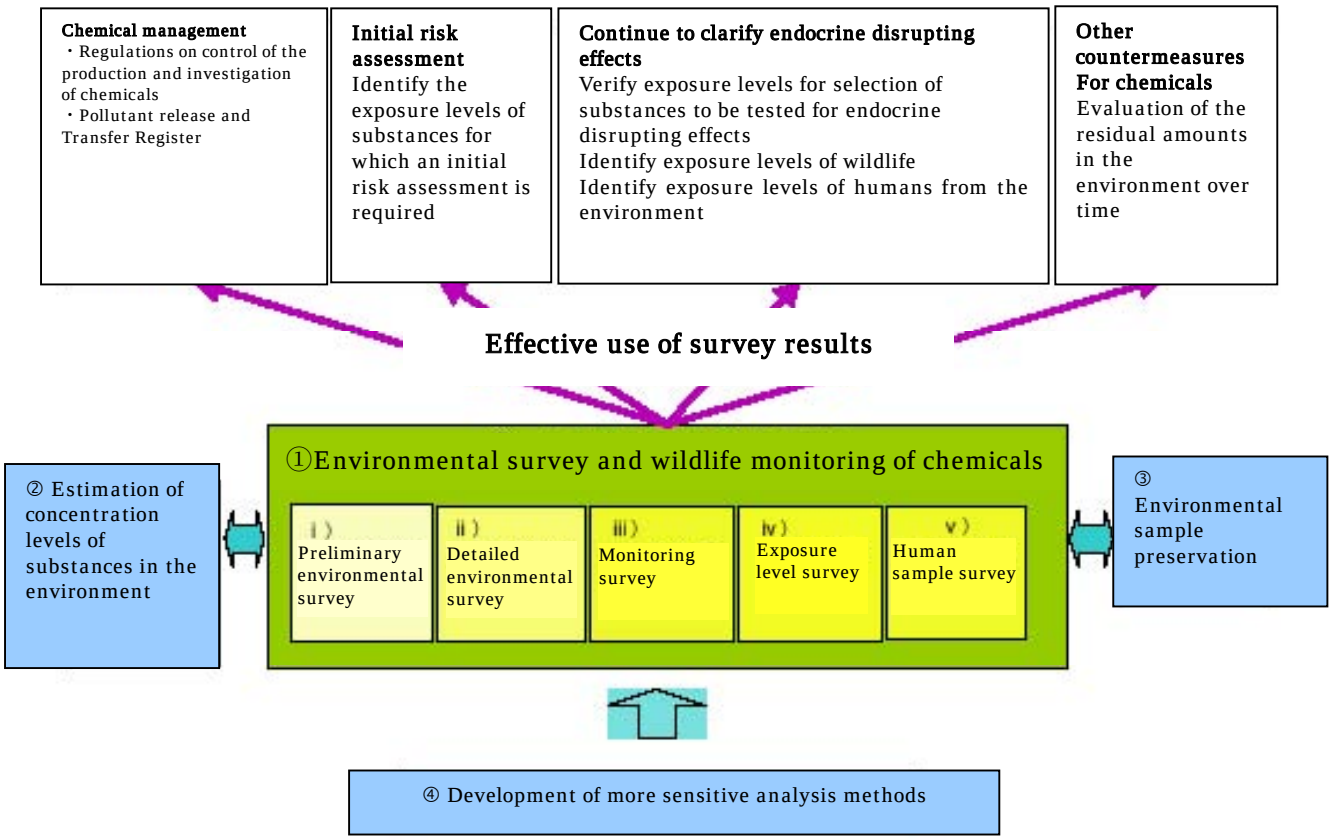

Figure 4 The structure of environmental survey and wildlife monitoring of substances

(3) Promotion of Basic Research

Compared to the situation in 1998, there is now a considerable accumulation of knowledge on endocrine disruption. However, there are still many issues to be resolved.

Understanding abnormalities of wildlife is not only an issue of endocrine disruptors, it is also important as a starting point for measures against chemicals from the viewpoint of preservation of the ecosystem. When changes in wildlife are observed, it should be determined, based on basic biological knowledge, whether the observed phenomenon is an abnormality of the organism (group) or not and what are the causes and mechanisms of the abnormality. And if there is a suggestion of relationship between changes and chemicals, investigations should be conducted including the evaluation of the involvement of endocrine disruption.

In order to ascertain whether an observed phenomenon is a direct effect of endocrine disruption or a secondary effect via endocrine disruption, knowledge on the mechanisms is indispensable. It is also necessary to clarify the relationships between hazardous effects on an individual level and changes on the cellular/molecular level.

Furthermore, it is necessary to establish a variety of test methods to estimate the impact on the ecosystem and the human health using the knowledge that is currently available. As for the development of test methods using fish and amphibians, it is important to continue to actively participate in the OECD activities. Figure 5 shows a feasible framework for basic research.

