# Further Actions to Endocrine Disrupting Effects of Chemical Substances

— EXTEND2016 —

(Tentative Translation)

June 2016

Ministry of the Environment, Japan (MOE)

Note: The subtitle of this program is named “EXTEND2016,” intending to follow the basic frameworks of former “EXTEND2010” and extend the program to address further regulatory issues.

EXTEND: Extended Tasks on Endocrine Disruption

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## Introduction

Beneficial aspects of chemical substances have contributed to our prosperous life. However, improper handling of chemical substances can lead to potential adverse effects on human health and ecosystem. This is why proper risk assessment and management of chemical substances have been recognized as a common international issue.

While many scientific uncertainties remain, endocrine disrupting effects of chemical substances on human health and wildlife have been widely regarded as an important issue for their potential transgenerational effects.

The Ministry of the Environment, Japan (MOE, formerly the Environment Agency before 2001), published "The Environment Agency's Basic Policy on Environmental Endocrine Disruptors – Strategic Programs on Environmental Endocrine Disruptors: SPEED'98 –" in May 1998 and subsequently "MOE's Perspectives on Endocrine Disrupting Effects of Substances – ExTEND2005 –" in March 2005, to collect and examine relevant information.

Following ExTEND2005, "Further Actions to Endocrine Disrupting Effects of Chemical Substances – EXTEND2010 –" was published in July 2010. In this program, a framework for testing and assessing endocrine disrupting effects of chemical substances was established. Necessary test methods were developed under international cooperation, contributing to the establishment of international standard test methods using fish, amphibians, and invertebrates. Under this framework, evaluation of existing information and new testing were done, allowing the MOE to accumulate data on the effects of over one hundred chemical substances.

Regarding international trends, in the United States, programs to assess endocrine disrupting effects of chemical substances have been administered, and in the European Union (EU), discussions on how endocrine disrupting effects should be dealt under various regulations are still on going. The World Health Organization (WHO) published an assessment report titled "State of the Science of Endocrine Disrupting Chemicals" in 2012. The Strategic Approach to International Chemicals Management (SAICM) added "endocrine-disrupting chemicals" to the "Emerging Policy Issues and Other Issues of Concern" to reinforce its approaches. In the Organisation for Economic Co-operation and Development (OECD), methods for testing and assessing endocrine disrupting effects have been developed intensively. EXTEND2010 is highly recognized at the international level and thus needs to continue with its active contribution.

Five-years after EXTEND2010 was launched, the MOE entrusted the "Task Force on Endocrine Disrupting Effects of Substances" and its sub-committees to review its achievements and discuss its directions and further actions, and has summarized the results as "Further Actions to Endocrine Disrupting Effects of Chemical Substances – EXTEND2016 –." In EXTEND2010, the MOE "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." The MOE will continue to follow this basic principle, and take appropriate actions to address the risks of endocrine disrupting effects.

June 2016

Environmental Health and Safety Division
Environmental Health Department
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# I Actions to Date

## 1. Activities under the Framework of SPEED'98 and ExTEND2005

The Ministry of the Environment (formerly 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. Therein, 67 chemicals (revised to 65 chemicals in November 2000) were identified as those having the highest priority in the survey and research in order to clarify the occurrence, the strength, and the mechanisms of endocrine disrupting effects. The program aimed to address this issue by 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.

Following this, the Ministry of the Environment (MOE) published "MOE's Perspectives on Endocrine Disrupting Effects of Substances – ExTEND2005 –" in March 2005. The program modified the previous approach, and instead of listing the high priority chemical substances, chemicals were selected and assessed based on their detection in the environment in Japan and existing knowledge. Basic principles of the program were 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.

### 1.1 Environmental Survey and Monitoring of Chemicals

In SPEED'98, listed chemicals were surveyed in four Japanese environmental media (water, sediment, soil and air), wildlife (fish, bivalves, amphibians, birds, and mammals), indoor air, and food samples during FY 1998 and FY 2004.

On the other hand, in ExTEND2005, the data from the Environmental Survey and Monitoring of Chemicals (Initial Environmental Survey, Detailed Environmental Survey, and Exposure Study) were utilized.

### 1.2 Promotion of Studies on Endocrine Disrupting Effects of Substances

In SPEED'98, several designated studies were done, and the effects on wildlife were surveyed with a focus on Rock Shell (*Thais clavigera*), a kind of marine snail. Abnormal sexual organ development, typically, the formation of male-type sex organs in females, was widely observed over wide coastal areas of Japan. This effect was related to organotin compounds such as tributyltin and triphenyltin in the marine environment.

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.

In ExTEND2005, Fundamental Studies and Research for Biological Observation of Wildlife was implemented from FY 2005. From FY 2006, new research themes were, in principle, publicly sought as proposals for designated research fields, and selected via critical review by 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"). Results of multi-year studies were also reviewed by both Sub-Committees every fiscal year to determine whether the study should be continued or not.

In ExTEND2005, the "Project for observation of familiar wildlife" had been implemented since FY 2005, aiming for the continuous observation of wildlife by children at the local level.

## 1.3 Effects Assessment

In SPEED'98, literature related to endocrine disrupting effects were searched, collected, and evaluated to select test chemicals. Selected chemicals were subjected to vitellogenin assays and partial life-cycle tests using Medaka (*Oryzias latipes*). If necessary, full life-cycle tests were also conducted. As a result, among 40 test chemicals, 4-nonylphenol (branched form) and 4-*t*-octylphenol were strongly suggestive of having endocrine disrupting effects on Medaka at environmentally relevant concentrations. Bisphenol A and *o,p'*-DDT were also suggestive of having endocrine disrupting effects on Medaka. For 37 chemicals, modified one-generation rat study was done, but no clear endocrine disrupting effects were recognized at doses estimated for human exposure.

In ExTEND 2005, projects on test method development, and selection and assessment of test chemicals were done. Here, test chemicals were not preliminarily listed as were in SPEED'98, but were selected based on their detection in the environment in Japan and evaluation of related information and knowledge on endocrine disrupting effects.

### (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 TG230 in 2009, and Fish Multi Generation Test have been developed under the framework of the Japan-US partnership.

For amphibians, *Xenopus laevis* Metamorphosis Assay and Amphibian Partial Life-Cycle Test were developed. *Xenopus laevis* Metamorphosis Assay was adopted as OECD TG231 in

2009, and Amphibian Partial Life-Cycle Test was developed and adopted for an OECD project.

For invertebrates, improvement on OECD TG211 *Daphnia magna* Reproduction Test was adopted in 2008 in the form of ANNEX 7 to OECD TG211. Under the framework of the Japan-US partnership, Daphnid Multi-Generation Assay was also investigated.

For *in vitro* assay, Japan, the UK and Sweden 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.

**(2) Selection of test chemicals and evaluation projects**

Chemicals were examined by *in vitro* assays (Medaka estrogen receptor α and β reporter gene assay, Medaka androgen receptor reporter gene assay, and Medaka thyroid hormone receptor binding assay) under the extended framework of SPEED'98 from FY 2005 to FY 2006.

Since FY 2007, selection of test chemicals and evaluation projects was carried out based on "Procedures for Selecting Chemical Substances for Testing and Assessment of Endocrine Disrupting Effects" under the framework of ExTEND2005.

## 1.4 Risk Assessment and Risk Management

The selection of test chemicals and the following effects assessment did not progress as expected. Therefore, chemical risk assessment including endocrine disrupting effects of chemical substances was not done, and the identification of chemicals for risk management was therefore not achieved.

## 1.5 Promotion of Information Sharing and Risk Communication

**(1) International symposium**

Since FY 1998, the MOE has hosted the International Symposium on Environmental Endocrine Disrupting Chemicals including participants from foreign governments and international organizations, and this was continued under ExTEND2005. From FY 2006 to FY 2008, the symposium was co-organized with the "International Symposium on Children's Environmental Heath." This symposium consisted of programs for both the general public and experts. Information was shared and opinions were exchanged on state-of-the-art research and actions taken in Japan and the world.

**(2) Preparation of webpages**

Through the webpage "Official Endocrine Disruption Website," the MOE has provided accurate and accessible information on endocrine disrupting effects of chemical substances mainly for the general public since FY 2005. On these webpages, topics related to health and chemicals were posted, and materials and references related to endocrine disrupting effects of

chemical substances were cited or linked.

### 1.6 International Cooperation

The MOE proposed new test methods and provided information including its test results for the OECD. The MOE also provided information including its approach and test results to the WHO.

The Japan-UK joint research started in 2000 under the agreement between the Minister of Environmental Agency (Japan) and the Environment Minister (UK) in March 1999. Four research themes were defined, and studies were undertaken by researchers in both countries. In addition, 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, and activities such as informational exchange about ecological effects assessment and test method development to assess effects on reproduction and propagation of fish, amphibians, and invertebrates were carried out at the partnership. These bilateral cooperation relationships are still ongoing.

## 2. Activities under the Framework of EXTEND2010

The MOE published "Further Actions to Endocrine Disrupting Effects of Chemical Substances – EXTEND2010 –" in July 2010. This program was created as a new program for approximately five years, adding necessary improvements to ExTEND2005 and succeeding parts of the framework as appropriate. The program aimed 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 as necessary.

EXTEND2010 was run 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

Implementing EXTEND2010, the MOE had 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"). The taskforce and

sub-committees reviewed the approaches to forward the program and evaluated research results and other actions every fiscal year (Figure 2).

**Figure 1 Conceptual Overview of Actions in EXTEND2010**

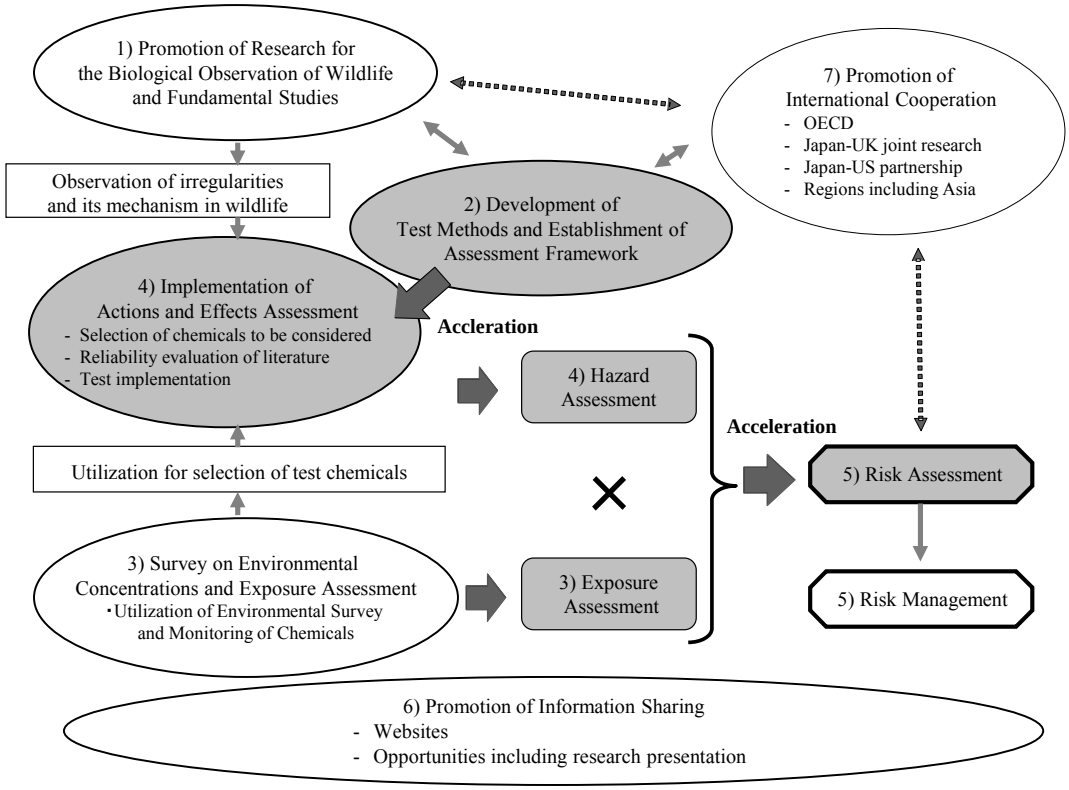
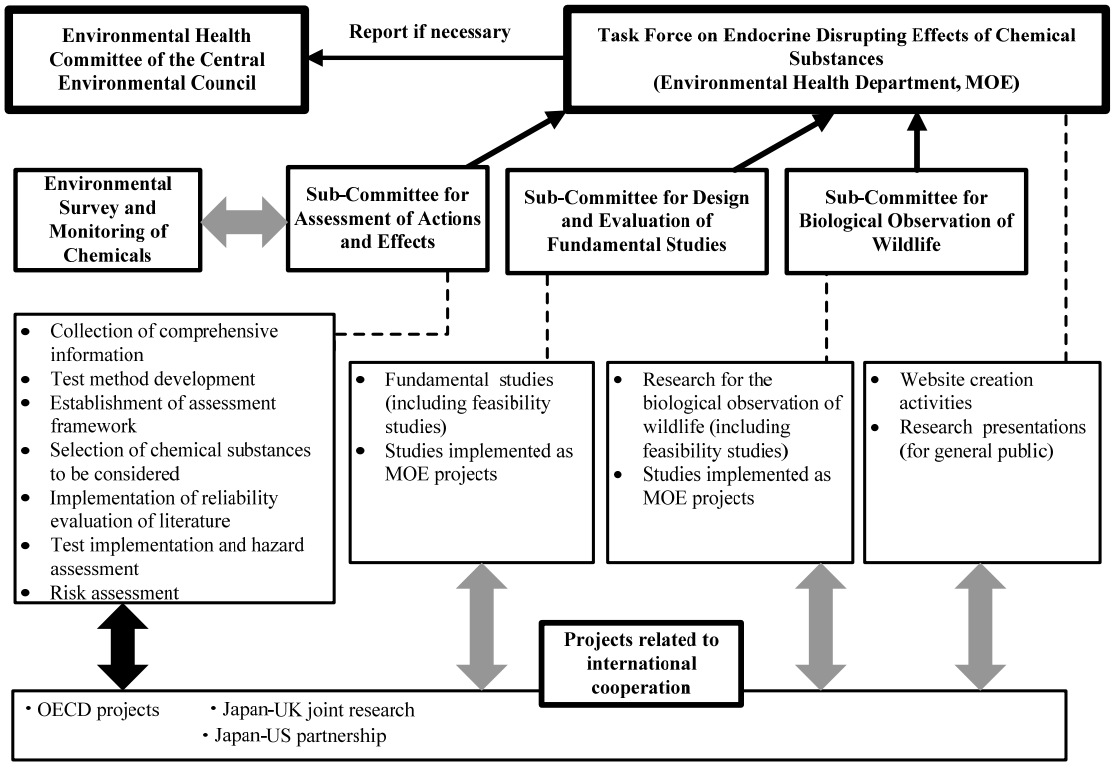



**Figure 2 EXTEND2010 Framework to Promote Research and Studies**



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

In EXTEND 2010, the framework of ExTEND2005 was basically succeeded, and research topics were publicly sought. Research topics were selected and the results were reviewed by experts of the Sub-Committee for Biological Observation of Wildlife and the Sub-Committee for Design and Evaluation of Fundamental Studies.

