Further Actions to Endocrine Disrupting Effects of Chemical Substances

— EXTEND 2010 —

(Tentative Translation)

July 2010

Ministry of the Environment, Japan (MOE)

Note: The subtitle of this program is named “EXTEND 2010”, intending to follow the basic frameworks of former “ExTEND2005” and extend the program to address further regulatory issues.

EXTEND: Extended Tasks on Endocrine Disruption

(formally ExTEND: Enhanced Tack on Endocrine Disruption)

Contents

Introduction

The useful properties of chemical substances have made our life comfortable. On the other hand, they have potential adverse effects on human health and the ecological system when they are not properly handled. Thus, proper risk assessment and management of chemical substances are common issues shared internationally.

Among these effects, endocrine disrupting effects of chemical substances on human health and the wildlife have been widely regarded as an important issue for their potential trans-generation effects although there remain many scientific uncertainties.

Consequently, the Environment Agency published "The Environment Agency's Basic Policy on Environmental Endocrine Disruptors – Strategic Programs on Environmental Endocrine Disruptors: SPEED'98 –" in May 1998 (revised in November 2000), and promoted activities including studies on chemical effects on endocrine systems, environmental surveys and monitoring of chemicals, as well as test development and implementation through the Millennium Project. This resulted in the identification of chemicals having potential endocrine disrupting effects on fish (Medaka).

Following this result, the Ministry of the Environment published "MOE's Perspectives on Endocrine Disrupting Effects of Substances – ExTEND2005 –" in March 2005, and promoted activities such as observation of wildlife, fundamental studies, assessment of chemical substances, information sharing and risk communication. Under this framework, fundamental studies on endocrine disrupting effects were conducted, and development of test methods using animals including fish was promoted under international cooperation.

On the other hand, as international activities, programs have been commenced in the United States and among the European Union to assess endocrine disrupting effects of chemical substances. Also in the Organisation for Economic Co-operation and Development (OECD), the workshop on testing, assessment and management of endocrine disrupters was held in Copenhagen in September 2009. This lead its member states to consider how endocrine disrupting effects of chemical substances should be assessed. Japan is also required to keep contributing to the OECD's activities based on the achievements including the development of test methods.

Under these circumstances towards the 5-year termination of EXTED2005 in March 2010, the MOE has reviewed its actions through the "Task Force on Endocrine Disrupting Effects of Substances" and its four sub-committees under the

framework of ExTEND2005 since November 2009, while considering principles for its future activities and identifying major issues to be addressed.

Based on these considerations, the MOE's further actions to endocrine disrupting effects of chemical substances have been summarized. Here, it is emphasized that the establishment of procedures to assess endocrine disrupting effects of chemical substances and their implementations should be accelerated, further aiming to conduct environmental risk assessment properly and to implement risk management if necessary in the national environmental administration. Based on this, the MOE intends to promote appropriate actions to endocrine disrupting effects.

July 2010
Environmental Health and Safety Division
Environmental Health Department
Ministry of the Environment, JAPAN

I Actions to date

1. Activities under the Framework of SPEED'98

In SPEED'98, published in May 1998, specific activities to the problem were (1) promotion of field investigations into the state of environmental pollution and effects on wildlife, (2) promotion of research and method development, (3) promotion of environmental risk assessment, environmental risk management, and information sharing, and (4) efforts to strengthen international networks. For the specific action planning, 67 chemicals were identified as those having the highest priority in the survey and research in order to clarify the presence, the strength, and the mechanisms of endocrine disrupting effects. Subsequently, this list was revised to 65 chemicals in November 2000, and the various activities have been facilitated.

1.1 Environmental Monitoring of Chemicals and Environmental Survey of Wildlife

Between FY 1998 and FY 2000, measurements of the concentrations of the chemicals listed in SPEED'98 have been conducted for four media (water, sediment, soil and air), wildlife (fish, bivalves, amphibians, birds, and mammals), indoor air, and food samples. These results have been utilized as the basic data to select the test chemicals and establish the test concentrations (doses) for hazard assessment.

In surveys of the effects on wildlife, abnormal sexual organ development with formation of male-type sex organs in females was widely observed in a kind of marine snail, the rock shell (*Thais clavigera*), over wide coastal areas of Japan. This effect was related to organotin compounds such as tributyltin and triphenyltin in the marine environment.

1.2 Tests Using Fish for Ecological Effects Assessment

Based on the list in SPEED'98, literature related to endocrine disrupting effects *in vivo* (including fish and mammals) and *in vitro* were searched and collected for each chemical, and the reliability evaluation was performed by experts. Based on the evaluation, test chemicals were selected and subjected to vitellogenin assays and partial life-cycle tests using Medaka. If necessary, full life-cycle tests were also conducted.

As a result, among 36 test chemicals, it was strongly suggested that 4-nonylphenol (branched form) and 4-*t*-octylphenol have strong endocrine disrupting effects on Medaka at the concentrations determined considering those found in the

environment. It was also suggested that bisphenol A and *o,p'*-DDT also have endocrine disrupting effects on Medaka. It was determined that no clear endocrine disrupting effects were recognized for the remaining 32 chemicals (Appendix 1).

1.3 Tests Using Mammalian for Health Effects Assessment

An enhanced one-generation test using rats was developed. It was determined that no clear endocrine disrupting effects were recognized for the above 36 chemicals at doses determined considering those estimated for human exposure (Appendix 2).

Also conducted were epidemiological surveys such as studies on congenital abnormalities, sex ratios at birth, effects on urogenital organs, and sperm formation. No clear region-specific changes were found in sex ratios at birth, and no relation between chemical exposure and abnormalities was found in other endpoints.

1.4 International Cooperation

The MOE has hosted the annual International Symposium on Endocrine Disruptors since 1998. In addition, bilateral joint research projects were conducted with the UK, the Republic of Korea, and the US. The MOE has proposed new test methods and provided information including its test results for the OECD. The MOE has also provided information including its approach status and test results for the WHO.

2. Activities under the Framework of ExTEND2005

With understandings from activities under the framework of SPEED'98, the MOE reviewed the program after 2003 and decided to take further actions after FY 2005 under the directions described below.

- ○ Detect changes in an individual (or a population) of organisms, determining whether the observed phenomena in scientific studies are normal or abnormal, based on continuous observation of wildlife in Japan.
- ○ Understand the presence or absence of exposure and the status of chemical substances in the environment, in order to detect effects of chemical substances in the environment on the ecological system and human health.
- ○ Promote fundamental studies on basic knowledge of the endocrine system of various species, mechanisms of various endocrine disrupting effects, and so on.
- ○ Establish a variety of test methods in order to estimate ecological effects and human health effects, utilizing the best knowledge at the present time. With the

MOE's high priorities on the establishment of test methods to assess ecological effects and on implementation of research, continue active cooperation toward test methods development by the OECD and others.

○ Pave the way for risk management via comprehensive risk assessment, based on not only the data related to endocrine disrupting effects, but also the data obtained considering various endpoints of hazard assessment as well as consideration for exposure status.

○ Provide wide and accurate information and promote risk communication based on information sharing and accurate understanding, in order not to incur irrational anxiety in the middle of current enormous uncertainties regarding endocrine disrupting effects.

For these perspectives, ExTEND2005 (Enhanced Tack on Endocrine Disruption) was designated with the following basic principles: (1) observation of wildlife, (2) Survey on environmental concentrations and measurement of exposure levels, (3) promotion of fundamental studies, (4) hazard assessment, (5) risk assessment, (6) risk management, and (7) promotion of information sharing and risk communication. The related research and studies were carried out under this framework.

In order to enhance these basic principles, the MOE set up the "Task Force on Endocrine Disrupting Effects of Chemical Substances" and its four sub-committees (Sub-Committee for Design and Evaluation of Fundamental Studies, Sub-Committee for Biological Observation of Wildlife, Sub-committee for Actions and Effects Assessment, and Sub-committee for Promotion of Risk Communication). Subjects such as direction of projects and evaluation of research results every fiscal year were discussed by this task force.

2.1 Observation of Wildlife

In ExTEND2005, the "Project for observation of familiar wildlife" had been implemented since FY 2005, aiming to foster human resources by inspiring interest in wildlife through wildlife observation. Those were expected to be able to consider ecological effects of chemical substances with the background of intimate understanding of natural life forms. It was presumed that the continuous observation of wildlife by regional levels should be followed by examination and consideration by experts in cases that biological changes including abnormality were observed (Appendix 3).

Under the existing organization of Kids' Eco Club, observation projects were

conducted from FY 2005 to FY 2007. A few clubs nominated on the advice of experts attended the Kids' Eco Club – National Festival and demonstrated an exhibition and explanation of natural life forms.

Wildlife-observing groups with children as the main active members were publicly recruited during FY 2008 and FY 2009. After receiving the advice of experts on observation methods in preparatory meetings, participant groups independently conducted observation. At the end of the fiscal year, presentations by 10 selected groups and an on-site tour in the National Institute for Environmental Studies were held.

This project played a certain role providing opportunities for children to enjoy the nature, look at wildlife ecology, and consider the relation between natural life forms and chemical substances, gaining good reputation by participants. However, there were no cases where wildlife observation by children could lead to studies and investigations by experts.

2.2 Surveys on Environmental Concentrations and Measurement of Exposure Levels

In ExTEND2005, test substances were to be selected after assessing the possibility of human and wildlife exposure and information concerning endocrine disrupting effects. In order to determine the possibility of human and wildlife exposure in the general environment in Japan, the results of the MOE's Environmental Survey and Monitoring of Chemicals were utilized.

In this survey and monitoring, the target chemicals are to be selected based on regulatory needs. Survey and monitoring of the following chemicals were requested to be conducted in order to understand their ambient concentrations for this program .

- Chemicals which were suggested to have potential effects on fish reproduction and development by the EU.
- Chemicals which had not been detected in SPEED'98, and thus should be surveyed with more sensitive detection limits.
- Chemicals reported to have potential effects on the endocrine system, and thus should be surveyed.

Including those requested chemicals, 257 chemicals (groups) were surveyed from FY 2005 to FY 2008 in the Environmental Survey and Monitoring of Chemicals (Initial Environmental Survey, Detailed Environmental Survey, and Exposure Study). Among them, 131 chemicals (groups) were detected (Appendix 4).

2.3 Promotion of Fundamental Studies

Fundamental Studies and Research for Biological Observation of Wildlife have been implemented since FY 2005.

(1) Overview of implemented Fundamental Studies and Research for Biological Observation of Wildlife

In FY 2005, the MOE selected 10 research themes from those implemented in SPEED'98. Additionally, seven themes were adopted for feasibility studies via recommendation by the members of the Sub-Committee for Design and Evaluation of Fundamental Studies, the Sub-Committee for Biological Observation of Wildlife, and the MOE.

After FY 2006, new research themes were recruited in principle under open information about recruitment fields and adopted for feasibility studies (FS: preliminary studies that can be implemented as research themes in the future) via the review of the Sub-Committee for Design and Evaluation of Fundamental Studies and the Sub-Committee for Biological Observation of Wildlife (hereinafter referred to as "both Sub-Committees") (Appendix 5). Study results were reviewed by both Sub-Committees every fiscal year to determine whether the study be adopted as a main theme of Fundamental Studies or Research for Biological Observation of Wildlife. Results of multi-year studies were also reviewed by both Sub-Committees every fiscal year to determine whether the study should be continued.

From FY 2005 to FY 2009, 38 themes in total were adopted and implemented as studies (Appendix 6).

(2) Main achievements and remaining subjects in projects on Fundamental Studies and Research for Biological Observation of Wildlife

1) Overview of main achievements in projects on Research for Biological Observation of Wildlife

- As several factors other than systemic insecticides might simultaneously affect the decreasing number of *Sympetrum* dragonflies, the same spots of numerous rice paddy fields were monitored. In addition to the potential effects of systemic insecticides, research results suggested the impact from the drying up during the off-season.
- As a plausible factor affecting the declining freshwater algae Charales, it was considered that a low level of pentachlorophenol (PCP) pesticide might cause

growth inhibition of the algae by photosynthesis inhibition. It was recognized that improvement of the environment and the withdrawal of PCP have contributed to the recovery of Charales.

2) Overview of main achievements in projects on Fundamental Studies

○ Studies on Invertebrates

・It was revealed that male juveniles appeared when *Daphnia* (only females can appear normally via parthenogenetic development) were exposed to juvenile hormone-like compounds including insect growth regulators. Gene clusters that showed changes accompanying the phenomena were also identified.

・In experiments to study the effects on nonylphenol on crustaceans (mysid), it was observed that slightly higher concentrations of nonylphenol than ambient concentrations could cause growth inhibition by retardation of mysid moulting.

・Nonylphenol, octylphenol, and bisphenol A have already been suggested to have potential endocrine disrupting effects on fish (Medaka). In experiments to study the effects of those chemicals on Ascidiacea, none of them inhibited embryogenesis of *Ciona intestinalis*.

○ Studies on Fish

・It was discovered that about 1% of wild Medaka (including species in China and the Republic of Korea) were individuals (sex-reversed individuals) showing a phenotypic sex different from genotypic sex. It was also revealed that the emergence of sex-reversed individuals could be attributed to genetic mutation.

・It was revealed that juvenile male Medaka are highly sensitive to female hormone-like compounds. The mechanism to generate ova in genotypic male was also clarified.