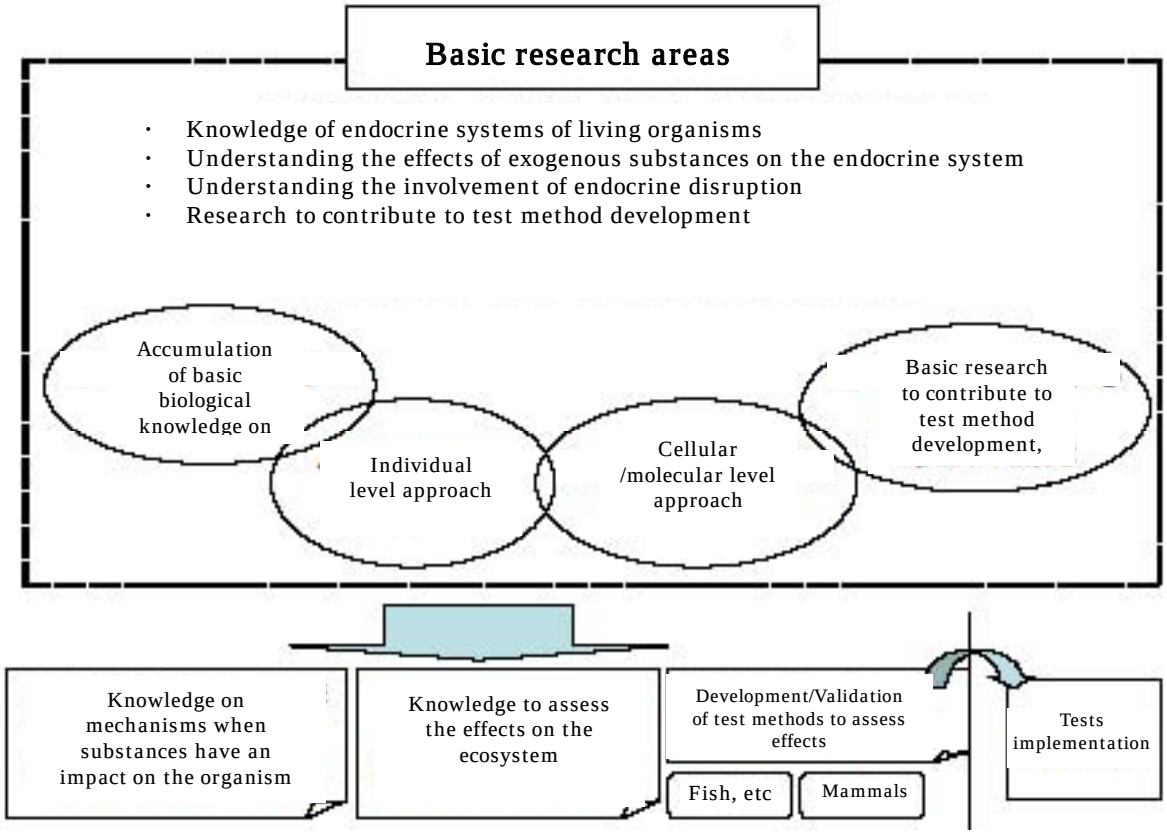

Figure 5 Basic research on endocrine disrupting effects of substances

① Accumulation of basic biological knowledge of wildlife

When changes or precursory changes are detected on an organism (or a group) through observation of wildlife, it is important to assess whether the change is abnormal or not, and to understand the mechanism of the change. To do this, it is necessary to have basic biological

knowledge of the species. Furthermore, when it is determined to be an abnormality, it is necessary to analyze the causes. This means, in addition to the measurement of concentrations of various substances in the habitat of the observation, it is required to accumulate information on the factors on the living organisms' side, that is, the factors regulating the differences in sensitivity relative to exposure to substances. Furthermore, an understanding of the environmental factors having an impact on the organism (such as physical factors of the environment, like changes in sunlight, temperature, and radiation) is not negligible. It is essential to continue to advance studies corresponding to the observed details.

② Individual level approach

The advantages of the individual level approaches, like *in vivo* experiments, are the ability to evaluate the absorption, distribution in the body, metabolism, and excretion of chemicals, the ability to comprehensively assess the entire endocrine system as well as other toxicity evaluation items, the ability to assess a wide range of mechanisms, and the historical evidence that toxicity tests at the individual level have been used for decades.[15]

It is necessary to investigate epidemiological methods that enable investigation of the causal relationship between human health effects at the individual level and exposure to substances in terms of endocrine disrupting effects.

i) Investigation of the dynamics of substances in the body

Investigation of the processes involving a substance in the body, including absorption, the distribution in the organs, metabolism, and excretion.

ii) Effects on organs and functions other than reproductive systems and effects on the endocrine system as a whole

For the assessment of the endocrine disrupting effects of a substance, basic knowledge based on a comprehensive understanding of biology will be collected, including the effects on target organs of the endocrine system other than the reproductive system, such as the thyroid and pituitary gland, as well as on non-endocrine/reproductive system effects, including effects on the nervous system and immune systems. It is also important to obtain the basic knowledge to evaluate and measure the effects between normal reactions and adverse reactions.