On the other hand, the following necessary revisions were made to obtain study results that meet regulatory needs.

- Select research topics putting high priority on potential contribution to environmental risk assessment
- Set research topics in the form of "designated research" as necessary

The study results were presented in seminars and made open to the public.

### (1) Overview of implemented Research for Biological Observation of Wildlife and Fundamental Studies (as of FY2015)

In EXTEND2010 that launched in FY2010, 56 research topics were submitted as proposals, and 13 of them were accepted for Feasibility Studies. Among them, 10 topics (three research topics as Research for Biological Observation of Wildlife and seven research topics as Research for Fundamental Studies) were continued for plural fiscal years.

Including the 10 research topics from ExTEND2005, 24 research topics (six on Research for Biological Observation of Wildlife, 12 on Research for Fundamental Studies, three Feasibility Studies, and three Other Related Studies) were pursued. (Of the three Feasibility Studies, two are still ongoing and one was terminated. Of the Other Related Study, one topic was originally submitted as a Feasibility Study but was adopted under this group.)

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

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

- Water samples were collected from coastal areas of Tokyo Bay and northern part of Kyushu Island, and analyzed for estrogenic compounds. Estrogenic compounds were detected in both areas. Nonylphenol was detected as a major component while the most estrogenic activity was attributed to estrone, a kind of natural estrogen. It was demonstrated that sewage water treatment plants are one of the main sources of estrogenic compounds found in environmental water and that the main constituents are natural

estrogens of human origin.

- Wild rats were collected nationwide and their body burden of environmental chemicals was analyzed. Accumulation in liver was not observed for chemicals such as polyaromatic hydrocarbons, pharmaceutical and personal care products and neonicotinoids, probably due to their fast metabolic rates. On the other hand, accumulation of organochlorine compounds and metals was observed. Stable isotope ratio analysis suggested that exposure source may differ among different regions.

2) Overview of main achievements in projects on Fundamental Studies

- Using transgenic *Xenopus laevis* larvae possessing a thyroid hormone responsive reporter gene system, a protocol was developed for a facilitated quantitative evaluation of potential endocrine disrupting chemicals at the individual level. Optimum conditions for the protocol to assess agonists and antagonists were arranged including exposure period, reference control, number of individuals, etc.
- Analysis using animal models of hyperreactivity (CIN85-deficient mice) and hypothyroidism (*rdw* rats) revealed that the fluctuation in amount of behavioral activity was due to the change of dopamine signal in the nigrostriatal system. It was shown that low-dose exposure of hydroxylated polychlorobiphenyl (OH-PCB) cause hyperreactivity via intensification of dopamine signal in the nigrostriatal system. It was also suggested that exposure to this chemical is a potential cause of hyperreactivity.

3) Overview of main achievements in projects on Other Related Studies

- With two-hybrid method, a screening procedure was established for rapid *in vitro* screening of chemicals with juvenile hormone activity in *Daphnia magna*. Diofenolan, a novel juvenile hormone like compound, was identified through this procedure. A reporter gene assay was also constructed, and the application using a juvenile hormone responsive element of *Tribolium castaneum* gave good results. A juvenile-hormone-exposure-independent male induction system was established by manipulating photoperiodic responses in *D. pulex*, and a juvenile hormone receptor was identified in daphnids as the first case among crustaceans.
- Chemical levels of brominated flame retardants (polybromodiphenyl ethers: PBDEs and hexabromocyclododecanes: HBCDs) and polychlorinated biphenyls (PCBs) were measured in birds (common cormorant), terrestrial mammals (palm civet cat, Japanese raccoon dog, and common raccoon), and aquatic mammals (finless porpoise) to assess their accumulation in wildlife. The result showed the tendency of age-dependent accumulation and biological concentration, indicating concerns for ecological risk and

effects on thyroid-hormone-mediated cerebral nervous systems.

## 2.2 Establishment of Assessment Framework and Development of Test Methods

### 2.2.1 Overview of establishment of assessment framework

In EXTEND2010, the basic principles was to promote establishment of assessment framework and implementation of assessment in order to assess the environmental risks posed by endocrine disrupting effects of chemical substances and manage them as necessary. Efforts were focused on ecological effects succeeding the achievements of ExTEND2005 such as test method development.

Of the test methods that have been well developed on the OECD Test Guideline Program, the following effects relevant to endocrine disruption were to be assessed using aquatic organisms (fish, amphibians, and invertebrates) as test species.

- Effects on reproduction: estrogenic effects, anti-estrogenic effects, androgenic effects, and anti-androgenic effects
- Effects on development (including metamorphosis): thyroid hormone effects and anti-thyroid hormone effects
- Effects on growth: juvenile hormone effects and molting hormone (ecdysone) effects

In order to assess the environmental risks of endocrine disrupting effects of chemical substances, not only the presence or absence of the effects on endocrine system, but also the presence or absence and the degree of adverse effects need to be investigated. Thus, a two-tiered system for testing and assessment was constructed involving ecological effect tests necessary to identify the presence or absence of these effects.

Chemical substances to be tested and assessed were selected, considering both the effect and the exposure of the chemical from the view of future environmental risk assessment. Chemicals detected from the Japanese environment were pooled as the preliminary group of chemicals for testing and assessment. From this group, chemicals which may pose endocrine disrupting effects were chosen based on existing knowledge.

In order to promote efficient assessment with limited resources and minimized number of testing animals, testing items were carefully selected based on existing knowledge, and *in vitro* tests were done to prioritize chemicals that shall to proceed to further *in vivo* tests.

The conceptual flow for testing and assessment of endocrine disrupting effects of chemical substances under EXTEND2010 is shown in Figure 3.

### 2.2.2 Selecting the preliminary group of chemicals to be tested and assessed

Succeeding the basic concept of ExTEND2005, chemicals detected in the environment in

Japan were chosen as the preliminary group of chemicals for testing and assessment. In addition to chemicals detected in Environmental Survey and Monitoring of Chemicals, those detected in Water Quality Survey of Public Water Areas and Research on the Existence of Chemical Substances have been also included since FY2011. Similarly, chemicals detected in Environmental Studies on the Pesticides and Class I Designated Chemical Substances of Law of Pollutant Release and Transfer Register (PRTR) system have been included since FY2013 and 2015, respectively.

### 2.2.3 Narrowing down chemicals by evaluating reliability of existing knowledge and information

By literature search with designated set of keywords, study reports on *in vivo* studies, *in vitro* studies, and epidemiological studies of the concerned chemical were obtained. Reliability evaluation of the collected literature was done under the basic understanding that endocrine disrupting chemical substances are defined as "exogenous chemicals that can cause impairment or adverse effects in living organisms by affecting endocrine systems."

Comprehensive judgement on whether the concerned chemical is a "Chemicals that can be subjected to test for endocrine disrupting effects" or not was made by evaluating if the literature shows evidence that the chemical should be subjected to testing based on the evaluation of whether "Materials and Methods" are well described so that "Results" can be substantiated and whether relation to endocrine disrupting effects can be noted or not.

### 2.2.4 Constructing two-tiered framework for testing and assessment

#### (1) Basic concept of two-tiered framework

Referencing "the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters" and the USA's "Endocrine Disruptor Screening Program (EDSP)," the following two-tiered framework for testing and assessment was constructed in order to implement assessment on endocrine disrupting actions and effects.

1) Tier 1
   - To identify chemical actions on endocrine system, Tier 1 test group was comprised of *in vitro* assays and short-term *in vivo* assays that can be relatively facile.
   - "Chemicals that can be subjected to tests for endocrine disrupting effects" identified through the reliability evaluation of existing knowledge were prioritized.
   - Tier 1 assessment was implemented after judging existing knowledge and test results.

2) Tier 2
   - To characterize hazardous property caused by endocrine disrupting effects, Tier 2 test

group was comprised of long-term *in vivo* assays.

- "Chemicals with actions relevant to endocrine disruption" judged through Tier 1 assessment were to be candidates for Tier 2 test group.

**(2) Selection of Test Method**

Under the framework of EXTEND2010, test methods that have been established in the OECD Test Guideline Program were preferably adopted, while other unestablished test methods were developed. For consistent assessment under the framework, same biological species were used in experiments, and the receptor used for *in vitro* test were basically selected from the same species as those used for *in vivo* test (The animals used in the testing were Medaka as fish, *Daphnia magna* as invertebrate, and *Xenopus laevis* or *Silurana tropicalis* as amphibian).

As Tier 1 *in vitro* test, reporter gene assay that indicate not only receptor binding but also the following transactivation was selected. As Tier 1 *in vivo* test, Fish Short Term Reproduction Assay (OECD TG229) using Medaka was used to identify mainly estrogenic, anti-estrogenic and androgenic activities. (If existing knowledge on 21-day Fish Screening Assay (OECD TG230) using Medaka was available, this was also referenced.)

**(3) Procedure for Testing and Assessment**

For testing and assessment in Tier 1, the following procedures were taken to facilitate efficient assessment, using the existing knowledge obtained through reliability evaluation effectively and avoiding exhaustive assessment from uniformly collected data.

1) Review existing knowledge identified in reliability evaluation under the following viewpoints to organize how sufficient the obtained information is prior to conducting Tier 1 test.
   - What type of endocrine system is the assumed target of endocrine disrupting effects?
   - Does the information equivalent to *in vitro* test in Tier 1 already exist?
   - Does the information equivalent to *in vivo* test in Tier 1 already exist?

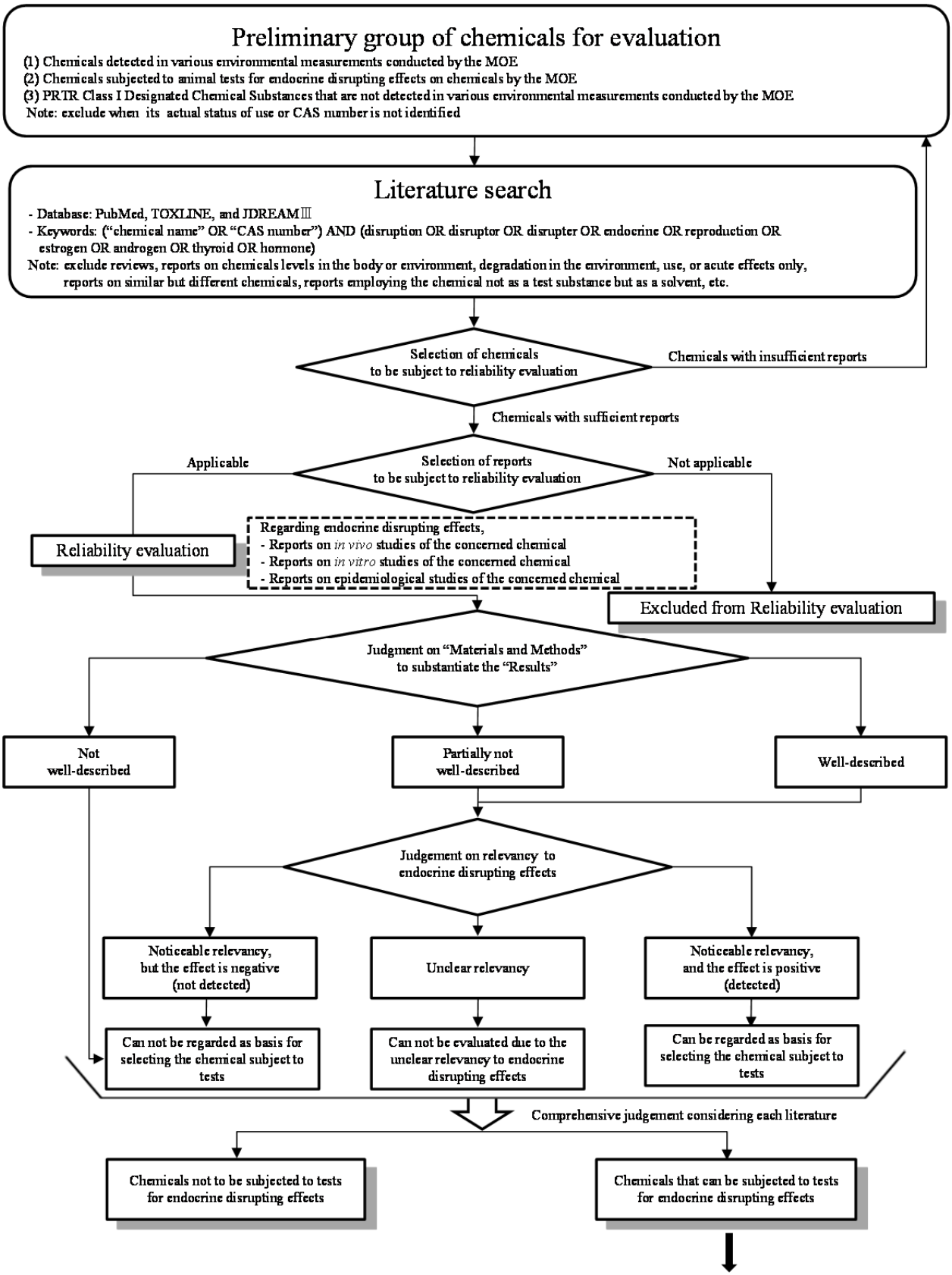
2) Conduct tests according to the following procedure, based on the result of review of existing knowledge as mentioned above.
   (i) Selection of the type of endocrine system to be tested
   (ii) Conduct *in vitro* test of a concerned chemical substance if the obtained information on the selected type of endocrine system is insufficient
   (iii) Conduct *in vivo* test of a chemical substance if the obtained information on the selected type of endocrine system is insufficient, in order of priority considering information from existing knowledge, results of *in vitro* testing, and overview of