・It was shown that estrogen receptor α from 9 fish species (Medaka, zebrafish, fathead minnow, stickleback, roach, carp, goldfish, bluegill, and guppy) has an almost identical response to estrogen (estradiol), but has interspecies difference toward DDT-related compounds, which were unused pesticides. Low sensitivity of carp and goldfish was also clarified.

○ Studies on Mammalian

・It was revealed that thyroid gland enlargement found in a certain strain of rat (Wistar Hannover GALAS) could be attributed to genetic mutation.

3) Remaining subjects in both projects

The following subjects were indicated by both Sub-Committees.

・In setting and adopting research themes, it is necessary to set themes that clearly

reflect the regulatory aims and needs. On the other hand, in order to encourage liberal ideas of scientists, it is necessary for recruited studies to have secured freedom of content to a certain degree as long as the theme is related to risk assessment for endocrine disrupting effects of chemical substances.

- In Research for Biological Observation of Wildlife, it is important to examine a wide variety of potential chemical effects on abnormalities found in wildlife. A step by step approach is also necessary to clarify the cause of abnormalities, mechanism of chemical effects, and endocrine disrupting effects.
- Achievements obtained by both research projects should be published in the forms convenient for utilization by both researchers and the general public.

2.4 Effects Assessments

Since FY 2005, projects have been implemented towards test method development, international cooperation, and selection and assessment of test substances as "Actions to Assess Actions and Effects." Here, test chemicals were not preliminarily listed, but assessment works were advanced with reported knowledge and understanding on the current status of environmental existence, while the principle and the procedure of how to select test chemicals were being clarified.

(1) Test method development

Since FY 2005, test methods have been developed for fish, amphibians, and invertebrates. For fish, 21-Day Fish Screening Assay and Fish Sexual Development Assay have been developed. 21-Day Fish Screening Assay was adopted as OECD TG 230 in 2009, and Fish Sexual Development Assay is now under validation for a test guideline. Also under the framework of the Japan-US partnership, Fish Full Life-Cycle Test is compared with Two-generation Reproduction Test proposed by the US.

For amphibians, *Xenopus laevis* Metamorphosis Assay and Amphibian Partial Life-Cycle Test were developed. *Xenopus laevis* Metamorphosis Assay was adopted as OECD TG 231 in 2009, and Amphibian Partial Life-Cycle Test was adopted for an OECD project of the same year.

For invertebrates, improvement on TG 211 *Daphnia magna* Reproduction Test was investigated, and this was adopted in 2008 in the form of ANNEX 7 to OECD TG 211. Also under the framework of the Japan-US partnership, *Daphnia* Multi-Generation Assay was investigated.

For *in vitro* assay, Japan, the UK and Sweden have proposed the investigation

of *in vitro* screening for endocrine disrupting chemicals using fish to the OECD, and worked together for the preparation of a review document.

Many test methods have been investigated and developed in this project, and the achievement was that some of them were proposed for test guidelines to the OECD and approved (Appendix 7). However, these methods have not been linked to practical tests for chemical substances, hazard assessment, or risk assessment. The necessity of these methods being linked to practical test, evaluation and assessment was pointed out.

(2) International cooperation

The Japan-UK joint research was started by the agreement between the Minister of Environmental Agency (Japan) and the Environment Minister (UK) in March 1999, and the second stage joint research (2004-2009) was agreed in FY 2004. The four themes (research to assess estrogen-like effects in waste water, research to develop assessment methods for androgen-like effects using three-spined stickleback, study on the mechanism of testis-ova induction in fish and research on interspecies difference of estrogen receptors in fish, and research to develop assessment methods for amphibian metamorphosis) were defined, and studies were promoted by researchers in both countries. In 2009, it was agreed to extend the period of the joint research for further five years from 2010.

The Japan-US partnership was agreed in the 12th Meeting of Japan-United States Joint Planning and Coordination Committee that was held in January 2004. Carried out at the meeting were activities such as informational exchange about ecological effects assessment and development of test methods to assess effects on reproduction and propagation of fish, amphibians, and invertebrates.

In addition, based on the agreement between the Minister of the Environment (Japan) and the Minister of Environment (Republic of Korea) in April 2001, the Japan-Korea joint research had been carried out in regards to the subjects including endocrine disrupting chemicals. In 2006, the theme of this joint research was switched to Persistent Organic Pollutants (POPs) and related compounds.

These international cooperative activities have resulted in several achievements including the test method development and the understanding of chemical effects on wildlife. On the other hand, it was pointed out in the Sub-Committee for Actions and Effects Assessment that the subjects including the project's stance and the research contents should be reviewed at the beginning of research planning on the Japan side.

(3) Selection of test chemicals and evaluation projects

Following SPEED'98, four chemicals were devoted to *in vitro* assay (Medaka estrogen receptor (ERß) reporter gene assay, Medaka androgen receptor reporter gene assay, and Medaka thyroid hormone receptor binding assay) under the framework of SPEED'98 from FY 2005 to FY 2006. No significant response was recognized for any of those chemicals (Appendix 8.1 & 8.2).

Since FY 2007, selection of test chemicals and evaluation projects have been carried out based on "Procedures for Selecting Chemical Substances for Testing and Assessment of Endocrine Disrupting Effects" under the framework of ExTEND2005 (Appendix 9.1). Literature search was performed for chemicals detected in the Environmental Survey and Monitoring of Chemicals, and the resultant reports were subjected to the reliability evaluation by the "Working Group for the Reliability Evaluation of the Literature Related to Endocrine Disrupting Effects of Chemical Substances". In addition, "chemicals that can be subjected to tests for endocrine disrupting effects" have been selected (Appendix 9.2). So far, seven chemicals have been selected as "chemicals that can be subjected to tests for endocrine disrupting effects", while three chemicals have been classified as "chemicals that are not presently subjected to tests." Reliability evaluation of the literature related to 17 chemicals is in progress (Appendix 10).

For "chemicals that can be subjected to tests for endocrine disrupting effects", the whole test framework and the selection of applicable test methods for each chemical are being reviewed in the "Working Group for the Review of Animal Test Methods for Ecological Effects Assessment."

In this project, reliability evaluation of the literature related to endocrine disrupting effects has not been advanced in a satisfactory way due to a number of literature yet to be evaluated, and the principle of how to impellent tests for evaluation is yet to be defined. Further activities should be accelerated.

2.5 Risk Assessment and Risk Management

As mentioned above, the selection of test chemicals and the following assessment of effects have not been advanced in a satisfactory way. Therefore, chemical risk including endocrine disrupting effects of chemical substances was not assessed, nor was the following identification of chemicals for risk management achieved.

2.6 Promotion of Information Sharing and Risk Communication

For the purposes of unbiased and accessible information sharing and risk communicating in regards to endocrine disrupting effects of chemical substances, websites were prepared and international symposiums were held mainly for the general public after FY 2005.

(1) Preparation of websites

Through the website "Official Endocrine Disruption Website", the MOE have provided unbiased and accessible information on endocrine disrupting effects of chemical substances mainly for the general public. On these websites, topics related to health and chemicals are cited, and materials and references in regards to endocrine disrupting effects of chemical substances are cited or linked.

With a large amount of traffic having been recorded, this website as a "neutral site independent of the MOE's formal website," seems to certainly be functioning as a medium providing information on endocrine disrupting effects of chemical substances. Further efforts are needed to improve the convenience for more general people and the quality of contents cited therein.

(2) International symposium

Since FY 2008, the MOE have hosted the International Symposium on Environmental Endocrine Disrupters with participants including international governments and international organizations, and this has been continued under ExTEND2005. From FY 2006 to FY 2008, this was co-hosted by the "International Symposium on Children's Environmental Heath." This symposium consisted of programs for both the general public and specialists. Information was shared and opinions were exchanged on state-of the art research and actions in Japan and the world (Appendix 11).

This international symposium has attracted many participants every year, and has become a beneficial place for information dissemination and information sharing. As large costs are required to host a symposium of this size, it is necessary to provide a place in a different form in the future.

II Overseas Activities

1. USA

The US Environmental Protection Agency (US EPA) is working under the framework of the Endocrine Disruptor Screening Program (EDSP). Established in 1999 under the stipulation of the Food Quality Protection Act and the Safe Drinking Water Act, the EDSP is a program to screen estrogenic pesticides and drinking water pollutants that can have adverse effects on human health.

(1) Development and validation of test methods

The EDSP adopts the two-step test system composed by the Tier 1 Screening and Tier 2 Test.

Tier 1 Screening comprises assays to detect chemical actions to the animal endocrine system, and composed by five *in vitro* assays (rat estrogen receptor binding assay, HeLa cell estrogen receptor transcriptional activation assay, rat androgen receptor binding assay, human cell steroidogenesis assay, and human cell aromatase assay) and six *in vivo* assays (rat uterotrophic assay, rat Hershberger assay, rat pubertal female assay, rat pubertal male assay, amphibian metamorphosis assay, and fish short-term reproduction assay). Validation of the test methods were investigated and published before October 2009.

Tier 2 Test comprises assays to identify adverse effects on animals. Validation of test methods is in progress and will be completed by the end of 2011, and it is expected that adoptable test methods will be determined.

(2) Implementation of Tier 1 Screening Assays

Judging whether information on human exposure existed or not, the chemicals selected in the first list for the Tier 1 Screening are 67 chemicals including 58 pesticide active ingredients (PAIs) and nine High Production Volume (HPV) chemicals used as pesticide inert ingredients. Further, among pesticides and pollutants detected in drinking water, at least 100 chemicals will be selected in the second list by the end of October 2010.

The EPA issued test orders to the registrants, manufactures, and importers of chemicals in the first list, requiring Tier 1 Screening between October 2009 and February 2010. Tests are to be completed by 2012, and the results will be published.

After the results of screening tests are evaluated considering

weight-of-evidence, chemicals for Tier 2 Test will be selected.

2. European Union (EU)

The European Commission commenced actions to endocrine disrupting substances in 1996. The Community Strategy for Endocrine Disrupters (COM(1999)706) was adopted in 1999, and this was later revised in 2004 (SEC(2004)1372) and in 2007 (SEC(2007)1635).

In this strategy, short-term action (establishment of a priority substances for further evaluation by information gathering exercises), mid-term action (test method development and research implementation), and long-term action (consideration of methodologies for risk assessment and risk management) have been continuously implemented, and it is expected that a report on the implementation of this strategy for endocrine disrupters will be compiled by 2010.

Also in European Union's REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) that entered into force on June 1, 2007, substances "having endocrine disrupting properties" that are also identified from scientific evidence as causing probable serious effects were mentioned as a condition to be authorized as a Substances of Very High Concern (SVHC). However, the details have not been clearly described.

3. Organisation for Economic Co-operation and Development (OECD)

(1) Historical outline

The Organisation for Economic Co-operation and Development (OECD) commenced a Special Activity on Endocrine Disrupter Testing and Assessment (EDTA) in the context of the Chemicals Programme in 1996, with the objectives of providing information and coordinating activities, developing new and revised existing Test Guidelines to detect endocrine disruptors, and harmonizing hazard and risk characterization approaches.

Under EDTA, the Conceptual Framework, as a framework for the testing and assessment, has been developed and revised to provide methodologies for the testing and assessment.

The following tests were newly developed or revised in the field of ecological toxicology, and partial or full life-cycle tests have been examined using fish, amphibians, and invertebrates.

・Fish Short-term Reproduction Assay (TG229): New
・21-Day Fish Assay: A Short-term Screening for Oestrogenic and Androgenic Activity, and Aromatase inhibition (TG 230): New (proposed by Japan)
・Amphibian Metamorphosis Assay (TG 231): New (proposed by Japan)
・Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment (TG233): New
・*Daphnia Magna* Reproduction Test (TG211): Revised (proposed by Japan)

Also, mammalian tests and *in vitro* assays have been widely investigated and developed, including:

・Repeated Dose 28-Day Oral Toxicity Study in Rodents (TG407): Revised
・Uterotrophic Bioassay in Rodents: A Short-term Screening Test for Oestrogenic Properties (TG440): New (proposed by Japan)
・Hershberger Bioassay in Rats: a Short-Term Screening Assay for (Anti) Androgenic Properties (TG 441): New
・Stably Transfected Human Estrogen-α Transcriptional Activation Assay for the Detection of Estrogenic Agonist-Activity of Chemicals (TG 455): New (proposed by Japan)

(2) Recent trends

In September 2009, the Workshop Regarding Testing, Assessment and Management of Endocrine Disrupters was held in Copenhagen for specialists of OECD member states to determine further directions of the OECD's actions. In the report of this workshop, subjects and needs that should be considered under the OECD are proposed. Particularly, the necessity of the evaluation of endocrine disruptors to be investigated was pointed out.

In response to this, preparation of a guidance document on the evaluation of endocrine disruptors has been discussed since 2010.

III Further Directions

1. Principles

(1) Aim and stance of this new program

In regards to endocrine disrupting effects of chemical substances, various research studies and development of test methods have been promoted in previous actions. With remaining uncertainties of the effects, it is considered to be important issues that the MOE should continuously address.

In reviewing the achievements of the research and studies implemented under ExTEND2005, there were scopes for improvement in some parts where progress has not fully been facilitated in the program, while overall revision of adopted principles and basic framework were not regarded as necessary.