③ Cellular/molecular level approach

The advantages of cellular/molecular level approaches, like *in vitro* experiments, are the efficiency and practicality of the testing (low cost, automation, short time periods), and that the information obtained contributes to the understanding of the specific mechanisms.[15]

i) Development of DNA micro-array

Recently, along with the progress in genomic technologies and the structural and functional studies of proteins, it has become possible to conduct a variety of investigations to assess the mechanisms of substances with data obtained using genomic technology. For example, in

[15] USEPA(1998)Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report

toxicogenomics, which uses genome analysis techniques for toxological assessments, a technology called the DNA micro-array is receiving a great deal of attention. The micro-array is characterized by the ability to comprehensively analyze the expression of multiple genes, and is a ground-breaking technology for clarifying the expression of hazardous effects at the genetic level.

However, the technology at the present stage is not necessarily sufficient in terms of quantification, reproducibility and sensitivity, so it is still not adequate to use this technique for analyzing the roles of multiple genes showing expression changes within an organism. Furthermore, it is not sufficient for analyzing the significance of genetic changes over time, or the effects of long-term exposure. At the present stage, it is most often used as a screening technique for genes (groups) of interest. It may become a valuable technology in the future, and application development as a technology for impact assessment is expected.

Furthermore, in order to properly assess the effect of a variety of substances, it is necessary to clearly identify the effects within the body for key species, such as medaka.

It is desirable to construct a database of the information on gene expression changes obtained using micro-arrays, and allow public access as far as reasonably possible.

ii) Identification of receptors and signal transmission systems

It is expected to identify the receptors that could be one effect point of substances, as well as the connected signal pathways, analyze the structure and expression of the receptors, and clone the relevant genes.

iii) Dynamics of receptors and transcription factors

It is necessary to clarify the sequences of mechanisms at the cellular/molecular level, including the binding of substances to receptors, and the turning ON/OFF of genes mediated by the subsequent activity/non-activity of the transcription factors.

iv) Investigation of whole organism control mechanisms not mediated by receptors

It is desirable to investigate mechanisms that contribute to metabolic effects, at the steroid hormone synthesis stage, without effecting the binding process.

v) Assessment of effects on the cellular/molecular level

When changes at the cellular/molecular level are observed, it is necessary to clarify what kinds phenotype could be observed at the individual level. On the other hand, when changes are observed at the individual level, it is important to understand the mechanisms and assess the changes at the cellular/molecular level.

Therefore, data obtained through cellular/molecular level approaches should be compared to the results of *in vivo* experiments. It is also anticipated that a database will be constructed to enable unification and assessment.

④ Basic research contributing to test methods development

i) Collection of basic data on test animals

Biological knowledge on the endocrine system of each test animal in the ecosystem is required.

This basic knowledge on the endocrine systems of various species is necessary to understand properly changes due to individual variations, species differences, breeding conditions differences, and to analyze the results. Without knowledge of the normal status, it is impossible to determine what is abnormal. Basic knowledge will be accumulated for each test animal to understand the range of variation from which a return to the original is possible through homeostasis, and also know about the genesis, development, sexual differentiation and reproduction of the test animals.

ii) Search for biomarkers

A biomarker that is already used for test methods is vitellogenin. Basic biological data and knowledge on the significance of vitellogenin production in the male is still inadequate, so further accumulation of knowledge is required.

It is also necessary to search for other highly-sensitive, highly-specific biomarkers besides vitellogenin.

iii) Investigation of relationships between *in vitro* test results and *in vivo* test results

It is important to investigate the relationship between the results of *in vivo* experiments and *in vitro* experiments, such as receptor binding tests. For example, it is necessary to investigate the correlations and commonalities between false positives/false negatives in screening assays and higher-tier tests.

iv) Development and validation of test methods

As a starting point of assessment of wide-ranging effects on the ecosystem, there is a need to develop methods to assess the endocrine disrupting effects on fish and amphibians. It is important to promote research to establish a variety of international test methods to estimate the effects on the ecosystem and effects on the human health, using knowledge that is currently available.

The OECD is currently investigating several test methods at a variety of levels. Japan has been playing a leader role in the development of test methods related to endocrine disruptors, and is expected to contribute to the validation of test methods. For special areas in the test method development, such as development of receptor binding assays, standardization of vitellogenin assays, and comparison of fish test methods using medaka, it is important to continue to build bilateral collaboration to exchange information with other countries conducting advanced research.