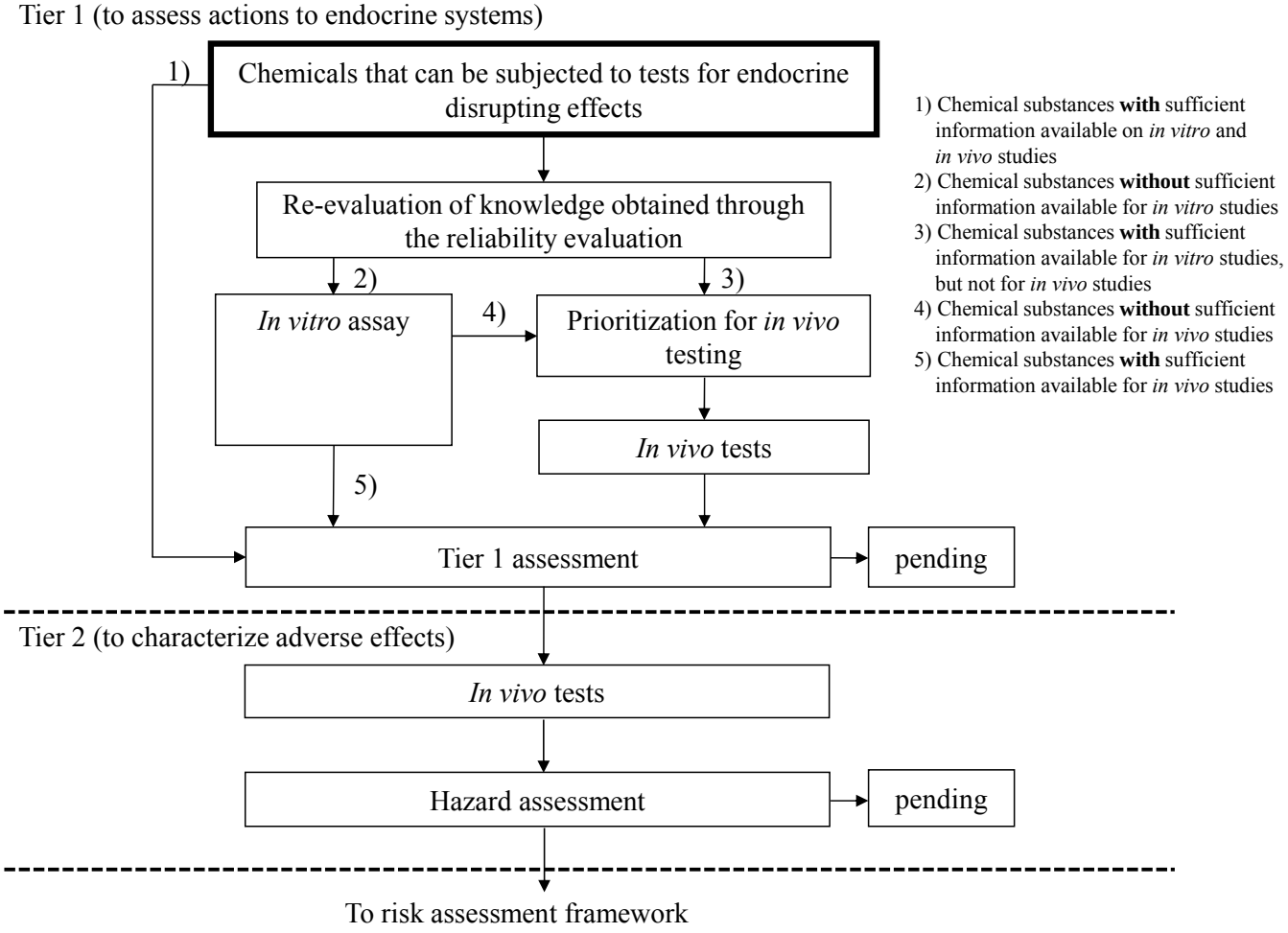
detection from the environment.

3) Implement Tier 1 assessment for chemical substances after gaining necessary information on *in vitro* and *in vivo* testing.

The procedure for Tier 2 testing and assessment was to be planned considering the advancement of test method development, accumulation of knowledge in Tier 1 testing and assessment, etc.

**Figure 3 Conceptual Flow for Testing and Assessment of Endocrine Disrupting Effects of Chemical Substances under EXTEND2010**




Tier 1 (to assess actions to endocrine systems)
1)
Chemicals that can be subjected to tests for endocrine disrupting effects
Re-evaluation of knowledge obtained through the reliability evaluation
2)
3)
In vitro assay
4)
Prioritization for in vivo testing
In vivo tests
5)
Tier 1 assessment
pending
Tier 2 (to characterize adverse effects)
In vivo tests
Hazard assessment
pending
To risk assessment framework
1) Chemical substances with sufficient information available on in vitro and in vivo studies
2) Chemical substances without sufficient information available for in vitro studies
3) Chemical substances with sufficient information available for in vitro studies, but not for in vivo studies
4) Chemical substances without sufficient information available for in vivo studies
5) Chemical substances with sufficient information available for in vivo studies

### 2.2.5 Test method development

#### (1) Development of fish test method

1) Fish Short Term Reproduction Assay (OECD TG229)

This assay, using fathead minnow (*Pimephales promelas*), was developed to identify not only estrogenic, androgenic, anti-estrogenic and aromatase inhibition activities but also effects on the hypothalamic–pituitary–gonadal axis, as well as endocrine disrupting effects on reproductive behavior, oogenesis, and spermatogenesis caused by chemical substances. While ExTEND2005 contributed to the development of 21-day Fish Screening Assay (OECD TG230), EXTEND2010 basically adopted OECD TG229 in Tier 1 due to its capability of detecting reproductive effects, and test conditions were reexamined for Medaka. In 2012, the OECD revised TG229 adopting the additional test conditions for Medaka proposed by Japan.

2) Medaka Anti-androgen Detection Assay (provisional title, currently under development)

Due to the difficulty in detection of anti-androgenic activity in Fish Short Term Reproduction Assay, a test method was developed aiming to detect the activity in Tier 1. This assay was designed to detect anti-androgenic effects of a test chemical on Medaka, focusing on the appearance of papillary processes of anal fin, a secondary sexual characteristic in male, as the major endpoint. Issues were reviewed and efforts were made to standardize experimental conditions such exposure period, numbers and week old age of Medaka. In 2015, the test was submitted as a new project to the OECD.

3) Medaka Extended One Generation Reproduction Test (OECD TG240)

This test is aimed to investigate the trans-generational effects caused by the transfer of chemicals from the parental body to egg, and assess chemical effects on fish (potential effects at population level) including endocrine disrupting effects (estrogenic, anti-estrogenic, androgenic, anti-androgenic, aromatase inhibition activities, effects on the hypothalamic–pituitary–gonadal axis, etc.). Chemical exposure is conducted over multiple generations from parental generation (F0) to hatching of third generation (F2), and adverse effects on survival, growth, development, and reproduction at each growth stage are observed.

The test was developed as “Medaka Life Cycle/Multi-generation Test” in the OECD, and was developed under the cooperation with the USA, with plans to utilize the test as Tier 2 *in vivo* test in EXTEND2010. In 2014, Japan and the USA co-submitted the draft of Medaka Extended One Generation Reproduction Test (MEOGRT) to the OECD. This draft was accepted in 2015 and published as Test Guideline 240 (OECD TG240). Thus, it is now possible to detect estrogenic, anti-estrogenic and androgenic effects by tests using Medaka at both stage of Tier 1 and Tier 2.

**(2) Development of amphibian test method**

- Larval Amphibian Growth and Reproduction Assay (LAGDA) (OECD TG241)

This assay is aimed to assess chemical effects on metamorphosis (hypothalamic–pituitary–thyroidal axis), reproduction, growth, etc. of amphibians (mainly *Xenopus laevis*) and is planned to be used in the Tier 2 test to identify adverse effects caused by thyroid hormone-like action or anti-thyroid hormone-like action. Test method development initiated in Japan-US partnership to establish amphibian life-cycle test. Japan and the USA co-submitted the draft of Larval Amphibian Growth and Reproduction Assay (LAGDA) to the OECD, and this was accepted in 2015 and published as Test Guideline 241 (OECD TG241).

**(3) Development of invertebrate test method**

1) Daphnid Juvenile Hormone Screening Assay (under development)

This assay detects juvenile hormone-like action using of egg-carrying daphnids as test organism, and the MOE aims to establish the method as Tier 1 *in vivo* assay. This was submitted as a new project to the OECD in 2015.

2) Daphnid Ecdysone (Screening) Assay (under development)

Similarly, this assay is aimed to be established as Tier 1 *in vivo* assay. The investigation on the basic experimental design (exposure periods, test organisms, endpoints, etc.) has just begun. As daphnids repeat molting to mature like insects, number of molting during a certain period is being examined as an endpoint to identify ecdysone-like action of a chemical.

3) Daphnid Multi-generational Test (under development)

This assay is aimed to detect chemical effects on daphnids at population level, observing fecundity of second generation which was exposed to chemicals in the parental body. The validity of the test methods, and methods to judge multi-generational effects were examined.

**Figure 4.1 Framework of Hazard Assessment of Endocrine Disrupting Effects**

**Effects on Reproduction**

(estrogenic, anti-estrogenic, androgenic actions, etc.)

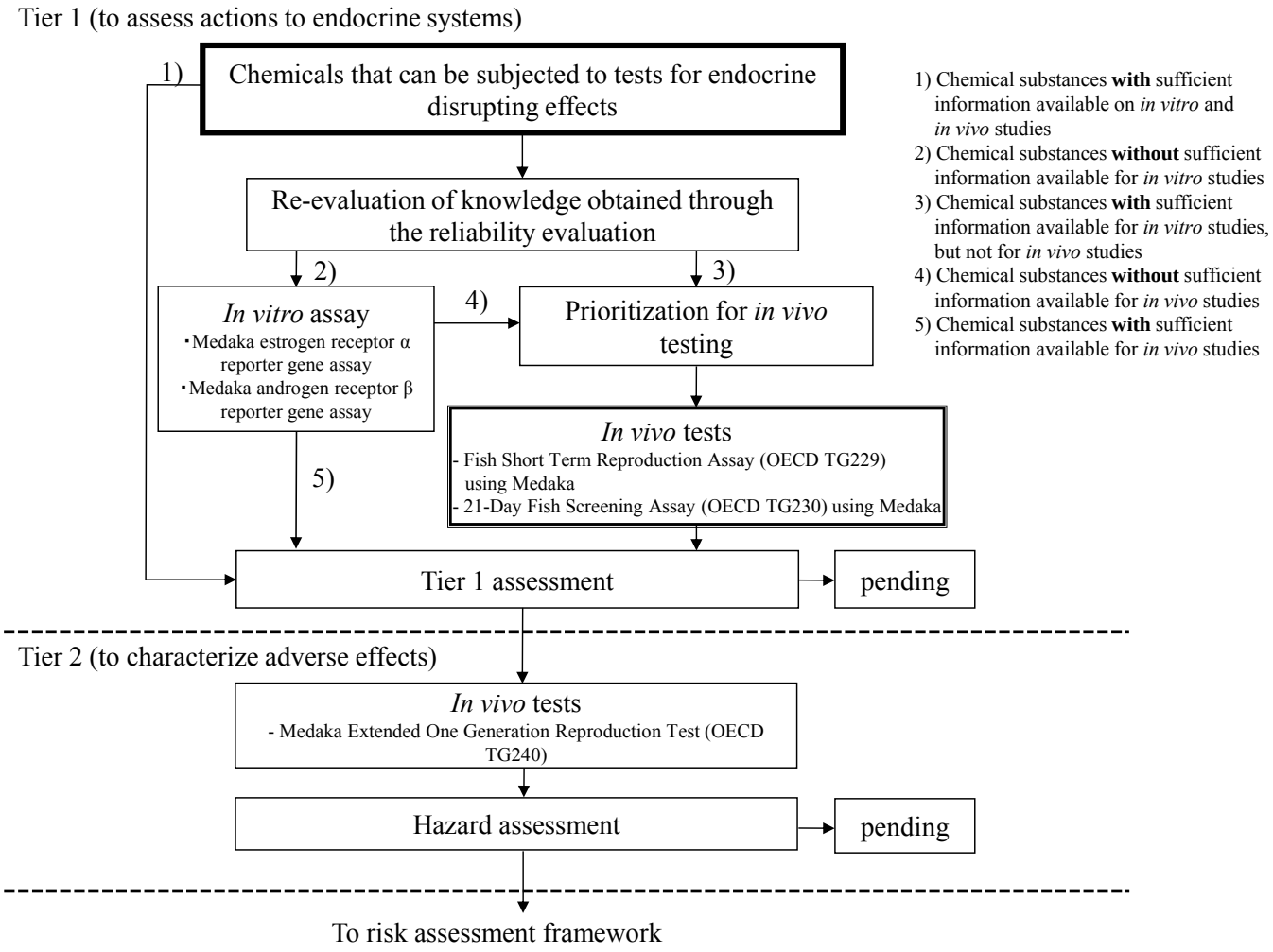
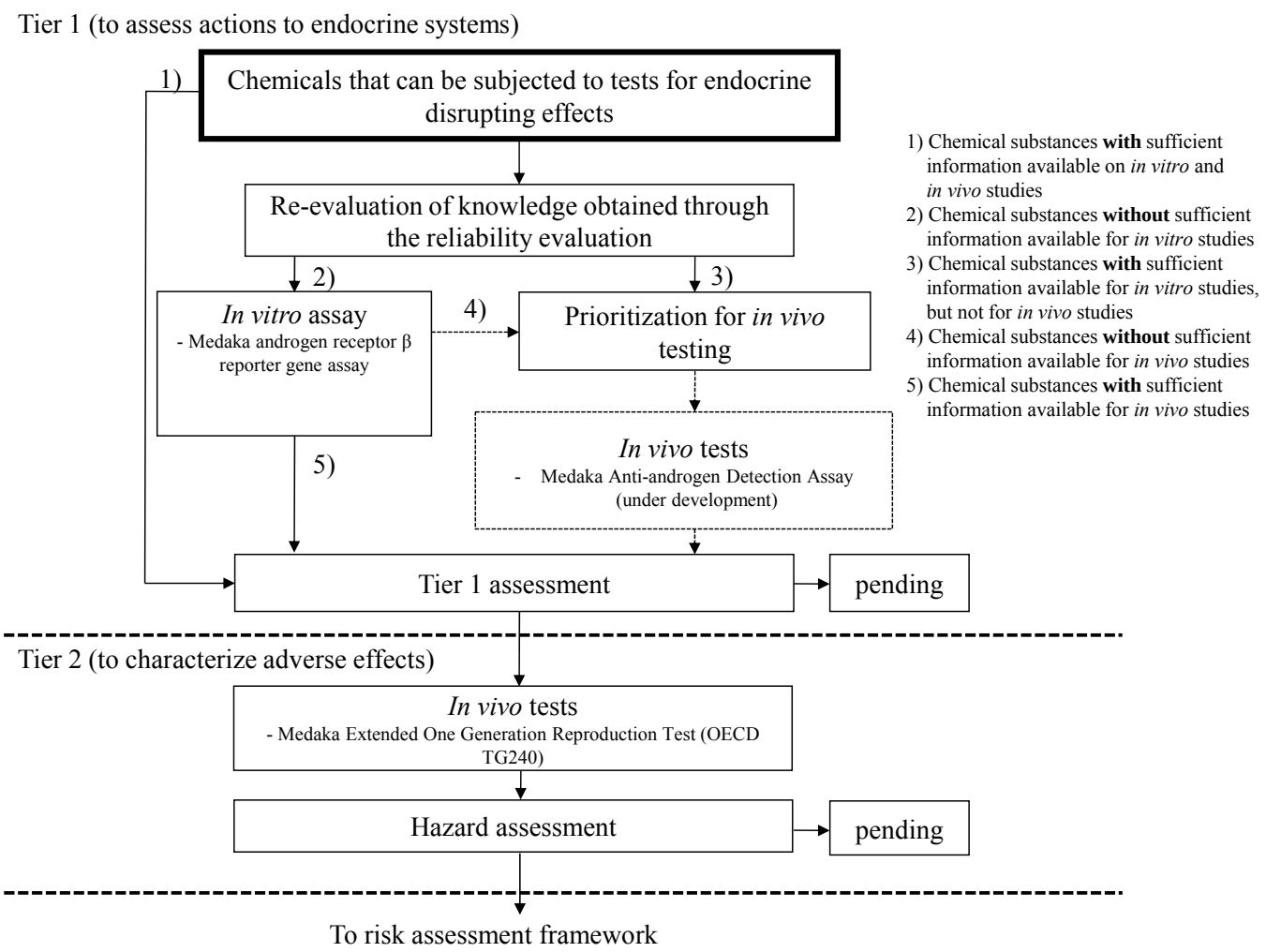