Therefore, the MOE decided to promote expansive actions toward endocrine disrupting effects by setting up a new program for approximately five years, adding necessary improvements to ExTEND2005, while maintaining appropriate parts of the framework. The new program is named EXTEND (Extended Tasks on Endocrine Disruption) 2010, aiming to accelerate the establishment and implementation of assessment methodologies toward the goal to properly assess the environmental risk of endocrine disrupting effects of chemical substances and to take management measures if necessary.

The MOE will maintain the high priority of actions on ecological effects and facilitate the establishment of methods for testing and assessment and their implementations, and will also consider human health risk caused by chemical substances in the environment, based on the MOE's role within the national government to,prevent the adverse effects on both human health and the ecological system via the environment.

Further, the MOE will continue to have an active involvement toward the establishment of testing and assessment methodologies through international cooperation such as with the OECD and bilateral cooperation, and to follow actions of other countries and international organizations including the OECD to take full advantages of their achievements.[1)]

[1)] In addition to documents such as WHO Global Assessment for ExTEND2005, the following documents and programs were consulted to extract further actions.
- OECD: Workshop Report on OECD Countries Activities regarding Testing, Assessment and Management of Endocrine Disrupters (22-24 September 2009, Copenhagen)
- US EPA: Endocrine Disruptors Screening Program (launched in 1999)
- EU: Community Strategy for Endocrine Disrupters (adopted in 1999, final review in 2007)

(2) The structures of this program

Based on the obtained achievements and the remaining subjects under ExTEND2005, EXTEND2010 (hereinafter referred to as “this program”) will be approached under the following structures (Figure 1).

1) Promotion of Research for Biological Observation of Wildlife and Fundamental Studies
2) Development of Test Methods and Establishment of Assessment Framework
3) Survey on Environmental Concentrations and Exposure Assessment
4) Implementation of Actions and Effects Assessment
5) Risk Assessment and Risk Management
6) Promotion of Information Sharing
7) Promotion of International Cooperation

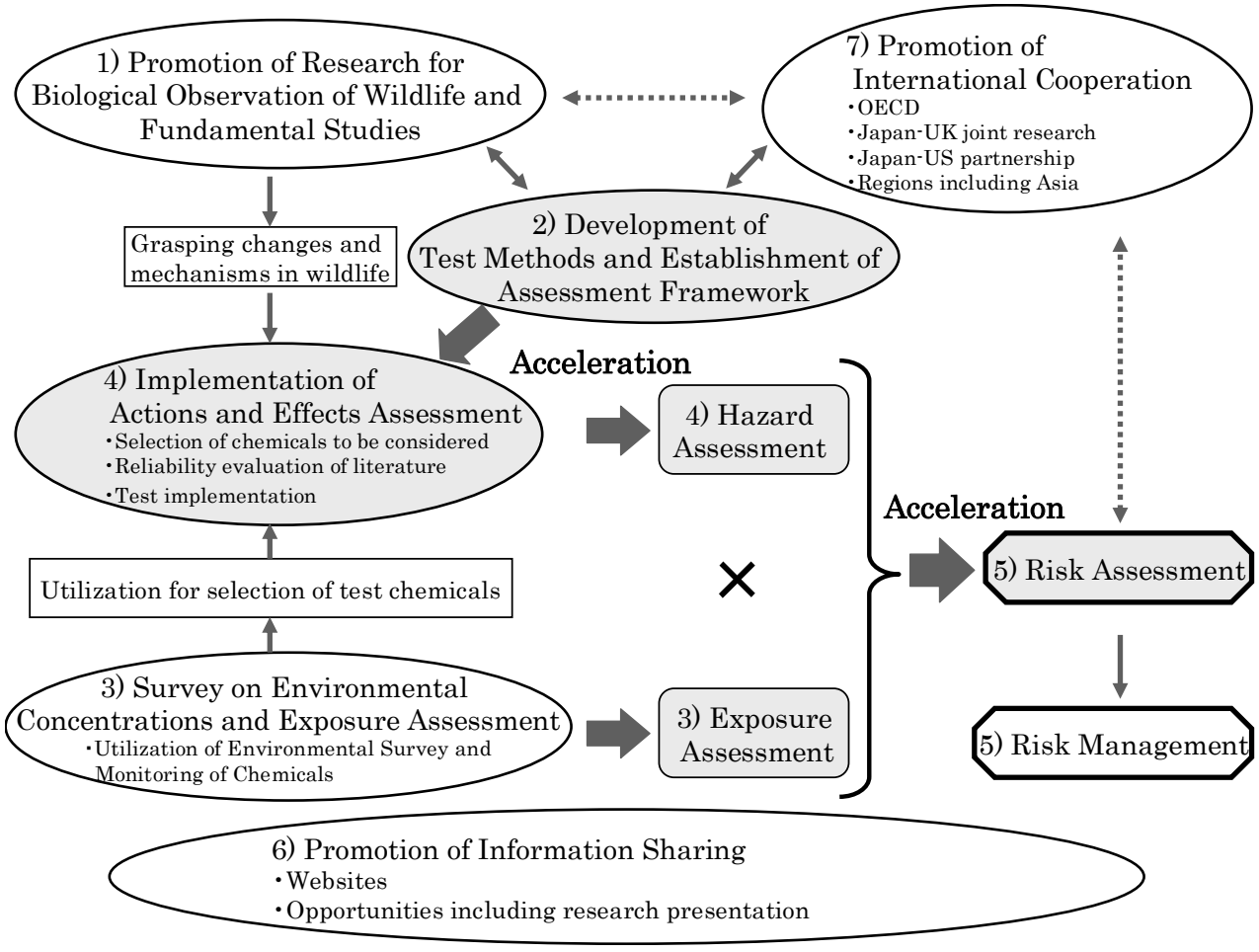

Figure 1 Conceptual Overview of Actions in EXTEND2010

2. Directions

2.1 Promotion of Research for Biological Observation of Wildlife and Fundamental Studies

In ExTEND2005, research themes were recruited in principle to promote Research for Biological Observation of Wildlife and Fundamental Studies. Adoption of each theme and review of study results were subjected by experts in the Sub-Committee for Biological Observation of Wildlife and the Sub-Committee for Design and Evaluation of Fundamental Studies. This framework should basically be maintained also in EXTEND2010, while necessary revision will be considered in implementation.[2)]

In this new program, the following points are to be considered to promote studies.

- In setting and adopting research themes, set themes that clearly reflect the regulatory aims and needs. Select research themes by putting high priority on potential contribution to environmental risk assessment, considering their utilization for regulatory purposes.
- For research themes related to risk assessment including endocrine disrupting effects of chemical substances, set themes in the form of "designated research" if necessary, while major research themes are recruited continuously.
- Determine whether the research theme should be continued or not by clear goal setting for each research theme and the objective evaluation on achievement.
- For themes such as basic research purely to elucidate phenomena, coordinate with other related research budgets.

(1) Research for Biological Observation of Wildlife

To detect and understand the changes observed in wildlife is an important subject when considering endocrine disrupting effects of chemical substances on the ecological system.

In order to judge if a change or sign found in wildlife at individual or group level is abnormal or not, it is important to understand causes and mechanisms, and it is essential to collect ecological data on the species. In order to analyze and verify

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2) In ExTEND2005, "Research for Biological Observation of Wildlife" was a part of "Fundamental Studies" while it was approached independently in the research program. Thus, here in EXTEND2010, "Research for Biological Observation of Wildlife" was to be treated independently. "Test method development", included in "Fundamental Studies" in ExTEND2005, was also decided to be treated independently in "Development of Test Methods and Establishment of Assessment Framework."

whether the chemical effects exist or not, it is also important to comprehend the current situation of the habitats, ambient and biological concentrations of various chemicals, sensitivity of the species to the chemicals, and so on.

In Research for Biological Observation of Wildlife, the MOE will enhance the studies to comprehensively grasp potential chemical effects among changes observed in wildlife. Therefore, not only endocrine effects but also other chemical effects are to be observed. There, clarification of the cause of the change, clarification of the mechanism of the chemical effects, and clarification of endocrine effects are to be approached in a stepwise way.

Animals for research are to be chosen by giving high priority to mammals and birds where chemicals are likely to be accumulated via the food chain, as well as those featured in risk assessment such as test animals used in ecotoxicological studies and their relative species.

While observation of wildlife by children and citizens under ExTEND2005 was regarded beneficial to motivate environmental awareness and risk communication, it seemed difficult to identify the biological effects of chemical substances in the environment. Since such effects are thought to be comprehended by research including observation by experts, observation of wildlife is basically to be promoted under "Research for Biological Observation of Wildlife."

(2) Fundamental Studies

The MOE will continuously enhance Fundamental Studies since there remain many unsolved subjects on endocrine disrupting effects of chemicals.

When endocrine disrupting effects of chemical substances are suspected in changes observed in wildlife individuals or populations, sufficient understanding of mechanisms of chemical substances in the bodies of wildlife individuals is essential in order to judge whether the observed individual-level change is a primary or secondary effect via endocrine disruption. It is also necessary to clarify the relationship between the significant individual-level changes and cell or molecular-level changes.

On the other hand, in order to deduce the endocrine disrupting effects of chemical substances on both the ecological system and human health, it is inevitable to establish various test methods (*in vivo* and *in vitro* assays) by utilizing available up-to-date knowledge and technology. Therefore, it is important to have clear basic knowledge of endocrine-related biological regulation function (homeostasis) of each test species, their variation ranges dependent of test conditions and individual

difference, and so on. It is also important to accumulate knowledge that can lead to the development of new test methods. This includes searches for biomarkers and endpoints that can be applicable to detect endocrine disrupting effects of chemical substances.

Since the MOE launched the Japan Environment & Children's Study (Japan Eco & Child Study) in FY 2010, it is important to comprehensively grasp the cause-effect relationship between chemical exposure and effects, relations to epigenetics[3], and so on. Consideration for developmental stages and effects on highly sensitive individuals, as well as comprehension of effects by combined chemical exposure, are also pointed out to be necessary.

Taking them into account, studies relate to the following items are to be promoted intensively.

1) Comprehension of chemical mechanism by individual- or population-level approach

Collect basic knowledge that is necessary for the assessment of individual- or population-level (adverse) effects including endocrine disrupting effects of chemical substances. Effects on the brain nerve system and immune system, as well as endocrine and reproductive system, are to be considered.

2) Cell or molecular level approach

As cell or molecular-level assessment system such as *in vitro* assays can be efficiently experimented, obtained knowledge can be beneficial to clarify a specific mode of mechanism. Clarify modes of mechanisms of chemical substances including epigenetics, while introducing useful technologies including genomics, proteomics, and metabolomics.

3) Fundamental studies conductive to test method development

During the course of test method development to assess the effects on wildlife in the environment, obtain basic knowledge that can support the effectiveness and validity of the test methods.

(3) Notes for management of the study program

Since it is preferable to promote Research for Biological Observation of Wildlife and Fundamental Studies in consideration of their mutual relations, procedures, including identification of vital research fields, adoption of research themes, advice

[3] Epigenetics is a mechanism that controls gene function without accompanying any changes in DNA sequence. It is suggested that endocrine disrupting effects can be triggered by changes in DNA methylation and subsequent changes in gene expression.

to promote research, and evaluation of research results, are to be promoted under the intimate cooperation between the Sub-Committee for Biological Observation of Wildlife and Sub-Committee for Design and Evaluation of Fundamental Studies. Due to the importance of the Fundamentals Studies intending future test method development, the MOE will promote actions by sharing information on the requirements of test method development, and actions in Japan and the world.

The following items are to be improved to manage the research program.

- In order to promote important subjects intensively, sizes of budgets are not to be divided excessively into small portions.
- In order to secure sufficient research periods, a research is to be started earlier than ever by facilitating procedures.

Achievements of research themes under this program are published not only in media for researchers including academic journals, but also in media including websites for general public in an accessible way.

2.2 Development of Test Methods and Establishment of Assessment Framework

In order to widely assess ecological effects, test methods were developed to assess endocrine disrupting effects in fish, amphibians, and crustaceans in ExTEND2005. Among various test methods, some are under consideration in the OECD at different levels of development. In the history of test method development in regards to endocrine disrupting effects, Japan is continuously required to play important roles as one of the leading countries. Based on the knowledge obtained from these tests, in reference to cases in Europe and America and further consideration for test methods developed in the OECD, it is necessary to establish without delay the framework to judge how risk assessment of the endocrine disrupting effects is to be implemented.

In such consideration, achievements obtained from Research for Biological Observation of Wildlife and Fundamental Studies are to be utilized if necessary.

(1) Test method development

The MOE will continuously promote test method development, putting emphasis on assessing effects on wildlife in the environment. Aiming at application for ecological assessment to be conducted as an environmental regulatory task, the MOE will intensively develop the test methods conductive to this purpose. From these points of view, the immediate priority is put on aquatic life, and test method development is to be promoted mainly for fish as well as crustaceans and

amphibians. At the same time, Japan is required to participate in international validation of test methods.

Also, in order to streamline screening methods, it is necessary to verify validity and effectivity of *in vitro* assays including receptor binding assay and receptor reporter gene assay, by collection of information on false positives and false negatives in assay results, in relation to *in vivo* assay results, shared properties, and so on.

The MOE will promote to develop these test methods efficiently and effectively in cooperation among OECD member states, while activating bilateral cooperation of Japan-US and Japan-UK.

(2) Establishment of assessment framework

In ExTEND2005, the reliability of extensively gathered existing literature that could be related to endocrine disrupting effects of each chemical substance was evaluated. Based on the results, whether the corresponding chemical can be classified as a "chemical that can be subjected to tests" or not was determined. Judging from the present state where discussion on what tests should be conducted for assessment has just been started, however, establishment of assessment framework is urgently required to start environment risk assessment and management following the test results.

Therefore, the actions of other countries and international organizations should always be followed to take full advantage of their achievements. For example, since actions have been commenced under the framework of the EDTA program in the OECD to assess endocrine disrupting effects of chemical substances, Japan will take part in the discussion and reflect its achievement. It is also necessary to facilitate consideration in Japan in full reference to the principles of assessment including the Endocrine Disruptor Screening Program in the US and REACH in the EU.

Notes for assessment framework are as follows.

- Make it clear in risk assessment what effects should be regarded as adverse effects caused by endocrine disruption.
- Classify tests into screening and definitive purposes in order to characterize the adverse effects.
- Make it systematic in hazard assessment how to combine available knowledge including information obtained from reliability evaluation of existing literature.
- Make efforts to reduce the number of test animals including mammals and fish,

considering the global animal welfare movement.

・Consider the possibility to assess the chemical effects on wild mammals, utilizing tests with mammals that have been used for human health effects assessment.

(3) Human health effects assessment

In ExTEND2005, establishment of methodologies to assess ecological effects and implementation of research were primarily considered. However, it is also necessary to accumulate knowledge for human health risk assessment of endocrine disrupting effects and to consider assessment methodologies since the human health risk assessment of effects caused by chemical substances in the environment should also be addressed by the MOE. Since relevant ministries and agencies have promoted investigation and development of test methods for human health effects assessment in Japan, the MOE will, in full reference to the progress of their consideration, approach the assessment applying their achievements if necessary.

2.3 Survey on Environmental Concentrations and Exposure Assessment

In order to assess environmental risk of chemical substances properly, it is essential to understand the current situation of their environmental levels as well as their hazard information. The MOE has implemented the Environmental Survey and Monitoring of Chemicals, and recently revised the system of this survey and monitoring including site selection procedures in order to provide essential and sufficient data in conjunction with PRTR data for regulatory risk assessment. The MOE will continuously utilize this survey and monitoring to understand the current situation of environmental levels of chemical substances. In measuring, sites are to be selected based on information including their production, use and PRTR data so that they can be employed in exposure assessment.

In exposure assessment, the MOE will take full advantage of other environmental survey results including those of Survey Items for water environment preservation. In addition to environmental survey data, based on exposure routes of chemicals in the environment to humans and wildlife, the MOE will collect and utilize relevant data for exposure assessment, and apply estimation models if necessary.

2.4 Implementation of Actions and Effects Assessment

In ExTEND2005, reliability evaluation of related reports was not advanced in a satisfactory manner, although the MOE promoted these actions based on objective

procedures and criteria. Therefore, the MOE will effectively promote evaluation in reference to actions in Europe and America. Evaluation procedures will be revised based on progress in "2.2. Development of Test Methods and Establishment of Assessment Framework" as well as this clause, while maintaining schemes under the framework of ExTEND2005 (Appendix 9.1 and 9.2).

(1) Selection of chemical substances to be considered

In ExTEND2005, considering all chemical substances detected in the Environmental Survey and Monitoring of Chemicals, the MOE have promoted reliability evaluation of literature related to a wide variety of effects in accordance with the principle that "substances specified in chemical regulations, substances specified of having an effect on/via the endocrine system in reports published by public organizations including international organizations, and so on, among all substances" are to be considered. From hereon, the MOE will choose about 100 chemicals for five years in order to efficiently screen chemicals suspected of potential environmental risk. The following points are to be noted.

- In selecting the chemicals for evaluations of literature, utilize the PRTR data such as releases, information of produced/imported amounts, as well as the results of the Environmental Survey and Monitoring of Chemicals.
- Judging from detection and other results in the Environmental Survey and Monitoring of Chemicals, narrow down considered chemicals.
- As candidates for chemical substances to be considered, include chemicals specified of having an effect on/via the endocrine system in reports published by public organizations including other governments and international organizations.

(2) Assessment of effects based on literature information (reliability evaluation)

On the basis of literature information selected by the most recent search at the time, the MOE will evaluate information of effects and events related to endocrine disruption, and narrow down considered chemical substances.

Although it is important to promote evaluation avoiding oversight, excessive emphasis on this point can rather spoil effective narrowing down of chemical substances suspected of endocrine disrupting effects. While keeping this in mind, it is required in reference to the progress of the establishment of the assessment framework to streamline a collection of literature and evaluation of their reliability, aiming to effectively identify chemical substances that require investigation for risk management.

The team for reliability evaluation is to be reinforced in order to facilitate assessment tasks.

(3) Test implementation and hazard assessment

When sufficient knowledge is not available from literature information, the MOE will consider test implementation for hazard assessment in accordance with the assessment framework that will be established in 2.2. When the MOE judges the necessity of test implementation, chemicals suspected of potential environmental risk should be considered a high priority in reference to their environmental existence so that the MOE will efficiently perform sufficient tests for evaluation. Hazard assessment for endocrine disrupting effects will be implemented by adding the test results to known data.

When sufficient knowledge for hazard assessment is obtained from literature information, the MOE will implement hazard assessment for endocrine disrupting effects based on this knowledge.

(4) Human health effects assessment

While (1), (2) and (3) will be promoted mainly for effects on wildlife, the MOE will, in reference to the progress concerning human health effects assessment for endocrine disrupting effects in Japan and the world, approach risk assessment of environmental chemicals on human health effects, applying their achievements if necessary.

2.5 Risk Assessment and Risk Management

(1) Risk assessment

Following the results of "2.4 Implementation of Actions and Effects Assessment," the MOE will promote risk assessment.

As indicated in ExTEND2005, it is not appropriate in risk assessment of environmental chemicals to assess endocrine disrupting effects independently. It is necessary to assess the effect as an aspect of various actions of the chemical substance or combined effects with other biological actions.

The MOE implements risk assessment in response to the following two levels.

- Initial risk assessment aiming to efficiently select chemical substances suspected of high environmental risk from numeral candidates, and screen candidates for detailed assessment toward the risk management.
- Detailed risk assessment aiming to judge the necessity for risk management by the

national environmental regulation including setting of standards and introduction of regulation by law.

The MOE will promote risk assessment, seeking possibility to add the assessment of endocrine disrupting effects to this system of risk assessment.

In risk assessment, the MOE will utilize the achievements of Research for Biological Observation of Wildlife if necessary.

(2) Risk management

The MOE will promptly consider proper risk management measures when risk assessment mentioned in (1) above has identified chemical substances in need of risk management.

2.6 Promotion of Information Sharing

Now that assessment methodologies and other actions are still in consideration in regards to endocrine disrupting effects of chemical substances, and assessment for endocrine disrupting effects is not adequately advanced also in other countries, risk communication is regarded continuously important. Information about the importance of considering endocrine disrupting effects of chemical substances needs to be addressed not only to specialists, but also to the general public.

Related information is to be disseminated through websites, and opportunities for research presentations are to be planned if necessary.

(1) Information sharing via websites

The MOE will promote information sharing through the website "Official Endocrine Disruption Website", improving the convenience for general people. The quality of the current contents is to be improved so that many more people can access and utilize it effectively. Study results are also to be provided for the general public. Additionally, while maintaining the feature as a "columns and essays" site to post various opinions, attention should be paid to encourage the general public to judge by their own ideas via such information.

(2) Hosting opportunities including research presentations

Based on the principle to actively host opportunities for research presentations instead of large international symposiums, the MOE will set up occasions to present achievements of studies and considerations as the host ministry or a co-host in conjunction with related academic societies, and induce contribution to academic

journals. The MOE will consider hosting sessions including symposiums where information such as progress of the program, achievement of research and studies, and international activities are presented to a general audience in an accessible way.

(3) Others

In the project for observation of wildlife as a part of risk communication, it was regarded meaningful to provide opportunity to consider chemical effects on wildlife, while a relation to endocrine disrupting effects of chemical substances was not clearly indicated. From hereon, this project will be transferred to similar projects within the MOE by adding the viewpoint of chemical effects. Under such a framework, the MOE will promote the actions including preparation of an advising manual for wildlife observation.

2.7 Promotion of International Cooperation

(1) Consideration in the OECD

In regards to activities including development of test methods and assessment methodologies, programs are promoted under the cooperation among OECD member states under the OECD Special Activity on Endocrine Disrupter Testing and Assessment (EDTA). The MOE will follow the acitivieis and maintain active contributions mainly to development of test methods for wildlife effects assessment.

(2) Japan-UK joint research

The MOE will promote the following four fields that were defined in November 2009 as core projects of the third term Japan-UK joint research.

1) Research to estimate the transport and fate of chemical substances suspected of endocrine disrupting effects in treated effluents and their receiving waters, and research to consider ways to reduce their environmental discharge.
2) Development of methodologies (test methods) to assess adverse effects on wildlife in order to assess potential environmental risk of endocrine disrupting chemicals.
3) Research related to evaluation of various endpoints (gene and molecular biological approach) in chemical testing to understand reproductive and developmental effects on animals including aquatic life.
4) Analysis of environmental risk for wildlife (including effects in population level) in the UK and Japan

(3) Japan-US partnership

The Japan-US partnership has been steadily promoted aiming to consider methodologies to assess developmental and reproductive effects on fish, amphibians, and invertebrates, towards test method development. The MOE will continuously promote research in this field along with regulatory information exchange under the cooperation with the US.

(4) Cooperation in regions including Asia

Concern about the effects of chemical substances on humans and wildlife is also growing in developing countries. International actions, including chemical risk reduction and information exchange, are in progress under the Strategic Approach to International Chemicals Management (SAICM) adopted in 2006. The MOE will also promote information sharing on chemical endocrine disruption based on accumulated knowledge.

3. Organization

Implementing this program, the MOE will set up the "Task Force on Endocrine Disrupting Effects of Chemical Substances" and its three Sub-Committees (Sub-Committee for Design and Evaluation of Fundamental Studies, Sub-Committee for Biological Observation of Wildlife, and Sub-Committee for Actions and Effects Assessment) where methods to promote projects and evaluation of research results and other actions are to be reviewed every fiscal year (Figure 2).

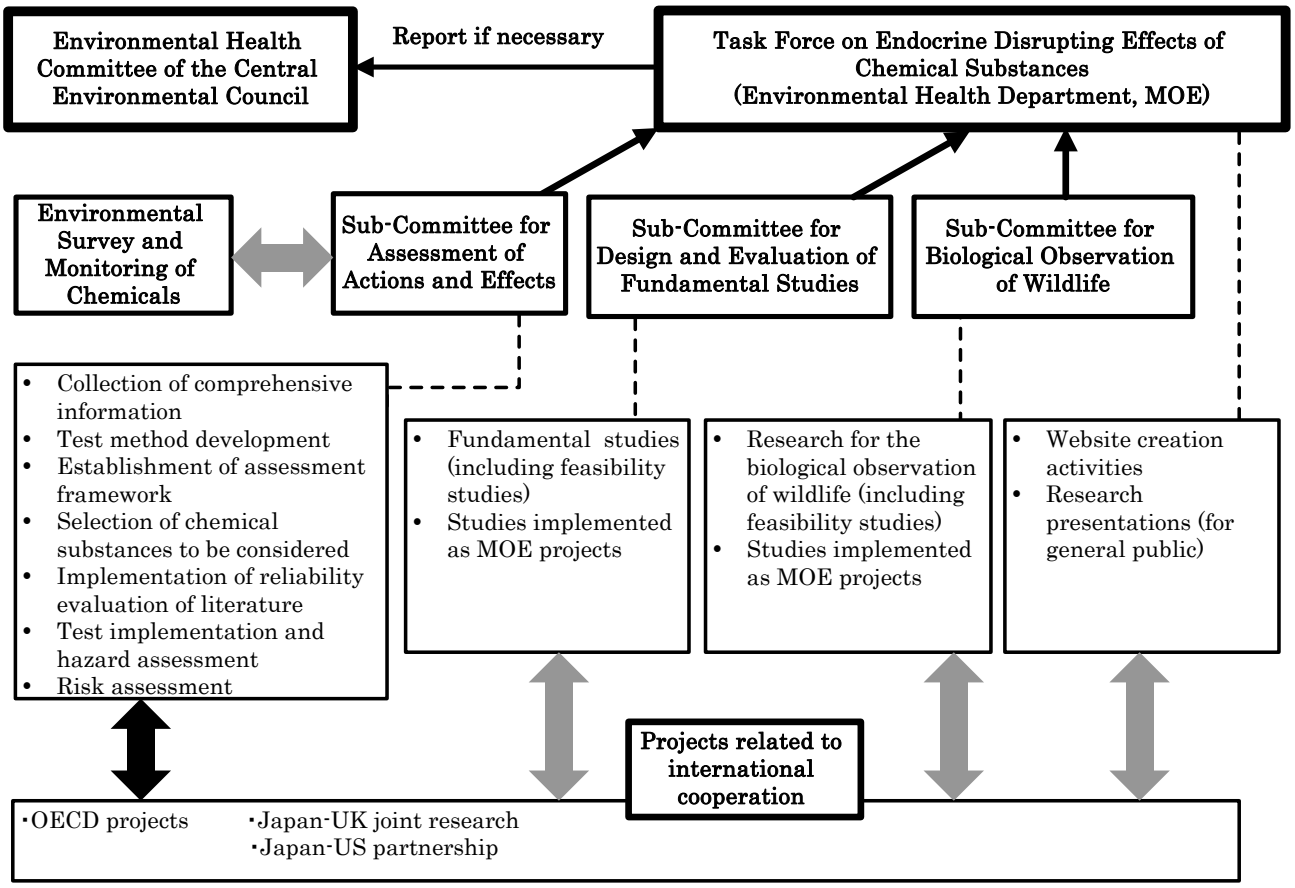


Figure 2　EXTEND2010 Framework to Promote Research and Studies

Concluding Remarks

Policies herein are the summary of the MOE's further actions to endocrine disrupting effects of chemical substances for approximately five years from 2010. As their intrinsic nature, they will be needed to be revised if necessary according to the progress of further studies and research.