**Figure 4.2 Framework of Hazard Assessment of Endocrine Disrupting Effects**

**Effects on Reproduction**

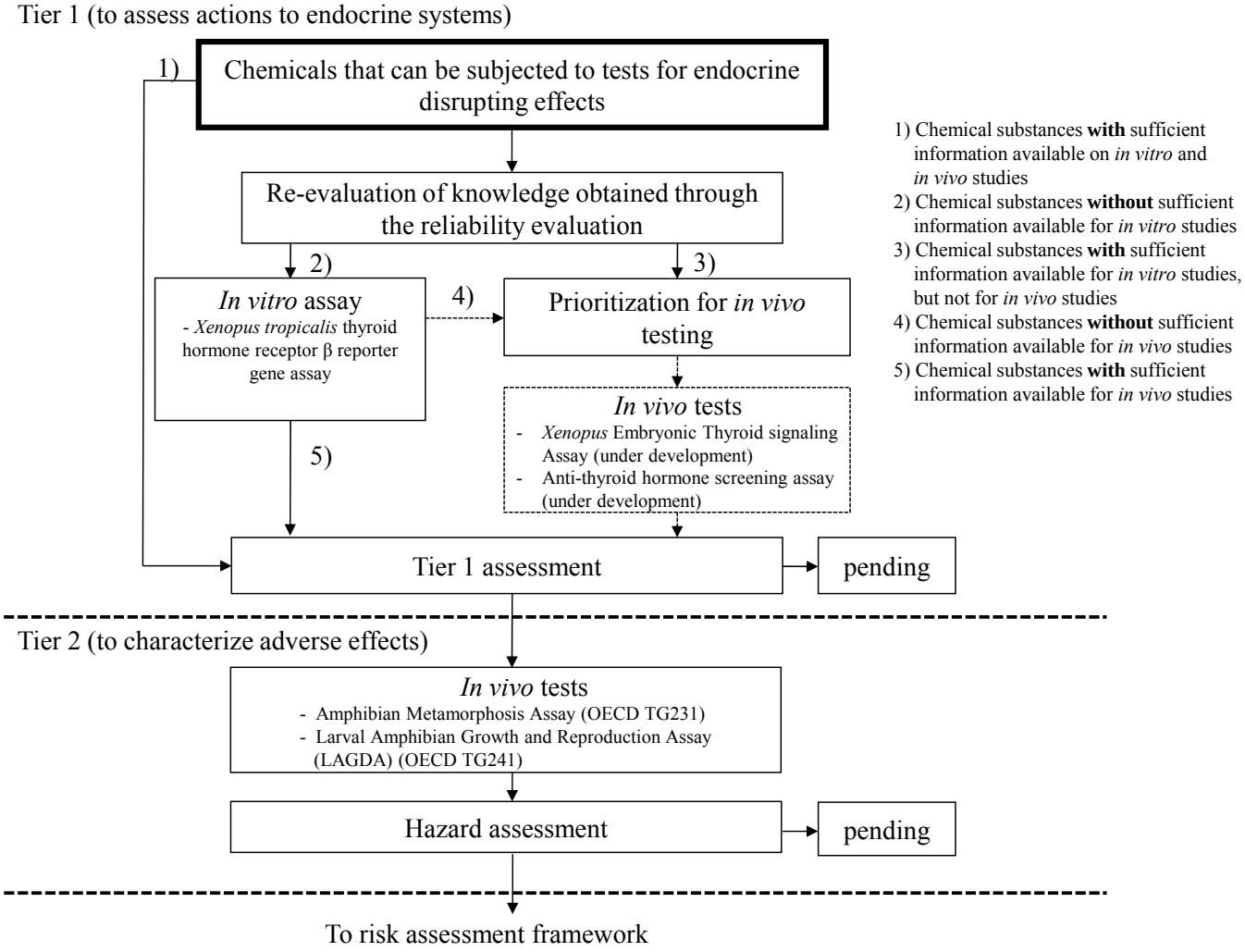
(anti-androgenic actions, etc.)



## Figure 4.3 Framework of Hazard Assessment of Endocrine Disrupting Effects

## Effects on Thyroid

(thyroid hormone-like, anti-thyroid hormone-like actions, etc.)



## Figure 4.4 Framework of Hazard Assessment of Endocrine Disrupting Effects

### Effects on Growth

(juvenile hormone-like, ecdysone-like actions, etc.)

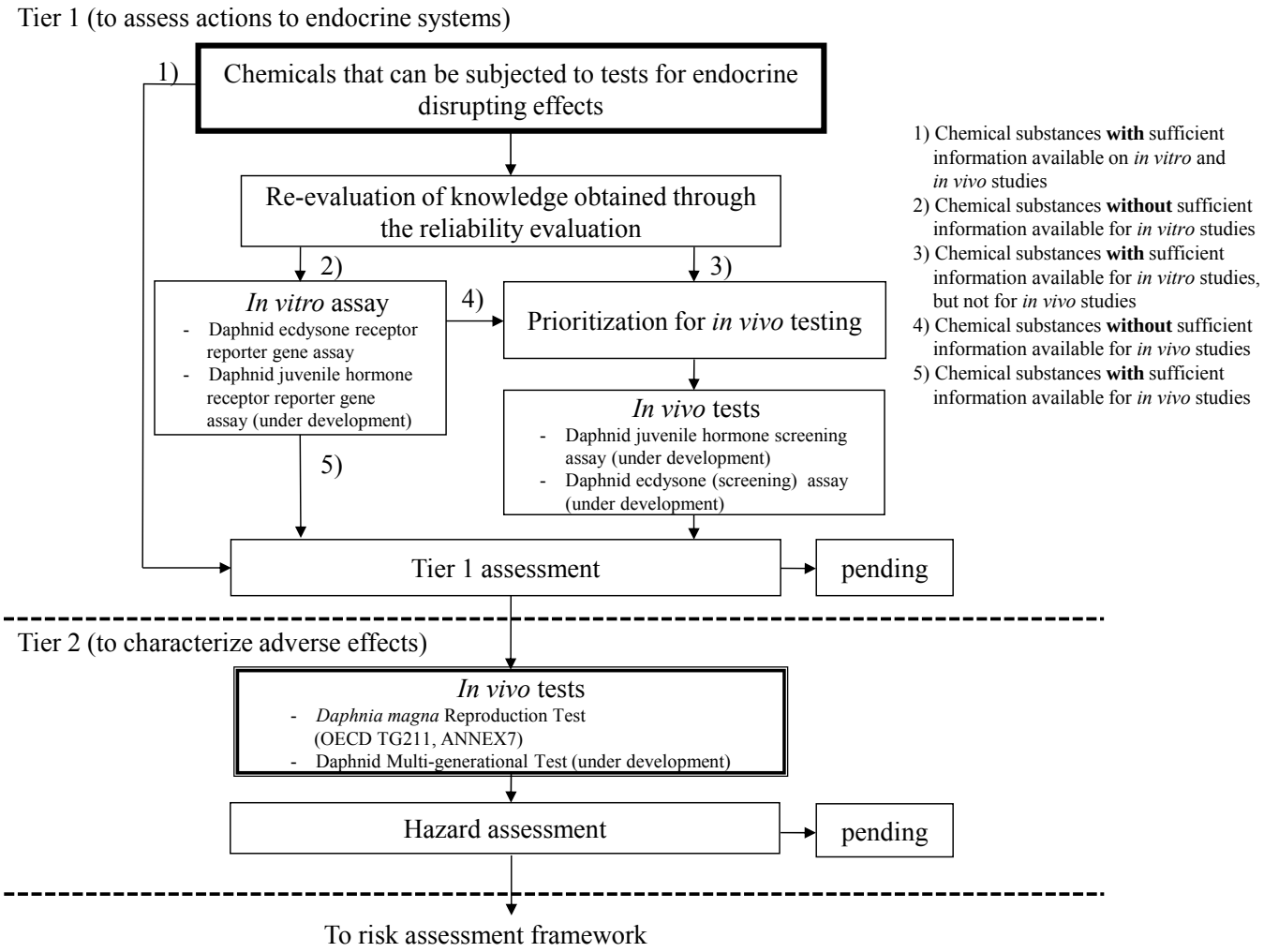


**Table 1 Progress of Test Method Development under EXTEND2010**

| Test Groups<br>Detectable actions | Tier 1 *in vitro* Tests<br>(screening tests) | Tier 1 *in vivo* Tests<br>(screening tests) | Tier 2 *in vivo* Tests<br>(definitive tests) |
|---|---|---|---|
| Estrogenic action<br>Anti-estrogenic action | * Medaka estrogen receptor α reporter gene assay | * Fish Short Term Reproduction Assay (OECD TG229) using Medaka<br>* 21-Day Fish Screening Assay (OECD TG230) using Medaka | * Medaka Extended One Generation Reproduction Test (MEOGRT) (OECD TG240) |
| Androgenic action | * Medaka androgen receptor β reporter gene assay | * Fish Short Term Reproduction Assay (OECD TG229) using Medaka<br>* 21-Day Fish Screening Assay (OECD TG230) using Medaka | * Medaka Extended One Generation Reproduction Test (MEOGRT) (OECD TG240) |
| Anti-androgenic action | **Medaka androgen receptor β reporter gene assay | **Medaka Anti-androgen Detection Assay (provisional title) | * Medaka Extended One Generation Reproduction Test (MEOGRT) (OECD TG240) |
| Thyroid hormone-like action<br>Anti-thyroid hormone-like action | * *Xenopus tropicalis* thyroid hormone receptor β reporter gene assay | ††*Xenopus* Embryonic Thyroid signaling Assay (XETA)<br>(Due to incapability to detect thyroid hormone-like action, other assays are being examined.) | * Amphibian Metamorphosis Assay (AMA)(OECD TG231)<br>* Larval Amphibian Growth and Reproduction Assay (LAGDA) (OECD TG241) |
| Juvenile hormone-like action<br>Anti- juvenile hormone-like action | † Daphnid juvenile hormone receptor reporter gene assay | **Daphnid juvenile hormone screening assay | * *Daphnia magna* Reproduction Test (OECD TG211, ANNEX7)<br>† Daphnid Multi-generational Test |
| Ecdysone-like action<br>Anti-ecdysone-like action | * Daphnid ecdysone receptor reporter gene assay | † Daphnid ecdysone (screening) assay | * *Daphnia magna* Reproduction Test (OECD TG211, ANNEX7)<br>† Daphnid Multi-generational Test |

*: Developed, **:Under development (close to completion), †:Under development, ††:Under consideration

## 2.3 Surveys on Environmental Concentration and Exposure Assessment of Chemical Substances

In EXTEND2010, environmental concentrations of chemical substances have been surveyed as in ExTEND2005, mainly utilizing the results of MOE's Environmental Survey and Monitoring of Chemicals. Since FY 2010, 20 chemicals have been requested to be included in the survey. Among them, 11 chemicals were measured and 7 chemicals (benzophenone, 4-(dimethylbenzyl)phenol, 4-*t*-octylphenol, bisphenol A, chlormadinone acetate, propylparaben, and 4-nonylphenol (branched)) were detected from surface water.

## 2.4 Assessment of Actions and Effects

### (1) Progress in reliability evaluation

EXTEND2010 aimed to "select about 100 chemicals in the next five years in order to efficiently screen chemicals." This goal was achieved, and 132 chemicals were selected for reliability evaluation. Among them, the process of reliability evaluation was completed for 122 chemicals, judging 85 as "Chemicals that can be subjected to tests for endocrine disrupting effects" and 37 as "Chemicals that are not presently subjected to tests."

### (2) Progress in Tier 1 *in vivo* tests

Among 85 "Chemicals that can be subjected to tests for endocrine disrupting effects," 49 chemicals were subjected to 134 Tier 1 *in vitro* tests, resulting in 19 positive and 30 negative results. Of the 85 chemicals, 19 chemicals were subjected to further consideration because the chemicals were presumed to have mechanisms that cannot be examined in the Tier 1 *in vitro* tests.

Among 19 positive chemicals screened in Tier 1 *in vitro* tests, 12 chemicals were subjected to Fish Short Term Reproduction Assay (OECD TG229) using Medaka as a Tier 1 *in vivo* test. The results indicated elevated vitellogenin levels in male liver for 5 chemicals (estrone, 4-*t*-octylphenol, 4-nonylphenol (branched), methyl 4-hydroxybenzate, and 4-*t*-pentylphenol), suggesting estrogenic effects. Decreased egg numbers spawned by fish were observed for 9 chemicals (estrone, cyanazine, diazinon, 1-naphotol, 4-nonylphenol (branched), methyl 4-hydroxybenzoate, fenitoin, 4-*t*-pentylphenol, and triphenyl phosphate). For one chemical (bisphenol A), elevation of vitellogenin levels in male liver was observed, but the result was regarded not conclusive due to accompanying high mortality rate.