In regards to endocrine disrupting effects of chemical substances, due to the accumulated knowledge particularly for test method development, collection of knowledge for selected chemicals and following regulatory consideration will possibly be promoted in Europe and America. Also in Japan, responses as environmental regulatory tasks should be considered in careful reference to activities that will be carried out in other nations.

In this program, keeping the above subjects in mind, the MOE will accelerate actions including establishing test methods and implementing assessments in order to promote environmental risk assessment for endocrine disrupting effects of chemical substances, seeking possibility to incorporate them into risk management systems if necessary.

Appendices

(Under the framework of SPEED'98)

1 Results of Tests Using Fish (Medaka) (FY 2000 - FY 2004)

2 Results of Tests Using Mammalian (Rat) (FY 2000 - FY 2004)

(Under the framework of ExTEND2005)

3 Overview of Implemented Projects on Observation of Familiar Wildlife (FY 2005 - FY 2009)

4 Results of Environmental Survey and Monitoring of Chemicals (Detected Chemicals) (FY 2005 - FY 2008)

5 Results of Recruitment and Adoption of Feasibility Studies (FY 2005 - FY 2009)

6 Overview of Implemented Fundamental Studies and Research for Biological Observation of Wildlife under the Framework of ExTEND2005

7 Overview of Test Methods Adopted by the OECD

8.1 Results of Tests Using Fish (Medaka) (FY 2005)

8.2 Results of Tests Using Mammalian (Rat) (FY 2005 - FY 2006)

9.1 Procedures for Selecting Chemical Substances for Testing and Assessment of Endocrine Disrupting Effects

9.2 Flowchart for Reliability Evaluation of Literature

10 Target Chemicals Subject to the Reliability Evaluation of the Reports on Endocrine Disrupting Effects of Chemicals (As at the end of FY 2009)

11 Overview of Previous International Symposium on the Environmental Risks of Chemicals (FY 2005 - FY 2008)

12 Members of the Task Force on Endocrine Disrupting Effects of Chemical Substances and its Sub-Committees

Appendix 1　Results of Tests Using Fish (Medaka)
(FY 2000 - FY 2004)

Test Chemicals	Test Results
Di-2-Ethylhexyl adipate	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Amitrole	Clear endocrine disrupting effects were not recognized.
Aldrin*	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, 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.
Endrin*	Clear endocrine disrupting effects were not recognized.
Octachlorostyrene	Clear endocrine disrupting effects were not recognized.
4-*tert*-Octylphenol	(1) Strong binding to the fish female hormone receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the male liver, (3) emergence of testis-ova, and (4) decreased number of eggs hatched and decreased fecundity rate were observed. It is postulated that there is a strong endocrine disrupting effect on fish.
cis-Chlordane	Clear endocrine disrupting effects were not recognized.
Kelthane*	Clear endocrine disrupting effects were not recognized.
2,4-Dichlorophenol	Clear endocrine disrupting effects were not recognized.
Dieldrin*	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
4-Nitrotoluene	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, 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 male liver, (3) emergence of testis-ova, and (4) decreased fecundity rate were observed. It is postulated that there is a strong endocrine disrupting effect on fish.
Bisphenol A	(1) Weak binding to the fish female hormone receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the male liver, (3) emergence of testis-ova, and (4) increased number of days of incubation (delayed hatching) were observed. 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	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Dicyclohexyl phthalate	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Di-*n*-butyl phthalate	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.

Test Chemicals	Test Results
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.
Butyl benzyl phthalate	Clear endocrine disrupting effects were not recognized.
β-Hexachlorocyclohexane	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Hexachlorobenzene	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Heptachlor*	Clear endocrine disrupting effects were not recognized.
Permethrin*	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Benzophenone	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized for low concentrations (comparatively low concentration for fish considering the estimated exposure dose obtained from literature).
Pentachlorophenol	Clear endocrine disrupting effects were not recognized.
Mirex*	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
Malathion*	Clear endocrine disrupting effects were not recognized.
p,p'-DDD	Testis-ova were observed in low appearance frequency to the extent that would not have caused adverse effects on fecundity. Thus, clear endocrine disrupting effects were not recognized.
p,p'-DDE	The indicators of endocrine disrupting effects did not show significant changes for the concentration range where lethal toxicity in fish was not recognized.
o,p'-DDT	(1) Weak binding to the fish female hormone receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the male liver, (3) emergence of testis-ova, (4) decreased fecundity rate, and (5) increased number of days of incubation (delayed hatching) were observed. It is postulated that there is a strong endocrine disrupting effect on fish.
p,p'-DDT	Clear endocrine disrupting effects were not recognized.

Note: In ExTEND2005, 28 test chemicals were listed. As shown in this table, eight more chemicals (added and marked with *) had been tested by FY 2004 and their test results were evaluated in FY 2005.

Appendix 2　Results of Tests Using Mammalian (Rat)
(FY 2000 - FY 2004)

Test Chemicals	Test Results
Amitrole	Clear endocrine disrupting effects were not recognized for doses (3 dose groups) determined considering estimated human exposure doses (obtained from literature).
Heptachlor*	
Benzophenone	
Di-2-Ethylhexyl adipate	Clear endocrine disrupting effects were not recognized for doses (4 dose groups) determined considering estimated human exposure doses (obtained from literature).
Aldrin*	
Triphenyltin chloride	
Tributyltin chloride	
Endrin*	
Octachlorostyrene	
4-*tert*-Octylphenol	
cis-Chlordane	
Kelthane*	
2,4-Dichlorophenol	
Dieldrin*	
4-Nitrotoluene	
trans-Nonachlor	
4-Nonylphenol (branched)	
Bisphenol A	
Diethyl phthalate	
Di-2-ethylhexyl phthalate	
Dicyclohexyl phthalate	
Dipropyl phthalate	
Dihexyl phthalate	
Dipentyl phthalate	
Butyl benzyl phthalate	
β-Hexachlorocyclohexane	
Hexachlorobenzene	
Permethrin*	
Pentachlorophenol	
Mirex*	
Malathion*	
p,p'DDD	
p,p'DDE	
o,p'DDT	
p,p'DDT	
Di-*n*-butyl phthalate	Clear endocrine disrupting effects were not recognized for doses (5 dose groups) determined considering estimated human exposure doses (obtained from literature).

Note: In ExTEND2005, 28 test chemicals were listed. As shown in this table, eight more chemicals (added and marked with *) had been tested by FY 2004 and their test results were evaluated in FY 2005.

Appendix 3

Overview of Implemented Projects on Observation of Familiar Wildlife (FY 2005 - FY 2009)

Year	Activities	Participants
FY 2005	○Under the previous organization of Kids' Eco Club, observation projects were conducted from FY 2005 to FY 2007. Before observation, participants received advice from experts in preparatory meetings. ○Reflecting expert opinions on the reports submitted by participants after observation, a few clubs were selected as representatives to join the Kids' Eco Club – National Festival. At this festival, special booths were set up. Exhibitions and explanations of animals were demonstrated, and panels were displayed in order to introduce the projects on observation of familiar wildlife and to report each club's observation results. Furthermore, guest speakers were invited to present lectures on the relations between wildlife in the ecosystem.	10 groups
FY 2006		28 groups
FY 2007		28 groups
FY 2008	○Wildlife-observing groups, where children are the main active members, were publicly recruited from FY 2008 to FY 2009. After receiving advice from experts on observation methods in preparatory meetings, participant groups independently conducted observations. The expert advices were being given to participant groups during the implementation period. Furthermore, the 10 best groups were selected and their presentations were held at the end of the fiscal year in order to encourage friendship between groups. ○Since FY 2008, as new actions, from the viewpoint of relations to endocrine disrupting effects of chemicals, opportunities such as research introductions by experts in preparatory meetings and on-site tours to learn the state-of-the-art research in the National Institute for Environmental Studies after presentations were held.	23 groups
FY 2009		23 groups

Appendix 4

Results of Environmental Survey and Monitoring of Chemicals (Detected Chemicals) (FY 2005 - FY 2008)

Survey Year (FY)	Survey Name	Surveyed Media (chemical numbers detected/surveyed)	Detected Chemicals
FY 2005	Initial Environmental Survey	Surface water (6/33)	17*β*-Estradiol Estrone 2,4,6-Tribromophenol Poly(oxyethylene) dodecyl ethers (polymerization degree 2 -14) among poly(oxyethylene) alkyl ethers (alkyl chain C12 - C15) Poly(oxyethylene) nonylphenyl ethers (polymerization degree 2 - 15) 2-Methoxy-5-methylaniline
		Sediment (6/13)	2,3-Epoxy-1-propanol *m*-Chloroaniline 3,3'-Dichloro-4,4'-diaminodiphenylmethane Short-chained chlorinated paraffins Linear alkylbenzene sulfonate (LAS, alkyl chain C10 - C14) 2,4-Toluenediamine (synonym: 2,4-Diaminotoluene)
		Wildlife (1/2)	Short-chained chlorinated paraffins
		Air (1/1)	*N*-(1,3-dimetylbutyl)-*N'*-phenyl-*p*-phenylenediamine
	Detailed Environmental Survey	Surface water (8/13)	4,4'-Isopropylidenediphenol (synonym: Bisphenol A) Ethylenediaminetetraacetic acid 4-(1,1,3,3-Tetramethylbutyl)phenol among *p*-Octyl phenols *o*-Dichlorobenzene* *p*-Dichlorobenzene *N,N*-Dimethylformamide Nonylphenol Perfluorooctanoic acid Perfluorooctanesulphonic acid
		Sediment (4/5)	Diisopropylnaphthalene Hydrazine Perfluorooctanoic acid Perfluorooctanesulphonic acid
		Wildlife (4/4)	Diisopropylnaphthalene Short-chained chlorinated paraffins Perfluorooctanoic acid Perfluorooctanesulphonic acid
		Air (1/1)	*N,N*-Dimethylformamide
	Exposure Study	Surface water (9/17)	Aniline 2-(2*H*-1,2,3-Benzotriazol-2-yl)-4,6-di-*tert*-butylphenol 2,4-Di-*tert*-butyl-6-(5-chloro-2*H*-1,2,3-benzotriazol-2-yl) phenol 2-Chloro-2',6'-diethyl-*N*-(2-propoxyethyl) acetanilide (synonym: Pretilachlor) Diisopropyl 1,3-dithiolan-2-ylidenemalonate (synonym: Isoprothiolane) *S*-(2,3-Dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-yl) methyl *O,O*-dimethyl dithiophosphate (Methidathion or DMTP) *O,O*-Dimethyl *O*-3-methyl-4-(methylthio)phenyl phosphorothioate (synonym: Fenthion OR MPP) *S*-Benzyl-*O,O*-diisopropyl phosphorothioate (synonym: Iprobenfos or IBP) *a,a,a*-Trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (synonym: Trifluralin) 1-Naphthyl methylcarbamate (synonym: Carbaryl or NAC)**

Survey Year (FY)	Survey Name	Surveyed Media (chemical numbers detected/surveyed)	Detected Chemicals
FY 2005	Exposure Study	Sediment (2/3)	Vinclozolin (synonym: *N*-3,5-Dichlorophenyl-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione) Methoxychlor
		Wildlife (2/8)	*O,O*-Diethyl *S*-(2-ethylthio ethyl) dithiophosphate (synonym: Ethyl thiometon) *a,a,a*-Trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (synonym: Trifluralin) 1-Naphthyl methylcarbamate (synonym: Carbaryl or NAC)**
		Food (2/2)	Acrolein Linear alkylbenzene sulfonate (LAS, alkyl chain C10 - C14)
		Indoor air (2/2)	Acrolein 3-Methyl-4-nitrophenol
FY 2006	Initial Environmental Survey	Surface water (13/49)	9,10-Anthracenedione (synonym: Anthraquinone) 2-Ethylamino-4-isopropylamino-6-methylthio-1,3,5-triazine (synonym: Ametryn) 5-Ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)- pyrimidinetrione (synonym: Phenobarbital) 1,2-Epoxybutane 2-(4-Chloro-6-ethylamino-1,3,5-triazine-2-yl)amino-2-methyl propiononitrile (synonym: Cyanazine) *a*-Cyano-3-phenoxybenzyl 2,2-dichloro-1- (4-ethoxyphenyl) cyclopropanecarboxylate (synonym: Cycloprothrin) Cyclohexanone Dichlorobromomethane 5,5-Diphenyl-2,4-imidazolidinedione (synonym: Phenytoin) 2-(Di-*n*-butylamino)ethanol 1,4-Dibromobutane Thallium and its compounds (as Thallium) Methyl 2-(4,6-dimethoxy-2-pyrimizinyloxy)-6-[1-(methoxyimino)ethyl] benzoate (synonym: Pyriminobac-methyl)
		Sediment (3/6)	Adipic acid Benzyl alcohol Poly(oxyethylene) alkyl (C12-15) ethers
		Wildlife (1/1)	Phenanthrene
		Air (7/28)	Indium and its compounds (as Indium) 1,2-Epoxybutane 2-Chloropropionic acid Mixture of 2,4-dinitro-6-octylphenyl crotonate and 2,6-dinitro-4-octylphenyl crotonate (octyl = 1-methylheptyl, 1-ethylhexyl or 1-propylpentyl) (synonym: Dinocap or DPC) Thallium and its compounds (as Thallium) Tellurium and its compounds (as Tellurium) Phenanthrene

Survey Year (FY)	Survey Name	Surveyed Media (chemical numbers detected/surveyed)	Detected Chemicals
FY 2006	Detailed Environmental Survey	Surface water (11/22)	*O*-Ethyl *O*-4-nitrophenyl phenylphosphonothioate (synonym: EPN) 2,6-Xylenol 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (synonym: Diuron or DCMU) 2,4-Di-*tert*-butyl-6-(5-chloro-2*H*-1,2,3-benzotriazol-2-yl)phenol *O,O*-Diethyl *O*-2-isopropyl-6-methyl-4-pyrimidinyl phosphorothioate (synonym: Diazinon) *O,O*-Dimethyl *O*-3-methyl-4-nitrophenyl phosphorothioate (synonym: Fenitrothion or MEP) 2-(2*H*-1,2,3-Benzotriazol-2-yl)-4,6-di-*tert*-butylphenol Methyl methacrylate 2-*sec*-Butylphenyl *N*-methylcarbamate (synonym: Fenobucarb or BPMC) Dimethyl 2,2-dichlorovinyl phosphate (synonym: Dichlorvos or DDVP) Tributyl phosphate
		Sediment (3/7)	2,4-Di-*tert*-butyl-6-(5-chloro-2*H*-1,2,3-benzotriazol-2-yl)phenol *N,N*-Dimethylformamide 2-(2*H*-1,2,3-Benzotriazol-2-yl)-4,6-di-*tert*-butylphenol
		Wildlife (5/11)	3-(3,4-Dichlorophenyl)-1,1-dimethylurea (synonym: Diuron or DCMU) 2,4-Di-*tert*-butyl-6-(5-chloro-2*H*-1,2,3-benzotriazol-2-yl)phenol Hydrogenated terphenyl Hydrazine 2-(2*H*-1,2,3-Benzotriazol-2-yl)-4,6-di-*tert*-butylphenol
		Air (6/7)	Isobutyl acetate 2,6-Dichlorobenzonitrile (synonym: Dichlobenil) Tetrahydrofuran 1-Butanol Furfural 2-(1-Methylethoxy)ethanol
		Food (2/2)	Hydrazine 2,3-Dihydro-2,2-dimethyl-7-benzo[b]furanyl *N*-methylcarbamate (synonym: Carbofuran)
FY 2007	Initial Environmental Survey	Surface water (8/17)	2,4-Xylenol Quinoline Dibenzyl ether (synonym: [(Benzyloxy)methyl]benzene) Dimethyl 4,4'-*o*-phenylene bis(3-thioallophanate) (synonym: Thiophanate-methyl) Vanadium and its compounds (as Vanadium) Phenanthrene Dimethyl phthalate Mercaptoacetic acid
		Sediment (3/3)	Dibenzyl ether (synonym: [(Benzyloxy)methyl] benzene) Phenanthrene Dimethyl phthalate
		Air (5/10)	1-Chloronaphthalene Dimethyl terephthalate Propane-1,2-diyl dinitrate Benzyl alcohol Triphenyl phosphate

Survey Year (FY)	Survey Name	Surveyed Media (chemical numbers detected/surveyed)	Detected Chemicals
FY 2007	Detailed Environmental Survey	Surface water (10/22)	Acrylic acid *S*-ethyl hexahydro-1*H*-azepin-1-carbothioate (synonym: Molinate) 2-Chloro-*N*- 2',6'-diethylphenyl-*N*-methoxymethyl acetamide (synonym: Alachlor) Diisopropylnaphthalene Cyclohexene 2,4-Dichlorophenoxyacetic acid (synonym: 2,4-D or 2,4-PA) Diphenylamine Dibenzyltoluene Hydrogenated terphenyl *O,O*-Dimethyl *O*-[3-methyl-4-methylthiophenyl] phosphorothioate (synonym: Fenthion or MPP)
		Sediment (5/8)	Diethylbiphenyl Cyclohexene Dibenzyltoluene Hydrogenated terphenyl 1,1-Bis(*tert*-butyl)-3,3,5-trimethylcyclohexane peroxide
		Wildlife (2/3)	Diethylbiphenyl Dibenzyltoluene
		Air (3/3)	Acrylic acid Naphthalene Biphenyl
FY 2008	Initial Environmental Survey	Surface water (4/9)	*p*-Aminophenol 4,6-Dinitro-*o*-cresol Methyl 4-hydroxybenzoate 6-Phenyl-1,3,5-triazine-2,4-diamine
		Sediment (1/5)	*o*-Nitroaniline
		Air (6/14)	9,10-Anthracenedione (synonym: Anthraquinone) Diethylene glycol Dibenzyl ether (synonym: [(Benzyloxy)methyl]benzene) *o*-Nitrotoluene 6-Phenyl-1,3,5-triazine-2,4-diamine 2-Propanol
	Detailed Environmental Survey	Surface water (8/11)	4-Amino-6-*tert*-butyl-3-(methylsulfanyl)-1,2,4-triazin-5(4*H*)-one (synonym: Metribuzin) and its degradation products 4-Chlorophenol (synonym: *p*-Chlorophenol) 4,4'-Diaminodiphenylmethane (synonym: 4,4'-Methylenedianiline) 1,4-Dimethyl-2-(1-phenylethyl)benzene Piperazine *p*-Bromophenol 1-Naphthyl methylcarbamate (synonym: Carbaryl or NAC) and its degradation products Methyl *N*-{[(methylamino)carbonyl]oxy}ethanimidothioate (synonym: Methomyl)
		Sediment (2/4)	1,4-Dimethyl-2-(1-phenylethyl)benzene 4-*tert*-Pentylphenol
		Air (4/8)	*n*-Butyl acrylate Acrolein Isobutanol Quinoline

*: *o*-Dichlorobenzene was detected in surface water (FY 1996, FY 1997, FY 1998, and FY 2002), sediment (FY 1996, FY 1997, FY 1998, FY 1999, FY 2000, FY 2001, and FY 2002) and air (FY 1999 and FY 2002).

** :1-Naphthyl methylcarbamate (synonym: Carbaryl or NAC) was detected in surface water in FY 1998 Survey on Environmentally Persistent Pesticides and FY 2000 Survey on Environmental Fate of Pesticides.

Appendix 5

Results of Recruitment and Adoption of Feasibility Studies

(FY 2005 - FY 2009)

Year (FY)	Adoption Procedure	Recruitment Themes	Number of Proposals	
			Applied	Adopted
2005	Considering issues remained in SPEED'98, the MOE selected 10 research themes. Six proposals for fundamental studies and four proposals for biological research on wildlife were adopted. Additionally, 10 themes were recommended by the members of the Sub-Committee for Design and Evaluation of Fundamental Studies, the Sub-Committee for Biological Observation of Wildlife, and the MOE. After consideration in the informal joint meeting of the two sub-committees, seven themes were adopted for feasibility studies.			
2006	New research themes were recruited, considered by the hearing in the joint meeting, and selected as research themes to be adopted.	Themes in accordance with the ideas shown in "Fundamental studies under the framework of ExTEND2005"	24	6
2007		In principle, themes should be the research that is in accord with ExTEND2005. ○ Collection of biological knowledge on wildlife When any individual- or population-level change or sign is found during the observation of wildlife, a review research is to be conducted to judge whether the change is abnormal or not and to understand its mechanism. In such a case, the review research should be a field study that can be linked to the research on endocrine disrupting effects of chemical substances. ○ Fundamental studies on endocrine disrupting effects of chemical substances.	7	4
2008		In principle, themes should be the research that is in accord with ExTEND2005. ○ Research for biological observation of wildlife A review research to judge whether or not whether any individual (population) -level change or sign found during the observation of wildlife is abnormal, and to understand its cause and mechanism. Recruited research themes were as follows. 1) Research on changes in amphibians 2) Research on changes in reptiles 3) Research on changes in birds 4) Research on changes in mammals	7	5
2008	New research themes were recruited, considered by the hearing in the joint meeting, and selected as research themes to be adopted.	○ Fundamental studies on endocrine disrupting effects of chemical substances Recruited research themes were as follows. 1) Studies related to clarification of newly recognized mechanisms of endocrine disrupting effects such as modification of genes (epigenetics) 2) Studies related to development of a screening system for chemicals by applying toxicogenomics to changes in Medaka 3) Studies related to development of a screening system for chemicals by applying QSAR to changes in test animals (e.g., Daphnia or Medaka)	7	5

Year (FY)	Adoption Procedure	Recruitment Themes	Number of Proposals	
			Applied	Adopted
2009		In principle, themes should be the research that is in accord with ExTEND2005. ○Research for biological observation of wildlife A review research to judge whether or not whether any individual (population) -level change or sign found during the observation of wildlife is abnormal and to understand its cause and mechanism. Recruited research themes were as follows. 1) Studies on changes (e.g., abnormal reproduction/development) in invertebrates (high priority on *Daphnia*, copepod, mysid and chironomid as OECD test species regarding endocrine disrupting effects of chemical substances) 2) Studies on changes (e.g., abnormal reproduction/development) in fish (high priority on sticklebacks as OECD test species regarding endocrine disrupting effects of chemical substances) ○ Fundamental studies on endocrine disrupting effects of chemical substances Recruited research themes were as follows. 1) Research related to endocrine disrupting effects of chemical substances on the immune system 2) Research related to development of a screening system for chemicals by applying QSAR to endocrine disrupting effects in OECD test species regarding endocrine disrupting effects of chemical substances (*Daphnia*, copepod, chironomid, mysid, Medaka, and sticklebacks)	6	4

Appendix 6

Overview of Implemented Fundamental Studies and Research for Biological Observation of Wildlife under the Framework of ExTEND2005

Classification in ExTEND2005	Objective	Chief Researcher	Subjects	FY 2005	FY 2006	FY 2007	FY 2008	FY 2009
(1) Observation of wildlife (3) Promotion of fundamental studies 1) Collection of fundamental biological knowledge of wildlife	1. Understanding of the status of abnormality found in wildlife	Tomoki Sunobe	1.1 Studies on sex change induction and social structure of hermaphrodite fish.	W	W	—	—	—
		Masatoshi Yui	1.2 Basic studies on biology and food-chain of "Osprey *Pandion haliaetus*," the fish-eating birds of prey.	—	—	—	FS	FS
	2. Clarification of causes of abnormality found in wildlife	Tetsuyuki Ueda	2.1 Survey on the declining population of Dragonfly *Sympetrum frequens* and elucidation of its causes.	—	FS	FS	W	W
		Masayuki Saigusa	2.2 Studies on screening of abnormality found in growth and sexual maturation of benthic crustacean and on environment effects assessment.	—	—	—	—	FS
		Toshihiro Horiguchi	2.3 Elucidation and factorial analysis of current status of ecological disturbance in Tokyo Bay.	—	FS	FS	W	—
		Minoru Takase	2.4 Incidence of testis-ova in amphibians in field and laboratory.	—	—	FS	FS	—
		Masumi Yamamuro	2.5 Feasibility study on the effect of herbicides on the distinction of Charophyte in Japan.	—	FS	—	—	—
		Yoshihiro Shiraiwa	2.6 Physiological and ecological studies on the effects of environmental impact chemicals to elucidate the factors of decreasing population of Charales.	—	—	FS	FS	W
	3. Clarification of mechanisms of abnormality found in wildlife	Takayuki Hanazato	3.1 Elucidation of current status and mechanisms of ecological disturbance of lake coasts and surrounding areas.	W	W	W	—	—
(2) Understanding of the status of concentrations and measurement of exposure	4. Understanding of the status of concentrations and measurement of exposure	Kiwao Kadokami	4.1 Comparison of dioxin levels in freshwater fish.	W	—	—	—	—
		Shinsuke Tanabe	4.2 Status of contamination by legacy and emerging POPs in wildlife from coastal and offshore waters of Japan and Korea.	W	etc	etc	etc	etc