## 2.5 Risk Assessment and Risk Management

Efforts were focused on expediting assessment of effects, and major progress was seen in test method development. However, risk assessment of endocrine disrupting effects and related actions of chemical substances is yet to be achieved. Consequently, no chemicals were identified for risk management.

## 2.6 Promotion of Information Sharing

### (1) Webpages

MOE's approaches to address endocrine disrupting effects of chemical substances were published in the following webpages.

http://www.env.go.jp/chemi/risk_assessment.html (in Japanese)

http://www.env.go.jp/en/chemi/index.html (in English)

### (2) Public Seminar

Every year since FY2010, "Public Seminar on Endocrine Disrupting Effects of Chemical Substances" was held as an opportunity to provide information on the results of recent research on endocrine disrupting effects of chemical substances, targeting a wide range of audience including experts and the general public. Seminar reports and presentation materials were published in the following webpage.

http://www.env.go.jp/chemi/end/extend2010/seminar.html (in Japanese)

## 2.7 Promotion of International Cooperation

### (1) Organisation for Economic Co-operation and Development (OECD)

Following ExTEND2005, test method development was promoted under the framework of the OECD Test Guideline Program with international cooperation. Japanese proposal to add experimental conditions for Medaka was adopted for Fish Short Term Reproduction Assay (OECD TG229) using Medaka. Medaka Extended One Generation Reproduction Test (MEOGRT) and Larval Amphibian Growth and Reproduction Assay (LAGDA) were co-submitted with the USA and adopted as Test Guideline 240 (OECD TG240) and 241 (OECD TG241), respectively. Xenopus Embryo Thyroid signaling Assay (XETA) was proposed by France, and Japan was involved in its international ring test. In 2015, Medaka Anti-androgen Detection Assay (provisional title) and Daphnid Juvenile Hormone Screening Assay (provisional title) were submitted as new projects to the OECD after years of development under EXTEND2010.

### (2) Japan-UK joint research on endocrine disrupting effects of chemical substances

Following ExTEND2005, the third-period co-project, agreed to be extended for another five years in FY2010, was carried out under EXTEND2010. This project, composed of four core-projects, was promoted by international cooperation between Japanese and UK researchers through annual workshops and other co-research activities, producing useful achievements for test method development, assessment of actions and effects, etc. The fourth-period co-project was agreed and signed between Japan and UK in 2016, and research is still continuing.

**(3) Japan-US partnership on endocrine disrupting effects of chemical substances**

Following ExTEND2005, the main objective of this partnership was to address technical issues found in methods and conditions in tests for assessing reproductive effects mainly on fish, amphibians, and invertebrates. Through efforts such as Japan-US joint research and validation, test method development was further promoted and protocols were submitted for OECD Test Guideline. Information exchange between Japan and US was also made regarding the progress of assessment projects in each country.

## 2.8 Summary

In EXTEND 2010, emphasis were put on "2) Development of Test Methods and Establishment of Assessment Framework" and "4) Implementation of Actions and Effects Assessment" aiming to facilitate test method establishment and actions and effects assessment.

For "Establishment of Assessment Framework," to promote testing and assessment effectively with limited resource, specific procedures were established to select chemicals and evaluate reliability of existing knowledge and information. In addition, two-tiered framework for testing and assessment was constructed by combining *in vitro* assays and *in vivo* assays. The framework will screen chemicals in Tier 1 through *in vitro* assays and short-term *in vivo* assays, and detect adverse effects in Tier 2 by long-term *in vivo* assays. This two-tiered concept is similar to the framework of the Endocrine Disruptor Screening Program (EDSP) in the USA (details in II 5).

For "Test Method Development," test methods essential for above-mentioned framework were developed, following ExTEND2005. Experimental conditions for Medaka were established for Fish Short Term Reproduction Assay in Tier 1. Medaka Extended One Generation Reproduction Test and Larval Amphibian Growth and Reproduction Assay were also established to be used in Tier 2. Development of other test methods essential for above-mentioned framework is underway using fish, amphibians, and invertebrates.

For "Implementation of Actions and Effects Assessment," the goal to "select about 100 chemicals in the next five years in order to efficiently screen chemicals" was achieved, from reliability evaluation of existing knowledge with priority on chemicals detected from the environment. Based on the judgment in reliability evaluation, "Chemicals that can be subjected to tests for endocrine disrupting effects" were subjected to Tier 1 *in vitro* and short-term *in vivo* assays. The test results were published in order after deliberation in Sub-Committee. Accumulating these test data, methodologies for Tier 1 assessment were examined.

"Promotion of International Cooperation" played an important role in implementing these actions. In the third-period Japan-UK joint research, useful findings were obtained for test method development, actions & effects assessment, etc. This led to the fourth-period joint research in 2015. In Japan-US partnership, joint-research on test method development

led to achievements such as completion of above-mentioned test method using Medaka and larval amphibians. These bilateral cooperation projects were also beneficial for information exchange on administrative actions. In OECD, two test methods developed as Japan-US joint research were co-submitted (both were adopted as new test guidelines), and active participation, such as proposal of two new short-term *in vivo* tests, was made.

As mentioned above, progress was made in the construction of methodology for testing and assessment, and actions and effects assessment was promoted mainly in Tier 1 tests. However, neither the implementation of Tier 1 assessment nor the initiation of Tier 2 test was accomplished because the establishment of methodology for testing and assessment took time. As a result, “Risk Assessment and Risk Management” were not reached. However, considerable progress to prepare for this stage was made through EXTEND2010.

## II Overseas Activities

### 1. World Health Organization (WHO)

In 2002, the International Programme on Chemical Safety (IPCS), the World Health Organization (WHO), the International Labour Organization (ILO) and the United Nations Environment Programme (UNEP) co-published a scientific review document titled "Global Assessment of the State-of-the-Science of Endocrine Disruptors." In this document, the definition of endocrine disruptor was described as follows: "An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations."

In 2012, WHO published "Endocrine disrupters and child health - Possible developmental early effects of endocrine disrupters on child health," a scientific review document on effects of endocrine disrupters on child health.

In 2013, UNEP and WHO co-published "State of the Science of Endocrine Disrupting Chemicals - 2012" as an update of the 2002 document. This scientific review document included indications mainly from epidemiological studies that were published after 2002.

* Global Assessment of the State-of-the-Science of Endocrine Disruptors.
  http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/
* Endocrine disrupters and child health — Possible developmental early effects of endocrine disrupters on child health
  http://www.who.int/ceh/publications/endocrine_disrupters_child/en/
* State of the Science of Endocrine Disrupting Chemicals — 2012
  http://www.who.int/ceh/publications/endocrine/en/

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

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

Under EDTA, the "Conceptual Framework for Testing and Assessment of Endocrine Disrupters", was proposed in 2002 (revised in 2012), and a variety of test methods to detect endocrine disrupting effects have been organized.

Test method development is currently underway to detect endocrine disrupting effects of chemical substances under the Test Guideline Programme. An advisory group on

endocrine disrupters testing and assessment has been organized in the programme, and new methodologies are being developed. The following documents were published in 2012:

* Guidance Document of Standardized Test Guidelines for Evaluating Chemicals for Endocrine Disruption
  http://www.oecd.org/chemicalsafety/testing/oecdguidancedocumentonstandardisedtestguidelinesforevaluatingchemicalsforendocrinedisruption.htm
* Detailed Review Paper on the State of Science on Novel *In vitro* and *In vivo* Screening and Testing Methods and Endpoints for Evaluating Endocrine Disruptors
  http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)23&doclanguage=en

## 3. United Nations (UN)

In 2002, the need for Strategic Approach to International Chemicals Management (SAICM) was decided at the United Nations Environment Programme (UNEP) Governing Council. In 2006, SAICM was adopted at the First session of the International Conference on Chemicals Management (ICCM1), and its Overarching Policy Strategy and Global Plan of Action were also declared. In 2012, "Endocrine-disrupting chemicals" was identified as one of SAICM Emerging Policy Issues at the Third session (ICCM3) with priorities for cooperative action.

* SAICM Emerging Policy Issues and Other Issues of Concern
  http://www.saicm.org/index.php?option=com_content&view=article&id=452&Itemid=685

## 4. European Union (EU)

### 4.1 European Commission (EC)

The European Commission (EC) has been commencing actions to address endocrine disrupting substances from 1996, and in 1999, The Community Strategy for Endocrine Disrupters (COM(1999)706) was adopted.

Following this Strategy, the EU legislation have individually established their criteria to identify substances with endocrine disrupting properties for plant protection products, biocidal products, REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) related chemicals, and cosmetics. However, the establishment of common criteria applicable for each regulation has taken time.

In June 2014, a roadmap toward defining criteria for identifying endocrine disruptors in the context of the implementation of the Plant Protection Product Regulation and Biocidal Products Regulation was published. In the Roadmap, several policy options were considered, and impact assessment was to be performed to judge the appropriate option.

Impacts on human health, environment, agriculture, socio-economy, and trade are currently being assessed.

* European Commission
  http://ec.europa.eu/environment/chemicals/endocrine/index_en.htm

### 4.2 European Environment Agency (EEA)

In 2012, European Environment Agency (EEA) published a scientific review document titled "The impacts of endocrine disrupters on wildlife, people and their environments – The Weybridge+15 (1996–2011) report."

* The impacts of endocrine disrupters on wildlife, people and their environments – The Weybridge+15 (1996–2011) report
  http://www.eea.europa.eu/publications/the-impacts-of-endocrine-disrupters

## 5. USA

The United States 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 has adopted a two tiered approach consisting of the Tier 1 Screening and Tier 2 Test.

Tier 1 Screening comprises assays to detect chemical actions to the animal endocrine system, and consists of 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). Validity of the test methods were investigated and test methods to be used by the industry were published before October 2009.

Tier 2 Test comprises assays to identify adverse effects on animals.

**(2) Implementation of Tier 1 screening assays**

In the Tier 1 Screening, 67 chemicals were selected in the first list, judged on the availability of information on human exposure (the number was later reduced to 52 chemicals due to reasons including voluntary cancellation of pesticide registration). In 2014,

109 pesticides and pollutants detected in drinking water were selected in the second list.

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. Tier 1 assessment was implemented considering the weight of evidence of submitted test data and existing knowledge (including unpublished data).

In June 2015, the USEPA released its reviews of the Tier 1 screening assay results for the 52 chemicals. 18 chemicals were selected to be tested in Tier 2, and the endocrine pathways that should be tested for each chemical was indicated.

* United States Environmental Protection Agency
  https://www.epa.gov/endocrine-disruption

# III The Program's Directions

## 1. Principles

### (1) Aim and scope of this new program

Various research studies and test method development have been done on the endocrine disrupting effects of chemical substances, and the MOE has implemented various measures in SPEED'98, ExTEND2005, and EXTEND2010. These actions have led to steady achievements including the establishment of framework for assessing endocrine disrupting effects of chemical substances and the development of several test methods that became authorized OECD test guidelines. Nevertheless, endocrine disrupting effects of chemical substances have not yet been fully elucidated. The issue of endocrine disrupting chemicals was also considered as a new emerging policy issue under SAICM in 2012. Under these circumstances, the MOE needs to continue addressing this issue.

The MOE reviewed the research and study done in EXTEND2010 and decided that the program's principles do not need fundamental modification, but there are some aspects that should be reorganized.

Thus, the MOE decided to launch a new five-year program by rearranging the framework and adding necessary improvements to EXTEND2010, to further steadily advance its actions toward endocrine disrupting effects. The new program, named EXTEND (Extended Tasks on Endocrine Disruption) 2016, aims to properly assess the environmental risk of endocrine disrupting effects of chemical substances and take management measures as necessary.

Based on the MOE's role within the national government, ecological effects will remain the highest priority on the assessment of priority chemicals, and the MOE will discuss approaches toward risk management, taking note of international activities. The MOE will also collect information on human health risk caused by chemical substances in the environment, and will explore on collaboration with other national programs such as the Japan Environment and Children's Study (JECS).

Furthermore, the MOE will promote international cooperation participating in test method establishment in the OECD, gathering knowledge and information via bilateral cooperation, and disseminating information on the nation's activity to the international community. The MOE will also closely follow actions in other countries and international organizations including the OECD to take full advantage of their achievements.

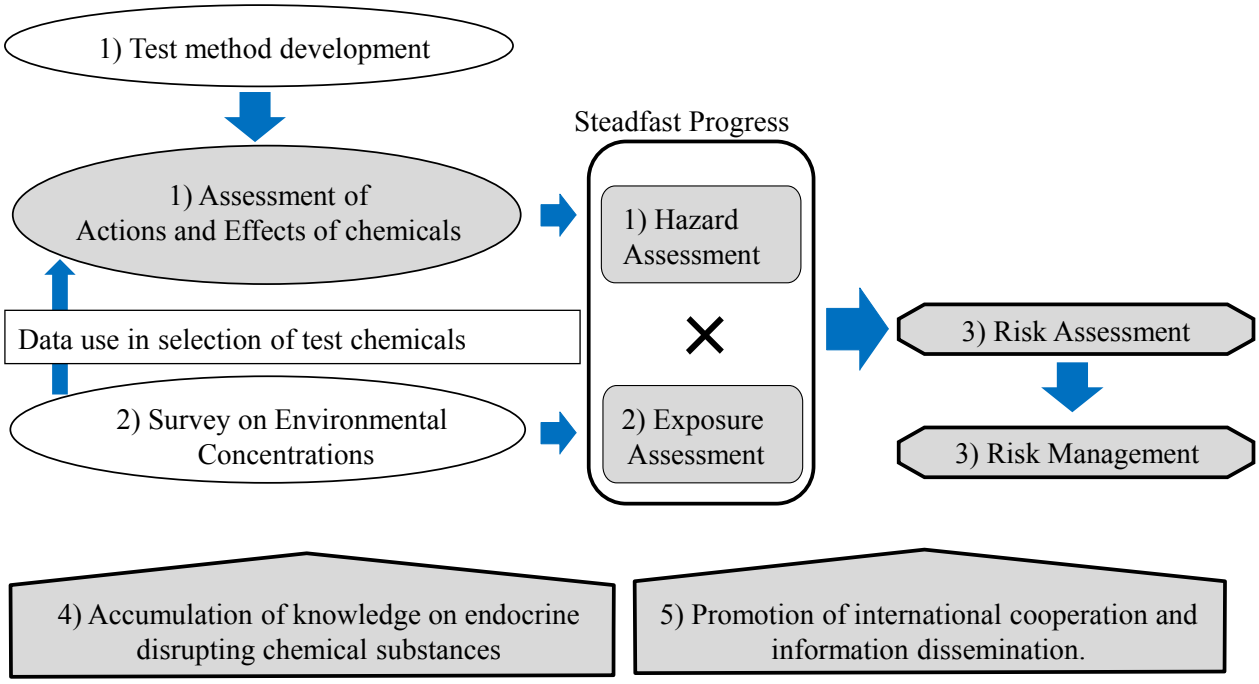
### (2) The program structure

Based on the obtained achievements and the remaining subjects under EXTEND2010, EXTEND2016 (hereinafter referred to as "this program") will be administered with the following structures (Figure 5).