Classification in ExTEND2005	Objective	Chief Researcher	Subjects	FY 2005	FY 2006	FY 2007	FY 2008	FY 2009
(3) Promotion of fundamental studies 2) Individual-level approach, and 3) Cell/molecular- level approach	5. Clarification of mechanisms that cause adverse effects on wildlife	Taisen Iguchi	5.1 Analysis of endocrine disrupting mechanisms in *Daphnia magna*.	F	etc	etc	etc	etc
		Norihisa Tatarazako	5.2 Search of juvenile hormone receptors of invertebrates and clarification of their mechanisms.	—	FS	—	—	—
		Minoru Koga	5.3 Observation and mechanism of abnormal reproduction/development in invertebrate (mysid).	—	—	—	—	FS
		Yoshitaka Nagahama	5.4 Studies on mechanism of endocrine disrupting effects of chemicals on reproductive endocrine system of Medaka.	F	F	F	F	F
		Toshinobu Tokumoto	5.5 Studies on endocrine disrupting actions of chemicals on membrane steroid receptors.	—	—	FS	FS	—
		Hisato Iwata	5.6 Development of comprehensive analysis of nuclear receptor ligands for risk assessment of wildlife.	—	FS	FS	F	F
		Shigeru Ohta	5.7 Metabolic activities and activation of endocrine disrupting chemicals in fetuses and neonates of rats.	FS	F	F	F	F
		Noriyuki Koibuchi	5.8 Mechanisms of environmental chemical action on nuclear receptor-mediated transcription.	FS	F	F	F	F
		Tsuyoshi Nakanishi	5.9 Effects of excessive estrogen exposure during the prenatal period on sexual development in mice.	FS	F	F	F	F
		Shuntaro Hara	5.10 Analysis of mechanism of endocrine disrupting effects of environmental chemicals by the observation of metabolic changes of arachidonate.	—	FS	—	—	—
	6. Understanding of newly recognized mechanisms	Shuntaro Hara	6.1 Studies of new mechanisms of endocrine disrupting effects of environmental chemicals via inhibition of phospholipase A2 in sperm.	—	—	FS	FS	—
		Seiichiro Osako	6.2 Verification of the DOHaD model by epigenetic modifications induced by chemicals.	—	—	—	FS	FS
(3) Promotion of fundamental studies 4) Fundamental studies qualified for test method development	7. Implementation of fundamental studies qualified for test method development	Masato Kinoshita	7.1 Examination of initial changes of reproductive lesion caused by endocrine disrupting chemicals in using transgenic Medaka.	FS	F	F	—	—
		Masaki Nagae	Evaluation of endocrine disrupting effects of chemicals using three-spined stickleback.	F	Under UK-Japan Joint Research			
		Makoto Nakai	7.2 Establishment of Medaka androgen receptor binding assay.	FS	—	—	—	—

Classification in ExTEND2005	Objective	Chief Researcher	Subjects	FY 2005	FY 2006	FY 2007	FY 2008	FY 2009
		Kazuichi Hayakawa	7.3 Evaluation of endocrine disrupting effects of polycyclic aromatic hydrocarbons in combustion exhaust gases.	FS	F	F	—	—
		Akihiko Kashiwagi	Studies on expression mechanism of endocrine disrupting effects on thyroid hormones in amphibians.	F	Under amphibian test methods development			
		Masaaki Kurasaki	7.4 Development of assay methods for ecological effects of endocrine disrupting chemicals.	FS	—	—	—	—
	8. Accumulation of knowledge of test animals for interpretation and evaluation of test results	Yoshinari Tanaka	8.1 Studies on new analytical approach by mathematical ecological methodology using improved Daphnia reproductive toxicity assay.	—	—	—	—	FS
		Satoshi Hamaguchi	8.2 Collection and analysis of basic information related to abnormal sex differentiation of wild Medaka.	FS	W	W	W	W
		Yoshinao Katsu	Analysis of induction mechanisms of testis-ova in fish by estrogenic chemicals.	F	Under UK-Japan Joint Research			
		Hiroaki Aoyama	8.3 Genetic analysis of intrinsic factors that may modulate the response to xenobiotics in mammalian species of animals.	F	F	F	F	F
	9. Development of a screening procedure for test chemicals / Consideration of inter-species differences between tested animals and others	Kaoru Azumi	9.1 Fundamental studies on toxicogenomics using marine invertebrate Ascidiacea.	—	—	—	FS	FS
		Koji Arizono	9.2 Effects of chemicals and their metabolites on reproduction of Medaka, and clarification of their mechanisms by toxicogenomics.	—	—	—	FS	FS
		Kazuichi Hayakawa	9.3 Studies on screening methods for chemicals using fish scales based on structure-activity relationship of the disrupting effects of polycyclic aromatic hydrocarbons.	—	—	—	FS	FS
		Taisen Iguchi	9.4 Establishment of *in vitro* screening systems for species and ligand-specificities using fish estrogen receptors.	—	—	—	etc	etc
		Toshinobu Tokumoto	9.5 Determination of chemical groups effecting steroid membrane receptors based on structure-activity relationship.	—	—	—	—	FS

W: Carried out as Research for Biological Observation of Wildlife.
F: Carried out as Fundamental Studies.
FS: Carried out as Feasibility Studies.
etc: Carried out as other studies.

Appendix 7　Overview of Test Methods Adopted by the OECD

Test Using Fish: 21-day Fish Screening Assay
Adopted as OECD Test Guideline 230

To observe sexual changes caused by sex hormones, using sexually mature, fertile male and female

In the case of Medaka (*Olyzias latipes*)

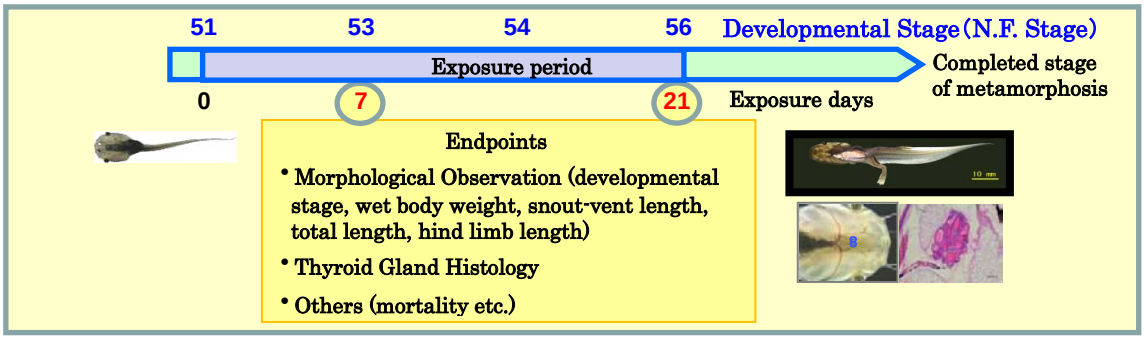
Exposure conditions

- Test type: flow-through
- Age of test organisms: 2～4 month of age after hatching.
- No. of treatments: 3 plus controls
- Exposure period: 3 weeks
- No of fish: 10 males and 10 females per test concentration
  No. of vessels: 2 (5 males and 5 females per test concentration)
- Monitoring of test solutions
  pH, DO, water temperature (24±1 deg C), and measurement of test concentrations once a week
- Feeding: Brine shrimp nauplii twice daily (ad libitum)

Measurement and observation

- Sampling: at termination of exposure (at day 21)
- Endpoints:
  - survival and physical appearance
  - secondary sex characteristics (papillary processes etc.)
  - measurement of vitellogenin (an egg yolk precursor protein) concentration in liver
- Data analysis: statistical test to detect significant difference from control

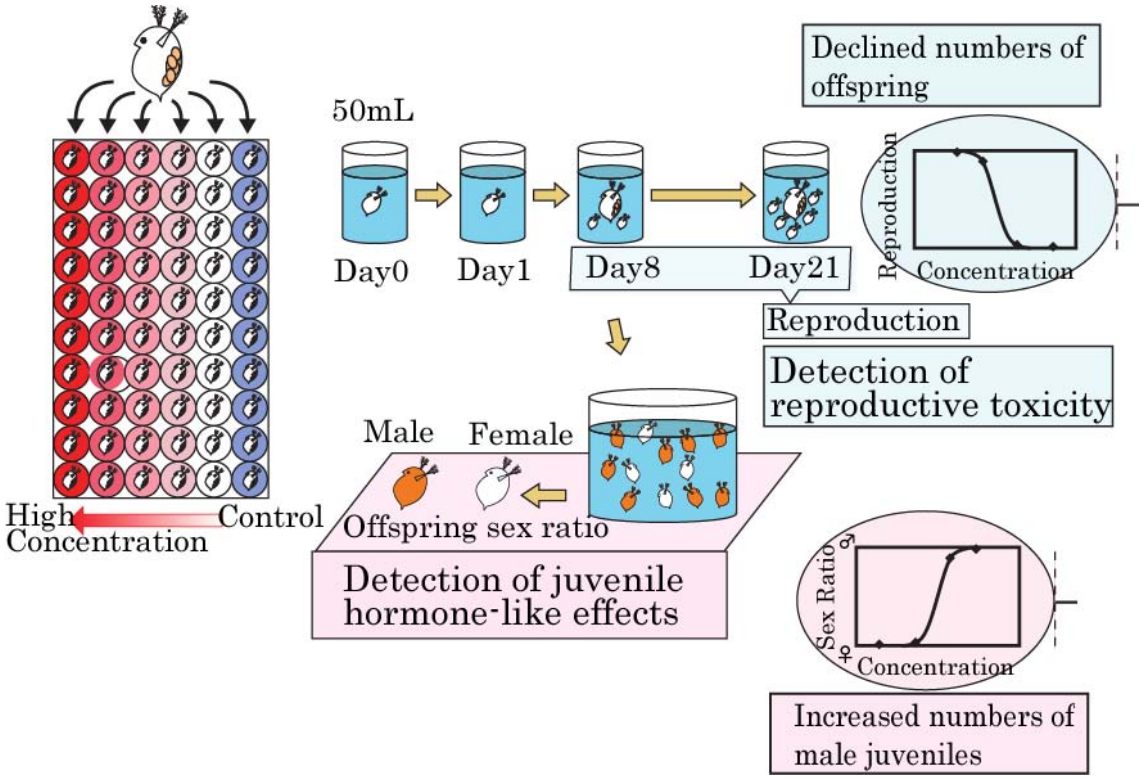
Test Using Amphibian (African Clawed Frog Metamorphosis Assay)
Adopted as OECD Test Guideline 231



Test species	African clawed frog (*Xenopus laevis*)
Exposure period	21 days from stage 51
Density of test animal	20 larvae/4 L/tank (including 5 sampled at day 7 for measurement)

Test Using Invertebrate (*Daphnia magna* Reproduction Test)
Adopted as ANNEX 7 of OECD Test Guideline 211

- Each juvenile aged less than 24 hours is exposed in each vessel.
- 21±1 deg C and 16L: 8D
- Living algal cells of Chlorella are supplied as the diet. The medium is renewed every other day.
- Test animal grows molting almost once a day and starts breeding on about 8 days of age.
- After breeding, the animal repeats molting and breeding in 2-3 days.
- Produced juveniles are removed and counted every day. Juvenile sex is also determined.



Appendix 8.1 Results of Tests Using Fish (Medaka)

(FY 2005)

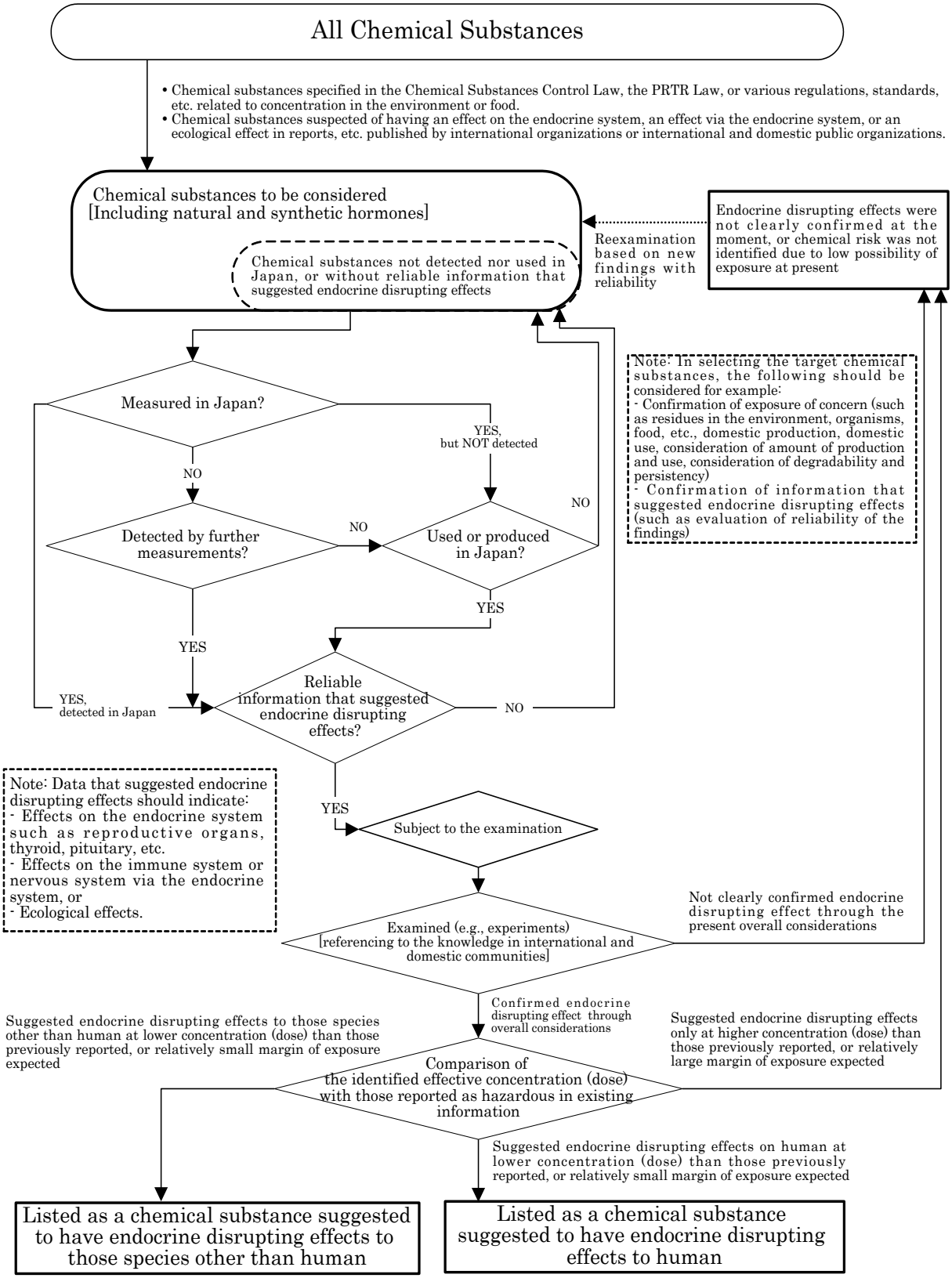
Test Chemicals	Test Results
4-*tert*-Butylphenol	In reporter gene assay for the Medaka estrogen receptor (ERα), some activities on ERα were observed, but no EC50 value was obtained. No significant effect was recognized in reporter gene assay for the Medaka estrogen receptor (ERβ), reporter gene assay for the Medaka androgen receptor, and binding assay for the Medaka thyroid hormone receptor.
Nonylphenol monoethoxylate	
Nonylphenol diethoxylate	
Nonylphenoxy acetate	