1) Actions and effects assessment and test method development

2) Survey on environmental concentrations and exposure assessment
3) Risk assessment and risk management
4) Accumulation of knowledge on endocrine disrupting effects of chemical substances
5) Promotion of international cooperation and information dissemination

**Figure 5 Conceptual Overview of Actions in EXTEND2016**



## 2. Directions

### 2.1 Action and Effect Assessment and Test Method Development

In EXTEND2010, based on the successful establishment of framework for assessing endocrine disrupting effects of chemical substances, the MOE selected candidate chemicals, evaluated reliability of existing knowledge, and conducted *in vitro* and *in vivo* testing, and then assessed the chemical substance. Since the MOE did not find any necessity to change this basic conceptual framework, the current direction will be succeeded in principle.

The MOE has developed methods to assess the endocrine disrupting effects of chemicals on fish, amphibian, and crustacean, and many tests have been adopted in the OECD as test guideline. As the MOE has played a leading role in developing test methods for endocrine disrupting effects, our continuous contribution is highly expected. Consequently, the MOE will continue its efforts on test method development to assess the endocrine disrupting effects targeted in this program.

From the above, regarding actions and effects assessment and test method development, the MOE considers that it is appropriate to basically succeed the directions of EXTEND2010. However, some improvements could be made in the selection process of chemicals for a more efficient assessment of actions and effects of chemical substances. Under these circumstances, the MOE decided to integrate “Implementation of Actions and Effects Assessment” and “Development of Test Methods and Establishment of Assessment framework”, which were two independent portion in the EXTEND2010 structure, into “Actions and effects assessment and test method development” to further promote the assessment of chemicals and to respond to future issues more flexibly.

#### (1) Selection of candidate chemicals

In EXTEND2010, the MOE assessed “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, etc., among all substances,” using the results of Environmental Survey and Monitoring of Chemicals and the like to prioritize the candidate chemicals. (Substances that have already been banned for production or import were excluded since the main objective of the program is to utilize the results for environmental administration). As a result, the MOE selected 132 chemical substances as candidates for assessment, surpassing the initial goal to “select about 100 chemicals in the next five years.”

At the moment, no established method exists to easily estimate the potential endocrine disrupting effects in assisting the prioritization of candidate chemicals. Thus, it is deemed appropriate to continue with the current selection process for the time being. However, it is important to reexamine the methodology for prioritization of potential endocrine disrupting chemicals to further forward the assessment. Information on new chemical selection methodologies such as *in silico* screening of chemicals will be

investigated for this purpose.

It is also important to constantly take note of the actions in other countries and international organizations and take full advantages of their achievements. For example, for chemical substances that have already been considered in the United States' EDSP or the European Union's REACH, and the like, the MOE will survey the underlining concept of the assessment to judge whether these chemicals should be considered in Japan as well.

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

In EXTEND2010, on the basis of literature information collected by the most recent literature search at the time, the MOE evaluated information on effects and biological events related to endocrine disruption, and narrowed down the candidate chemicals for testing. As the MOE considers it appropriate to succeed this direction of EXTEND2010, the MOE will continue to collect literature data and conduct reliability evaluation effectively.

**(3) Testing and actions and effects assessment**

The MOE will conduct testing of potential endocrine disrupting chemicals indicated through literature, under the framework for actions and effects assessment. When prioritizing chemicals for testing, other information such as their occurrence in the environment and the progress of test method development will be taken into account to conduct testing efficiently.

The assessment of endocrine disrupting actions and effects will be done comprehensively, based not solely on the test results but also other information such as existing knowledge from literature and the results of the assessment and discussion done in the United States' EDSP, the European Union's REACH and other relevant overseas programs.

In cases where data equivalent to the test method adopted in Japan are obtained through literature or information sharing with other nations, etc., the MOE will use these data to assess the endocrine disrupting actions and effects.

**(4) Test method development**

The MOE will continue with test method development, with an emphasis on the effects on wildlife. Based on the assessment framework established in EXTEND2010, the MOE will develop the required test methods and participate in their international validation as necessary.

These test methods will be developed efficiently and effectively in cooperation among OECD member states, and through bilateral cooperation of Japan-US and Japan-UK.

## 2.2 Survey on Environmental Concentrations and Exposure Assessment

In order to assess environmental risk of chemical substances properly, it is essential to determine the environmental occurrence as well as their hazard information. The MOE considers appropriate to succeed the direction of EXTEND2010 to continue its survey on environmental concentrations utilizing the MOE's Environmental Survey and Monitoring of Chemicals.

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

## 2.3 Risk Assessment and Risk Management

### (1) Risk assessment

In EXTEND2010, no chemicals were subjected to risk assessment, because a longer period was necessary to develop the test method for actions and effects assessment. Nevertheless, the Medaka Extended One Generation Reproduction Test (MEOGRT), which was developed under this program to assess estrogenic effects, was adopted as Test Guideline 240 (OECD TG240) in July 2016. The MOE will steadily advance its risk assessment through the actions and effects assessment as mentioned 2.2 above.

In the environmental risk assessment of chemicals, it is not appropriate to assess the endocrine disrupting effects independently. It is necessary to assess the effect as one aspect of the chemical's various actions or in combination with other biological actions.

In the MOE, risk assessment is done at the following two levels.

- Assessment aiming to efficiently select potential environmentally high risk chemical substances from numerous candidates, and screen candidate chemicals for detailed assessment for risk management.
- Detailed risk assessment aiming to judge the necessity for risk management by the environmental administration including the establishment of standards and introduction of regulation based on law.

EXTEND2010 envisaged to seek the possibility of adding the assessment of endocrine disrupting effects to this system of risk assessment in its risk assessment program. In EXTEND2016, the MOE will continue with this direction in principle. If the action mentioned in (2) bellow calls for necessary modification, the MOE will take the matter into consideration as appropriate.

### (2) Risk management

As in (1) above, risk management was also not discussed in EXTEND2010. Nevertheless, steady progress of risk assessment is expected in EXTEND2016. As risk

management of endocrine disrupting effects of chemical substances are already being examined in other nations, the MOE must soon start the discussion on the appropriate direction for risk management, taking note of other international activities.

## 2.4 Accumulation of Knowledge on Endocrine Disrupting Effects of Chemical Substances

In EXTEND2010, to address the still largely unknown endocrine disrupting effects of chemical substances, new research topics were sought under the scheme of "Promotion of Research for Biological Observation of Wildlife and Fundamental Studies" and information were collected on specific topics. However, the MOE will modify this approach, and will use the Ministry's competitive research funding scheme in order to seek for a wider range of research topics (This does not apply to the research topics already underway at the start of this program). To utilize the competitive research funding scheme effectively, the MOE will disclose topics that needs to be pursued as its regulatory needs in a timely manner.

On the other hand, thorough collection of national and international information will become essential for future implementation of actions and effects assessment and further approach to risk management. This will also become a prerequisite for delivering regulatory needs that the MOE hopes to be pursued in competitive research funding as mentioned above. Taking these points into account, the MOE decided that the scheme, titled as "Promotion of Research for Biological Observation of Wildlife and Fundamental Studies" in EXTEND2010, be revised as "Accumulation of knowledge on endocrine disrupting effects of chemical substances" in order to collect broader range of information that can be utilized in this program.

## 2.5 Promotion of International Cooperation and Information Dissemination

The MOE has actively participated in international meetings and discussions and made contributions including test guideline developments in OECD. Japan-UK joint research and Japan-US partnership are also currently under way to address this topic at the international level. International cooperation is beneficial not only for global progress toward endocrine disrupting effects of chemical substances but also for harmonization of our actions with the international community. Consequently, it is important to continue with these approaches.

On the other hand, information on the MOE's current actions to endocrine disrupting effects of chemical substances has been disseminated only in Japanese. Although many scientific uncertainties remain, progress has been made in the assessment of endocrine disrupting effects of chemicals in Japan and other nations. Taking these points into account, it is insufficient to disseminate our achievement inside Japan only, and thus our current progress should be disseminated both nationally and internationally. In addition, from the view point of promoting efficient chemical assessment, there is a need to share test results

for actions and effects assessment with the international community.

Under these circumstances, the scheme separately titled as “Promotion of Information Sharing” and “Promotion of International Cooperation” in EXTEND2010, will be integrated as “Promotion of international cooperation and information dissemination” to disseminate information more actively.

**(1) Contribution to OECD**

In regards to activities including test method development 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 take note of the activities and maintain active contributions mainly to test method development for wildlife effects assessment.

**(2) Japan-UK joint research**

The MOE will promote the following four fields that were defined in April 2015 as core projects of the fourth term of the Japan-UK joint research.

1) Research to estimate the transport and fate of chemical substances suspected of having endocrine disrupting effects in treated effluents and their receiving waters, and research to consider ways to reduce their environmental discharge.
2) Research to establish transgenic fish necessary for the development of screening system to detect endocrine disrupting effects of chemical substances, and research to elucidate molecular mechanism of various endocrine disrupting effects.
3) Research related to evaluation of various endpoints in chemical testing to understand reproductive and developmental effects on animals including aquatic life.
4) Simulation of effects of suspected endocrine disrupting chemicals on population and analysis of environmental risk of these chemicals on wildlife in UK and Japan.

**(3) Japan-US partnership**

The Japan-US partnership will continue to cooperatively make efforts in developing new test methods, sharing test data, exchanging new knowledge regarding chemical assessment methodology, etc.

**(4) Information dissemination via webpages**

The MOE will review its webpages on endocrine disrupting effects of chemical substances, and organize the information suitable for easy navigation and update.

In addition, the MOE will prepare English webpages and update it to provide

information on how Japan is addressing the issue of endocrine disrupting effects of chemical substances.

**(5) Seminars**

In EXTEND2010, the MOE hosted open seminars to provide state-of-the-art information on endocrine disrupting effects of chemical substances to the public. The MOE will continue to hold such seminars as necessary, and in addition, will consider other ways to better organize them, such as co-hosting with other chemical seminars. The MOE will also examine the seminar agenda in order to reach not only experts but also the general public.

## 3. Steering Organization

In EXTEND2010, the program was run under the auspices of "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"). These committees discussed the direction of the program and evaluated the results of research projects every fiscal year. However, since the approach regarding "Promotion of Research for Biological Observation of Wildlife and Fundamental Studies" was modified, the MOE has decided to reorganize the overall steering organizations of the program.

In this program, the Sub-Committees will be integrated into "Task Force on Endocrine Disrupting Effects of Chemical Substances" and this single task force will continue with the actions and effects assessment of chemicals, etc. Furthermore, the results will be periodically reported to the Environmental Health Committee of the Central Environmental Council, and the program will advance, taking in comments received.

## Conclusion

Policies herein are the summary of the MOE's future actions to address endocrine disrupting effects of chemical substances for the next approximately five years starting from 2016. The program succeeds the basic principles of EXTEND2010, however, the principle should be flexible, and revisions shall be made as necessary reflecting the progress from further research and accumulation of knowledge.

In Europe and the United States, approaches for endocrine disrupting effects of chemical substances are expected to accelerate from the knowledge accumulated, particularly from test method development. While it has become increasingly important to take note and understand such activities carried out in other nations, it is also crucial for Japan to disseminate the achievements from EXTEND as Japan's contribution to the world.

In this program, keeping the above in mind, the MOE will accelerate its actions with the establishment of evaluation methods and assessment of chemicals in order to assess the environmental risk of endocrine disrupting effects of chemical substances, and in the process, explore the possibility of incorporating them into the framework for environmental risk management as necessary.

# Appendices

## Appendix 1 Fish (Medaka) Test Results (FY 2000 - FY 2005)

| Test Chemicals | Test Results |
|---|---|
| Di-2-Ethylhexyl adipate | Testis-ova were observed at low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Amitrole | No clear endocrine disrupting effects were found. |
| Aldrin | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Triphenyltin chloride | No clear endocrine disrupting effects were found. |
| Tributyltin chloride | No clear endocrine disrupting effects were found. |
| Endrin | No clear endocrine disrupting effects were found. |
| Octachlorostyrene | No clear endocrine disrupting effects were found. |
| 4-*tert*-Octylphenol | (1) Strong binding to the fish estrogen receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the male liver, (3) emergence of testis-ova, and (4) decrease in number of eggs hatched and fertilization rate were observed. Endocrine disrupting effects on fish were strongly suspected. |
| *cis*-Chlordane | No clear endocrine disrupting effects were found. |
| Kelthane | No clear endocrine disrupting effects were found. |
| 2,4-Dichlorophenol | No clear endocrine disrupting effects were found. |
| Dieldrin | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| 4-Nitrotoluene | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| *trans*-Nonachlor | No clear endocrine disrupting effects were found. |
| Nonylphenoxyacetic acid | No significant effects were observed in Medaka ERα reporter gene assay. |
| 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 fertilization rate were observed. Endocrine disrupting effects on fish were strongly suspected. |
| Nonylphenol monoethoxylate | No significant effects were observed in Medaka ERα reporter gene assay. |
| Nonylphenol diethoxylate | No significant effects were observed in Medaka ERα reporter gene assay. |
| Bisphenol A | (1) Weak binding to the fish estrogen receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the male liver, (3) emergence of testis-ova, and (4) increased number of days for incubation (delayed hatching) were observed. Endocrine disrupting effects on fish were postulated. |
| Diethyl phthalate | No clear endocrine disrupting effects were found. |
| Di-2-ethylhexyl phthalate | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. Thus, no clear endocrine disrupting effects were found. |
| Dicyclohexyl phthalate | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Di-*n*-butyl phthalate | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Dipropyl phthalate | No clear endocrine disrupting effects were found. |
| Dihexyl phthalate | No clear endocrine disrupting effects were found. |
| Dipentyl phthalate | No clear endocrine disrupting effects were found. |
| Butyl benzyl phthalate | No clear endocrine disrupting effects were found. |
| 4-*tert*-butylphenol | No significant effects were observed in Medaka ERα reporter gene assay. |
| *β*-Hexachlorocyclohexane | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Hexachlorobenzene | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Heptachlor | No clear endocrine disrupting effects were found. |
| Permethrin | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Benzophenone | Testis-ova were observed at low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found at low concentrations (concentrations comparatively low for fish considering the estimated exposure dose obtained from literatures). |

| | |
|---|---|
| Pentachlorophenol | No clear endocrine disrupting effects were found. |
| Mirex | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| Malathion | No clear endocrine disrupting effects were found. |
| *p,p′*-DDD | Testis-ova were observed in low frequency to the extent that would not have caused adverse effects on fertilization rate. No clear endocrine disrupting effects were found. |
| *p,p′*-DDE | No significant changes were observed in the indicators of endocrine disrupting effects at the fish's non-lethal concentration range. |
| *o,p′*-DDT | (1) Weak binding to the fish estrogen receptors, (2) elevated vitellogenin (egg yolk protein precursor) concentration in the male liver, (3) emergence of testis-ova, (4) decreased fertility rate, and (5) increased number of days for incubation (delayed hatching) were observed. Endocrine disrupting effects on fish were postulated. |
| *p,p′*-DDT | No clear endocrine disrupting effects were found. |

## Appendix 2 Mammalian (Rat) Test Results (FY 2000 - FY 2006)

| Test Chemicals | Test Results |
|---|---|
| 4-*tert*-butylphenol | No clear endocrine disrupting effects were found at doses (2 dose groups) determined from estimated human exposure doses based on literatures. |
| Amitrole | No clear endocrine disrupting effects were found at doses (3 dose groups) determined from estimated human exposure doses based on literatures. |
| Heptachlor | |
| Benzophenone | |
| Di-2-Ethylhexyl adipate | No clear endocrine disrupting effects were found at doses (4 dose groups) determined from estimated human exposure doses based on literatures. |
| 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 | |
| $\beta$-Hexachlorocyclohexane | |
| Hexachlorobenzene | |
| Permethrin | |
| Pentachlorophenol | |
| Mirex | |
| Malathion | |
| *p,p'*-DDD | |
| *p,p'*-DDE | |
| *o,p'*-DDT | |
| *p,p'*-DDT | |
| Di-*n*-butyl phthalate | No clear endocrine disrupting effects were found at doses (5 dose groups) determined from estimated human exposure doses based on literatures. |

## Appendix 3 Overview of Research Topics Submission and Selection (FY 2005 -FY 2015)

| FY | No. of Research Topics Submitted | No. of Research Topics Selected | No. of Research Topics Selected and Continued over Multiple Years |
|---|---|---|---|
| 2005 | Was not sought | W: 5<br>F: 13 | W: 4 (one subject was reclassified as Others in 2006)<br>F: 6 (one subject was reclassified as Others in 2006) |
| 2006 | 24 | W: 3<br>F: 3 | W: 1<br>F: 5<br>Others: 2 |
| 2007 | 7 | W: 1<br>F: 3 | W: 0<br>F: 0 |
| 2008 | 7 | W: 1<br>F: 4<br>Others: 1 | W: 2<br>F: 1<br>Others: 1 |
| 2009 | 6 | W: 1<br>F: 3 | W: 1<br>F: 0 |
| 2010 | 16 | W: 1<br>F: 3 | W: 1<br>F: 5 |
| 2011 | 17 | W: 2<br>F: 3 | W: 1<br>F: 2 |
| 2012 | Was not sought | — | W: 1<br>F: 1 |
| 2013 | 13 | W: 0<br>F: 2 | W: 0<br>F: 2 |
| 2014 | Was not sought | — | — |
| 2015 | 10 | W: 1<br>F: 1 | —<br>— |
| Total | 100 | W: 15<br>F: 35<br>Others: 1 | W: 11<br>F: 22<br>Others: 3 |

W: Selected as Research for Biological Observation of Wildlife
F: Selected as Fundamental Studies
Others: Selected as Other Related Studies

## Appendix 4 Overview of Implemented Studies (FY 2005 -FY 2015)

| Chief Researcher Affiliation | Research Topics | Study Period in FY (years continued) |
|---|---|---|
| Research for Biological Observation of Wildlife | | |
| Kiwao Kadokami Kitakyushu City Institute of Environmental Sciences | Comparison of dioxin levels in freshwater fish. | 2005 (1) |
| Tomoki Sunobe Natural History Museum and Institute, Chiba | Studies on sex change induction and social structure of hermaphrodite fish. | 2005-2006 (2) |
| Takayuki Hanazato Shinshu University | Elucidation of current status and mechanisms of ecological disturbance of lake coasts and surrounding areas. | 2005-2007 (3) |
| Satoshi Hamaguchi Niigata University | Collection and analysis of basic information related to abnormal sex differentiation of wild Medaka. | 2005-2009 (5) |
| Toshihiro Horiguchi National Institute for Environmental Studies | Elucidation and factorial analysis of current status of ecological disturbance in Tokyo Bay. | 2006-2008 (3) |
| Tetsuyuki Ueda Ishikawa Prefectural University | Survey on the declining population of Dragonfly *Sympetrum frequens* and elucidation of its causes. | 2006-2010 (5) |
| Yoshihiro Shiraiwa University of Tsukuba | Physiological and ecological studies on the effects of environmental impact chemicals to elucidate the factors of decreasing population of Charales. | 2007-2010 (4) |
| Masayuki Saigusa Okayama University | Studies on screening of abnormality found in growth and sexual maturation of benthic crustacean and on environment effects assessment. | 2009-2011 (3) |
| Seiichi Uno Kagoshima University | Exposure and risk assessment of estrogenic compounds via marine sediments. | 2010-2011 (2) |
| Mayumi Ishizuka Hokkaido University | Genomic stress caused by environmental chemicals on wild sentinel species Rattus sp. and its adaptation. | 2011-2014 (4) |
| Kiyoshi Soyano Nagasaki University | Clarification of biological effects on grey mullet, Japanese common goby, and bivalves caused by sediment-accumulative chemicals in Japanese coastal areas. | 2011-2014 (4) |
| Fundamental Studies | | |
| Akihiko Kashiwagi Hiroshima University | Studies on expression mechanism of endocrine disrupting effects on thyroid hormones in amphibians. | 2005 (1) Under UK-Japan Joint Research since 2006 |
| Yoshinao Katsu National Institutes of Natural Sciences | Analysis of induction mechanisms of testis-ova in fish by estrogenic chemicals. | 2005 (1) Under UK-Japan Joint Research since 2006 |
| Masaki Nagae Nagasaki University | Evaluation of endocrine disrupting effects of chemicals using three-spined stickleback. | 2005 (1) Under UK-Japan Joint Research since 2006 |
| Masato Kinoshita Kyoto University | Examination of initial gonadal changes in transgenic Medaka caused by endocrine disrupting chemicals and its recovery. | 2005-2007 (3) |
| Hiroaki Aoyama Institute of Environmental Toxicology | Genetic analysis of intrinsic factors that may modulate the response to xenobiotics in mammalian species of animals. | 2005-2009 (5) |
| Shigeru Ohta Hiroshima University | Metabolic activities and activation of endocrine disrupting chemicals in fetuses and neonates of rats. | 2005-2009 (5) |
| Noriyuki Koibuchi Gunma University | Mechanisms of environmental chemical action on nuclear receptor-mediated transcription. | 2005-2009 (5) |
| Tsuyoshi Nakanishi Osaka University | Gain of function of estrogen signal during fetal period and its reversibility of sexual differentiation | 2005-2009 (5) |

| Chief Researcher<br>Affiliation | Research Topics | Study Period in FY<br>(years continued) |
|---|---|---|
| Yoshitaka Nagahama<br>National Institutes of Natural Sciences | Studies on the mechanism of endocrine disrupting effects of chemicals on reproductive endocrine systems in medaka. | 2005-2009 (5) |
| Hisato Iwata<br>Ehime University | Development of comprehensive analysis of nuclear receptor ligands for risk assessment of wildlife. | 2006-2009 (4) |
| Kaoru Azumi<br>Hokkaido University | Fundamental studies on toxicogenomics using marine invertebrate Ascidiacea. | 2008-2010 (3) |
| Kazuichi Hayakawa<br>Kanazawa University | Studies on screening methods for chemicals using fish scales based on structure-activity relationship of the disrupting effects of polycyclic aromatic hydrocarbons. | 2008-2010 (3) |
| Seiichiro Osako<br>University of Tokyo | Verification of the DOHaD model by epigenetic modifications induced by chemicals. | 2008-2012 (4) |
| Minoru Koga<br>Prefectural University of Kumamoto | Observation and mechanism of abnormal reproduction/development in invertebrate (mysid). | 2009-2012 (4) |
| Yoshinari Tanaka<br>National Institute for Environmental Studies | Studies on new analytical approach by mathematical ecological methodology using improved *Daphnia* reproductive toxicity assay. | 2009-2012(4) |
| Noriyuki Koibuchi<br>Gunma University | Studies on identification of hyperactivity - related factors in endocrinological systems and effects of endocrine disrupting chemicals. | 2010-2012 (3) |
| Hiroki Inoue<br>Rakuno Gakuen University | Studies on disposition of easily metabolized chemicals and its effects on the next generations. | 2010-2013 (4) |
| Sonoko Ogawa<br>University of Tsukuba | Fundamental studies on assessment of behavioral effects caused by endocrine disrupting chemicals and its mechanistic analysis in the brain. | 2011-2012 (2) |
| Akihiko Kashiwagi<br>Hiroshima University | Development of screening system for thyroid hormone disrupting substances using *Xenopus* metamorphosis assay. | 2011-2013 (3) |
| Kei Nakayama<br>Ehime University | Monitoring of natural or synthetic glucocorticoids and their activities in effluents of sewage treatment plants and evaluation of their effects on fish. | 2011-2013 (3) |
| Hironori Aramaki<br>Daiichi University of Pharmacy | Clarification of endocrine disrupting mechanism targeting the second estrogen receptor ERβ. | 2013-2015 (3) |
| Koji Arizono<br>Prefectural University of Kumamoto | Elucidation of contamination status caused by equilins from pregnant mares and its ecological effect assessment. | 2013- (3) |
| Feasibility Studies | | |
| Masaaki Kurasaki<br>Hokkaido University | Development of assay methods for ecological effects of endocrine disrupting chemicals. | 2005 (1) |
| Makoto Nakai<br>Chemicals Evaluation and Research Institute | Establishment of Medaka androgen receptor binding assay. | 2005 (1) |
| Kazuichi Hayakawa<br>Kanazawa University | Evaluation of endocrine disrupting effects of polycyclic aromatic hydrocarbons in combustion exhaust gases. | 2005-2007 (3) |
| Norihisa Tatarazako<br>National Institute for Environmental Studies | Search of juvenile hormone receptors of invertebrates and clarification of their mechanisms. | 2006 (1) |
| Shuntaro Hara<br>Showa University | Analysis of mechanism of endocrine disrupting effects of environmental chemicals by the observation of metabolic changes of arachidonate. | 2006 (1) |

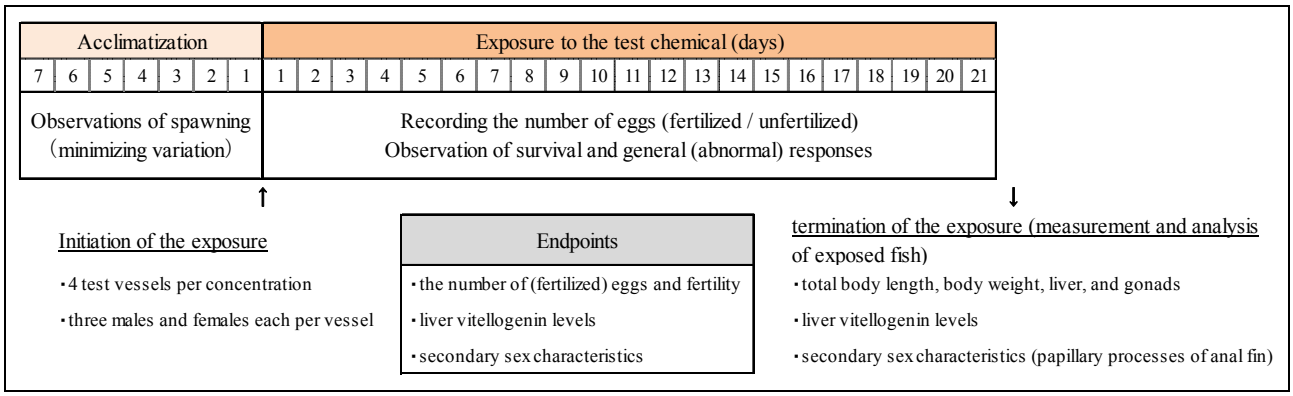
| Chief Researcher<br>Affiliation | Research Topics | Study Period in FY<br>(years continued) |
|---|---|---|
| Masumi Yamamuro<br>National Institute of Advanced Industrial Science and Technology | Feasibility study on the effect of herbicides on the distinction of Charophyte in Japan. | 2006 (1) |
| Minoru Takase<br>Hiroshima University | Incidence of testis-ova in amphibians in field and laboratory. | 2007-2008 (2) |
| Toshinobu Tokumoto<br>Shizuoka University | Studies on endocrine disrupting actions of chemicals on membrane steroid receptors. | 2007-2008 (2) |
| Shuntaro Hara<br>Showa University | Studies of new mechanisms of endocrine disrupting effects of environmental chemicals via inhibition of phospholipase A2 in sperm. | 2007-2008 (2) |
| Koji Arizono<br>Prefectural University of Kumamoto | Effects of chemicals and their metabolites on reproduction of Medaka, and clarification of their mechanisms by toxicogenomics. | 2008-2009 (2) |
| Masatoshi Yui<br>Iwate Prefectural University | Basic studies on biology and food-chain of "Osprey Pandion haliaetus," the fish-eating birds of prey. | 2008-2009 (2) |
| Toshinobu Tokumoto<br>Shizuoka University | Determination of chemical groups effecting steroid membrane receptors based on structure-activity relationship. | 2009 (1) |
| Tohru Kobayashi<br>University of Shizuoka | Studies on mechanisms of endocrine disrupting effects using fish reproductive capacity as an indicator. | 2010 (1) |
| Tatsuya Kunisue<br>Ehime University | Studies on accumulation features and risk assessment persistent property of emerging POPs and candidate POPs in stranded whales. | 2015- (1) |
| Shinichi Miyagawa<br>National Institutes of Natural Sciences | Construction of *in vitro* assay system focusing on fish metabolic disruption. | 2015- (1) |
| Adopted as Other Related Studies | | |
| Taisen Iguchi<br>National Institutes of Natural Sciences | Analysis of endocrine disrupting mechanisms in *Daphnia magna*. | 2005-2015 (11),<br>As a Fundamental Research in 2005, and Other Related Study since 2006 |
| Shinsuke Tanabe<br>Ehime University | Elucidation of status of contamination by bioaccumulative endocrine disrupting chemicals in Japanese wildlife. | 2005-2015 (11), As a Research for Biological Observation of Wildlife in 2005, and Other Related Study since 2006 |
| Taisen Iguchi<br>National Institutes of Natural Sciences | Development of screening method to detect endocrine disrupting effects of chemical substances applying toxicogenomics | 2008-2011 (4) Under UK-Japan Joint Research since 2012 |

Note: Seven underlined subjects were presented in the "Public Seminars on Endocrine Disrupting Effects of Chemical Substances (EXTEND2010)"

## Appendix 5 Summary of Test Methods adopted as OECD Test Guidelines

### 1. Fish Short Term Reproduction Assay (OECD TG229)

The test design of Fish Short Term Reproduction Assay (FSTRA) using Medaka (*Oryzias latipes*) is outlined in the following figure. Sexually reproductive, fertile male and female Medaka are placed in a test vessel (three male and female fish each), and exposed to the test chemical for 21 days. Eggs spawned by female during the exposure period are collected to assess the number and fertility of eggs. At the end of the exposure, liver vitellogenin levels and secondary sex characteristics (the number of the joint plates with papillary processes of anal fin) are measured in the survived fish. From the responses of each endpoint (increase or decrease), effects of the test chemical on Medaka are evaluated for estrogenic effects, anti-estrogenic effects, androgenic effects, aromatase inhibition (steroid synthesis inhibition) and/or hypothalamic-pituitary-gonadal axis.