Appendix 8.2 Results of Tests Using Mammalian (Rat)

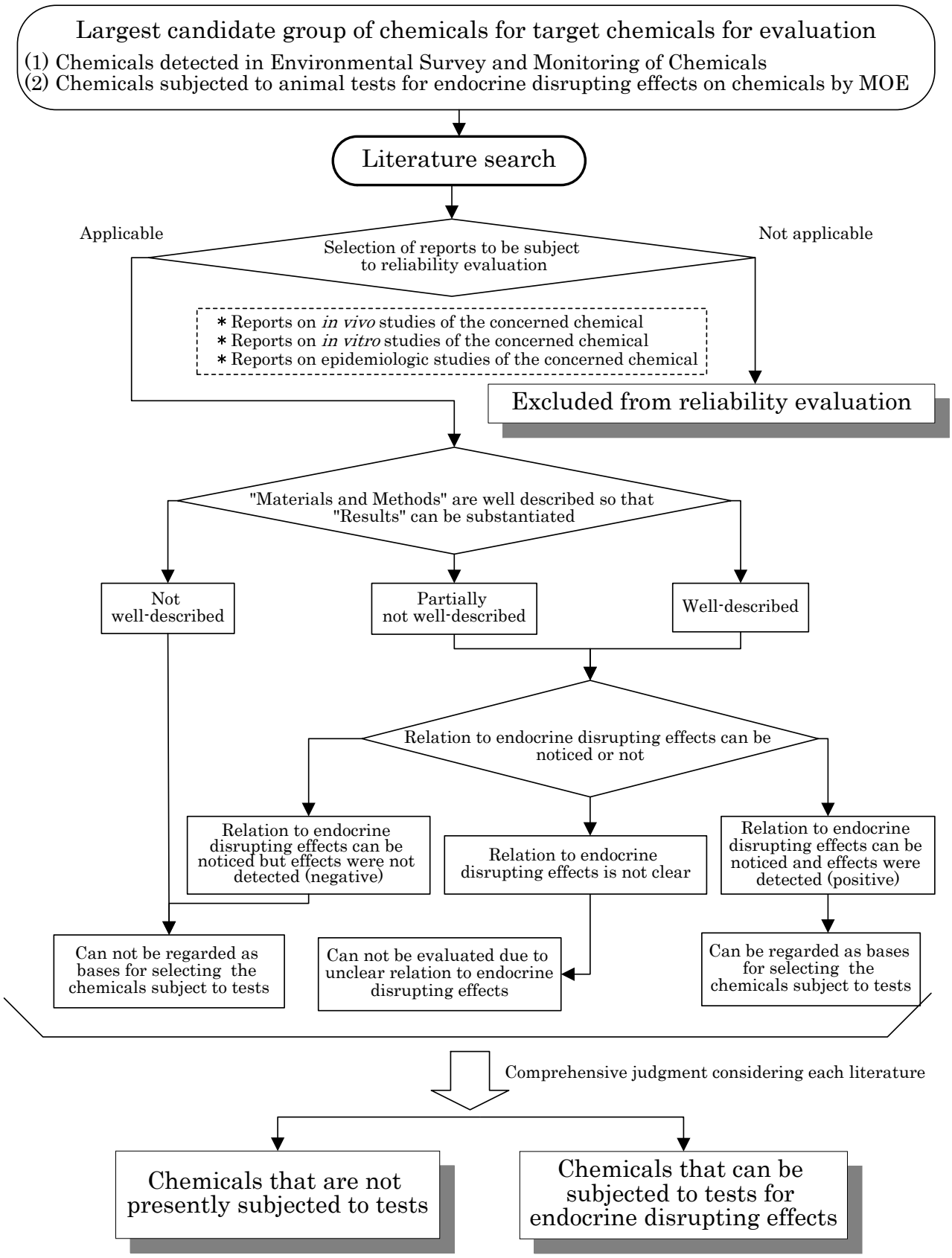
(FY 2005 - FY 2006)

Test Chemicals	Test Results
4-*tert*-Butylphenol	Clear endocrine disrupting effects were not recognized for doses (2 dose groups) determined considering estimated human exposure doses obtained from literature.

Appendix 9.1 Procedures for Selecting Chemical Substances for Testing and Assessment of Endocrine Disrupting Effects



Appendix 9.2 Flowchart for Reliability Evaluation of Literature



Appendix 10　Target Chemicals Subject to the Reliability Evaluation of the Reports on Endocrine Disrupting Effects of Chemicals

(As of March 2010)

Estrone*, *p*-Dichlorobenzene*, *N,N*-Dimethylformamide*, 2,4,6-Tribromophenol*, 2,4-Toluenediamine*, Hydrazine*, Fenthion*, *o*-Dichlorobenzene**, Linear alkylbenzene sulfonate and its salts**, Trifluralin**, Adipic acid, Carbaryl (NAC), Carbofuran, Cyanazine, Diuron, Dichlorvos, Dichlorobromomethane, Diazinon, Phenanthrene, phenytoin, Fenitrothion, Phenobarbital, 1-Butanol, Perfluorooctanoic acid, Benzyl alcohol, Methyl methacrylate, and EPN

*: Chemicals that can be subject to tests for endocrine disrupting effects.

**: Chemicals that are not presently be subject to tests.

Other: Chemicals whose reliability evaluation is in progress.

Appendix 11　Overview of Previous International Symposium on the Environmental Risks of Chemicals

(FY 2005 - FY 2008)

Year (FY) (Number) Date	Venue	Main Theme Theme of panel discussion as a program for the general public	Number of Total Participants
FY 2005 (8th) December 4-6	Naha City, Okinawa	Information sharing and exchange of opinions in respect of foreign and domestic state-of-the art research/approach What is happening now in the environment? – How can we protect the ecological system from endocrine disrupting effects?	About 530 (3 days)
FY 2006 (9th) November 12-14	Kushiro City, Hokkaido	Endocrine disrupting effects of chemicals, actions for risk management of chemicals, and children's environmental health from the viewpoint of health risks from chemicals. How should we get together with the chemicals? - Consideration of risks and merits.	About 400 (3 days)
FY 2007 (10th) December 9-10	Saitama City, Saitama	Actions for risk management of chemicals and children's environmental health from the viewpoint of health risks from chemicals. General overview of the endocrine disrupting effects of chemicals.	About 850 (2 days)
FY 2008 (11th) December 14-15	Koto-ku, Tokyo	Environmental and health effects surrounding children and future prospects and actions against endocrine disrupting effects of chemicals.	About 930 (2 days)

Appendix 12
Members of the Task Force on Endocrine Disrupting Effects of Chemical Substances and its Sub-Committees

(○: Chair)

Members of the Task Force on Endocrine Disrupting Effects of Chemical Substances (FY 2009 and FY 2010)

	Yoshiko Arita	Environment Section Manager, The Federation of Consumer Organization "SHUFUREN"
	Tohru Inoue	Pharmaceuticals and Medical Devices Agency
	Masako Ueji	Technical Adviser, Japan Plant Protection Association
	Masatoshi Ogura	Executive Director, Japan Chemical Industry Association (until June 1, 2010)
○	Masaru Kitano	Professor, Department of Applied Chemistry, School of Science and Technology, Meiji University
	Shigeyuki Koide	Senior Editor, Tokyo Head Office, The Yomiuri Shimbun
	Yuko Sakita	Journalist and Environment Counselor
	Hiroshi Satoh	Professor, Tohoku University School of Medicine
	Fumiaki Shono	Executive Director, Japan Chemical Industry Association (since June 2, 2010)
	Michihito Takahashi	Representative, Pathology Peer Review Center
	Ryo Tachikawa	Director, Ehime Prefectural Center for Environmental Creation
	Chiharu Tohyama	Professor, Graduate School of Medicine, The University of Tokyo
	Makoto Watanabe	Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba

(Observers)

Takanori Abe	Environmental Information Center
Taisen Iguchi	Professor, Okazaki Institute for Integrative Bioscience, National Institute of Natural Sciences
Chisumi Eto	IDEA Consultants, Inc.
Yuta Onishi	IDEA Consultants, Inc.
Yukio Kawashima	JAPAN NUS, Co., Ltd.
Norihisa Tatarazako	National Institute for Environmental Studies

Members of the Sub-Committee for Design and Evaluation of Fundamental Studies (FY 2009)

	Yasuyoshi Okuno	Chair, New Chemical Issues Working Group, Japan Chemical Industry Association
	Jiro Koyama	Professor, Education and Research Center for Marine Resources and Environment, Faculty of Fisheries, Kagoshima University
○	Hiroshi Satoh	Professor, Tohoku University School of Medicine
	Chiharu Tohyama	Professor, Graduate School of Medicine, The University of Tokyo
	Akira Naganuma	Professor, Laboratory of Molecular and Biochemical Toxicology, Department of Life Science, Tohoku University Graduate School of Pharmaceutical Sciences
	Satoshi Hagino	Manager, Environmental Toxicology Group, Environmental Health Science Center, Sumika Technoservice Corporation, Japan
	Shigeki Masunaga	Professor, Graduate School of Environment and Information Sciences, Yokohama National University
	Chiho Watanabe	Professor, Department of Human Ecology, School of International Health, Graduate School of Medicine, The University of Tokyo

Members of the Sub-Committee for Biological Observation of Wildlife (FY 2009)

	Takashi Iwamatsu	Professor emeritus, Aichi University of Education
	Yoshitaka Tsubaki	Professor, Center for Ecological Research, Kyoto University
	Kenji Hata	Professor, Center of Field Sciences, Akita Prefectural University
	Yukio Murata	Senior Officer, World Wildlife Fund for Nature Japan
○	Makoto Watanabe	Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba

Members of the Sub-Committee for Actions and Effects Assessment (FY 2009)

	Shoji Saito	Group Manager, Chemical Safety Assessment Group, Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.
	Hiroaki Shiraishi	Director, Research Center for Environmental Risk, National Institute for Environmental Studies
	Yoshio Sugaya	Senior Researcher, Research Center for Environmental Risk, National Institute for Environmental Studies
○	Chiharu Tohyama	Professor, Graduate School of Medicine, The University of Tokyo
	Akihiko Hara	Dean, Graduate School of Fisheries Sciences, Hokkaido University
	Kazunori Fujii	Chief, Ecotoxicology Section, Environmental Chemistry Division, National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency

Members of the Sub-Committee for Promotion of Risk Communication (FY 2009)

	Yoshiko Arita	Environment Section Manager, The Federation of Consumer Organization “SHUFUREN”
	Ayumu Inoue	General Manager, Public Relation, Japan Chemical Industry Association
	Iwao Uchiyama	Professor emeritus, Kyoto University
○	Masaru Kitano	Professor, Department of Applied Chemistry, School of Science and Technology, Meiji University
	Toshiko Kikkawa	Associate Professor, Faculty of Business and Commerce, Keio University
	Rie Masho	Senior Researcher, Research and Development, Center for Environmental Information Science
	Yukio Murata	Senior Officer, World Wildlife Fund for Nature Japan
	Hiroo Yamagata	Commentator and Translator
	Hiroo Wakimori	Japan Crop Protection Association

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Environmental Health and Safety Division, Environmental Health Department, Environmental Policy Bureau
Ministry of the Environment, Japan
1-2-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-8975 JAPAN
TEL: +81-3-5521-8261　FAX: +81-3-3580-3596　E-mail: ehs@env.go.jp