The test design of Fish Short Term Reproduction Assay (FSTRA) using Medaka

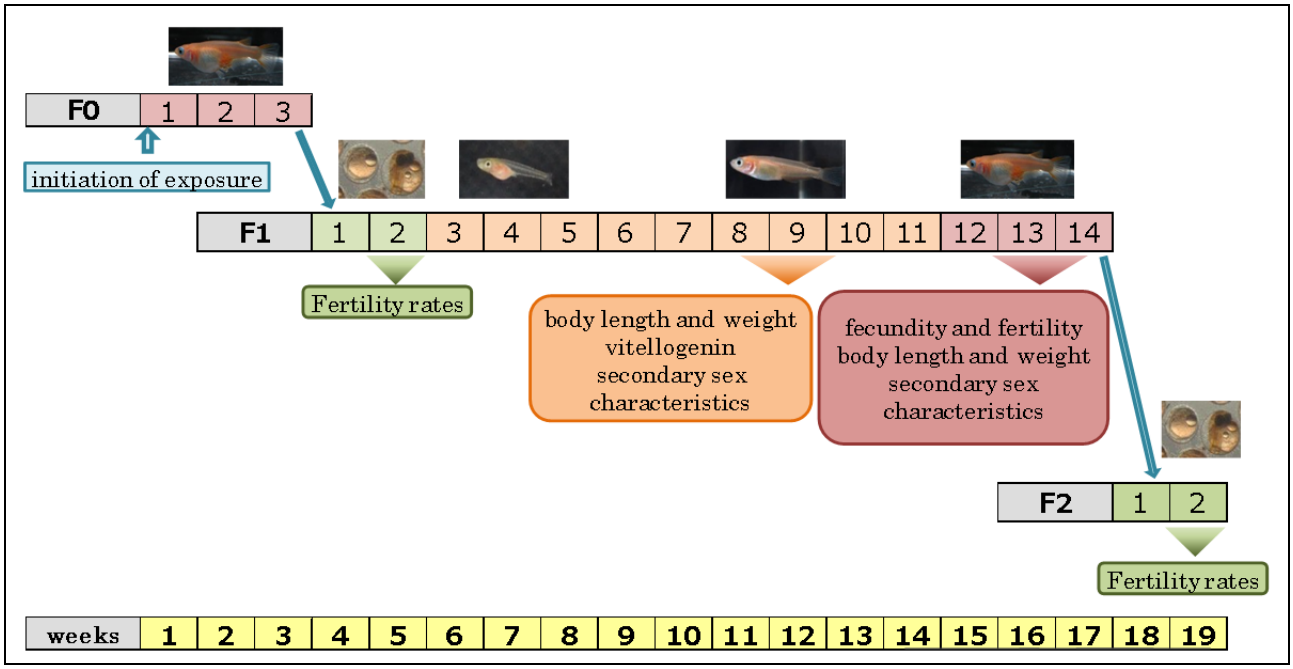
### 2. Medaka Extended One-Generation Reproductive Toxicity Test (OECD TG240)

Medaka Extended One-Generation Reproductive Toxicity Test (MEOGRT) uses five chemical concentrations plus control(s). Six replicate test chambers per chemical concentration are used, and twelve replicates are set as control (and solvent control). During the reproductive phase of F1 generation, the number of replicates in the controls are doubled (24 replicates) or quadrupled (48 replicates). Water temperature is maintained at 25 (±1) °C and a 16 h light:8 h dark photoperiod is applied over the exposure period. Fish are fed with ration (24-hour-old nauplii of brine shrimp, *Artemia* spp.) two or three times daily during the exposure period.

The test starts with a female-male pair of sexually reproductive individuals (Medaka) more than 12 weeks post fertilization (wpf) per chamber. After 3 weeks of exposure on this parental generation (F0), fertilized eggs are collected on the first day of Test Week 4 as the first next generation (F1), and exposure on F1 generation is initiated (20 eggs per chamber). Fish density of F1 is adjusted to 12 fish per chamber after hatching. At Test Weeks 8 to 9, genetic sex of every fish

is determined, and randomly selected XX-XY breeding pairs (12 pairs per chemicals concentration and 24 pairs in controls) are further exposed. The remaining non-paired fish are subjected to endpoint measurements. During Test Weeks 12 through 14, spawned eggs of each pair are collected daily for 21 consecutive days and assessed for fecundity and fertility. On the first day of Test Week 15, fertilized eggs are collected as the second next generation (F2), and exposure on F2 generation is initiated. After initiation of F2 exposure, each pair of F1 generation is subjected to endpoint measurements. Exposure is terminated when hatching of F2 generation is observed. The total exposure duration is 19 weeks from F0 generation.

Endpoints of F1 and F2 generations are hatching success and time to hatch of fertilized eggs. Endpoints of F1 are survival rates until 4 weeks post fertilization (wpf); survival rates, growth (length and weight), vitellogenin (mRNA and/or protein level), secondary sex characteristics (papillary processes of anal fin papillae), external sex ratio, and time to first spawn at 9-10 wpf (at the sub-adult sampling); reproduction (fecundity and fertility) at 12-14 wpf; and survival rates, growth, secondary sex characteristics, and histopathological observation (kidney, liver and gonad) of adult fish at 15 wpf (at the end of reproductive phase). These endpoints data are analyzed separately for each genotypic sex for the calculation of mean values and other statistical analysis.

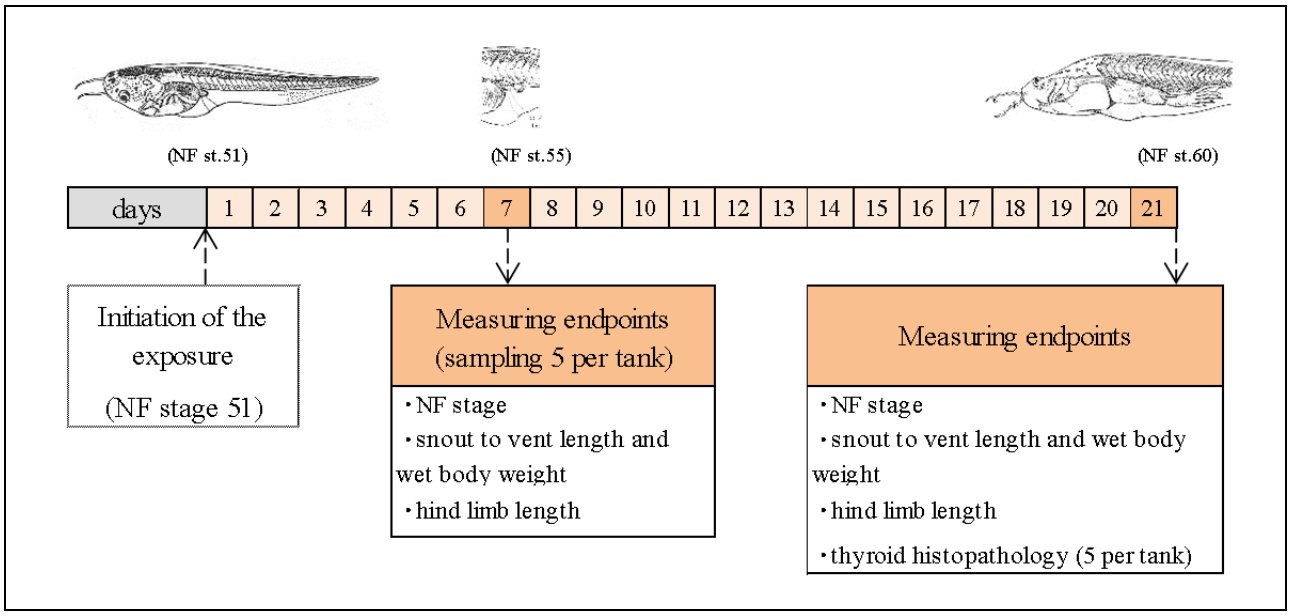



The test design of Medaka Extended One-Generation Reproductive Toxicity Test

### 3. Amphibian Metamorphosis Assay (OECD TG231)

In Amphibian Metamorphosis Assay (AMA), Nieuwkoop and Faber (NF) stage 51 *Xenopus laevis* tadpoles are used as test animal, and the duration of chemical exposure is for 21 days. On day

7 of exposure, five tadpoles are removed from each test tank to determine developmental stage, snout to vent length, hind limb length, and body weight. At test termination, all the remaining survived animals are assessed in the same manner as done on day 7. Additionally, animals selected for thyroid histopathology (5 from each tank) is evaluated for the frequency and severity of abnormalities to assess effects of the test chemical on the hypothalamic-pituitary-thyroid axis.




The test design of Amphibian Metamorphosis Assay (AMA)

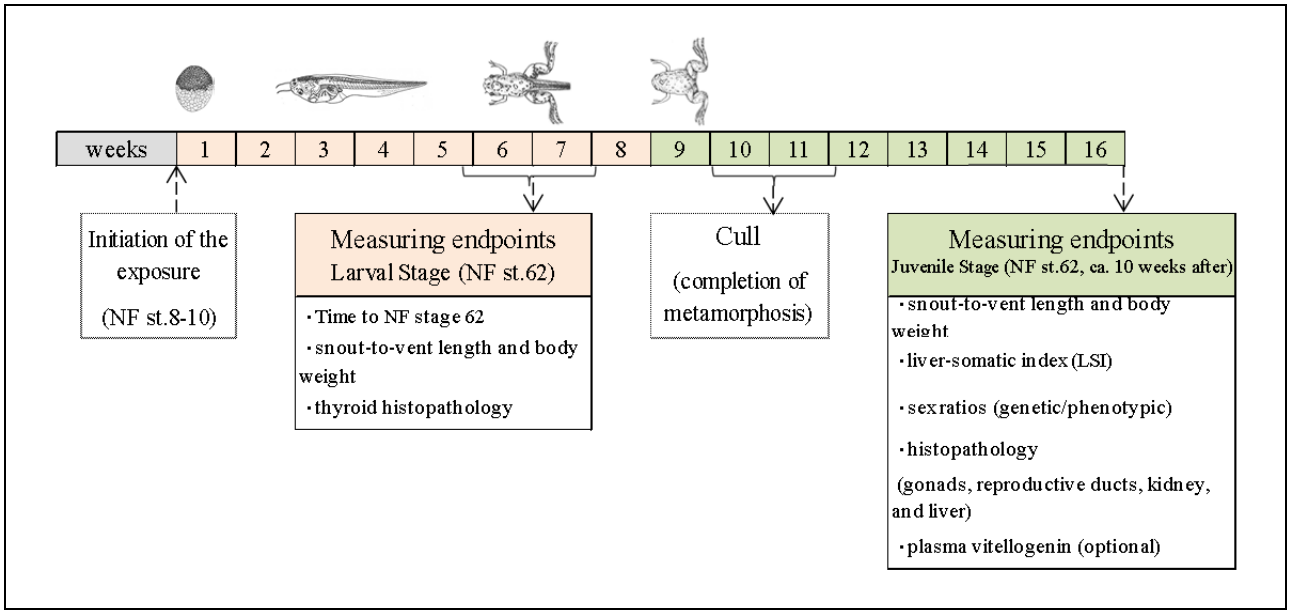
## 4. Larval Amphibian Growth & Development Assay (OECD TG241)

In Larval Amphibian Growth & Development Assay (LAGDA), *Xenopus laevis* tadpoles are used as test animal. A minimum of four chemical concentrations and appropriate controls (including solvent controls, if necessary) are used. Four replicate tanks per test concentration and eight replicates for the controls (and solvent control, if needed) are used. Water temperature is maintained at 21 (±1) °C and a 12 h light: 12 h dark photoperiod is applied over the entire exposure period. Animals are fed a mixture of formula feed and algae, live larval *Artemia*, etc, daily during exposure period.

The exposure begins with newly spawned NF stage 8 -10 *Xenopus laevis* embryos (20 embryos per tank). After ca. 5 weeks from the assay initiation, NF stage of each animal are observed and the number of animals that reached NF stage 62 are recorded on each day of exposure. A portion of animals at NF stage 62 are sampled for the measurement of Larval Stage endpoints. Remaining animals that reached NF stage 62 are separated from other larvae within a same tank and exposure is continued. After all animals have reached NF stage 66 (completion of metamorphosis) or after 70 days from the assay initiation, a cull is conducted to reduce the number of animals to 5

per tank. The remaining animals continue exposure until 10 weeks after the median time to NF stage 62 in the control. At test termination, all surviving animals are sampled for the measurement of Juvenile Stage endpoints.

Endpoints for Larval Stage are time (day) to NF stage 62, thyroid histology and growth (snout-to-vent length and body weight). Endpoints for Juvenile Stage are growth (snout-to-vent length and body weight), liver-somatic index (LSI), sex ratios (gap between genetic and phenotypic), histopathology (gonads, reproductive ducts, kidney, and liver), and plasma vitellogenin (optional). For the measurement of Juvenile Stage endpoints, genetic sex is also assessed for each animal. These endpoints data should be analyzed separately for each genotypic sex for the calculation of mean values and other statistical analysis.



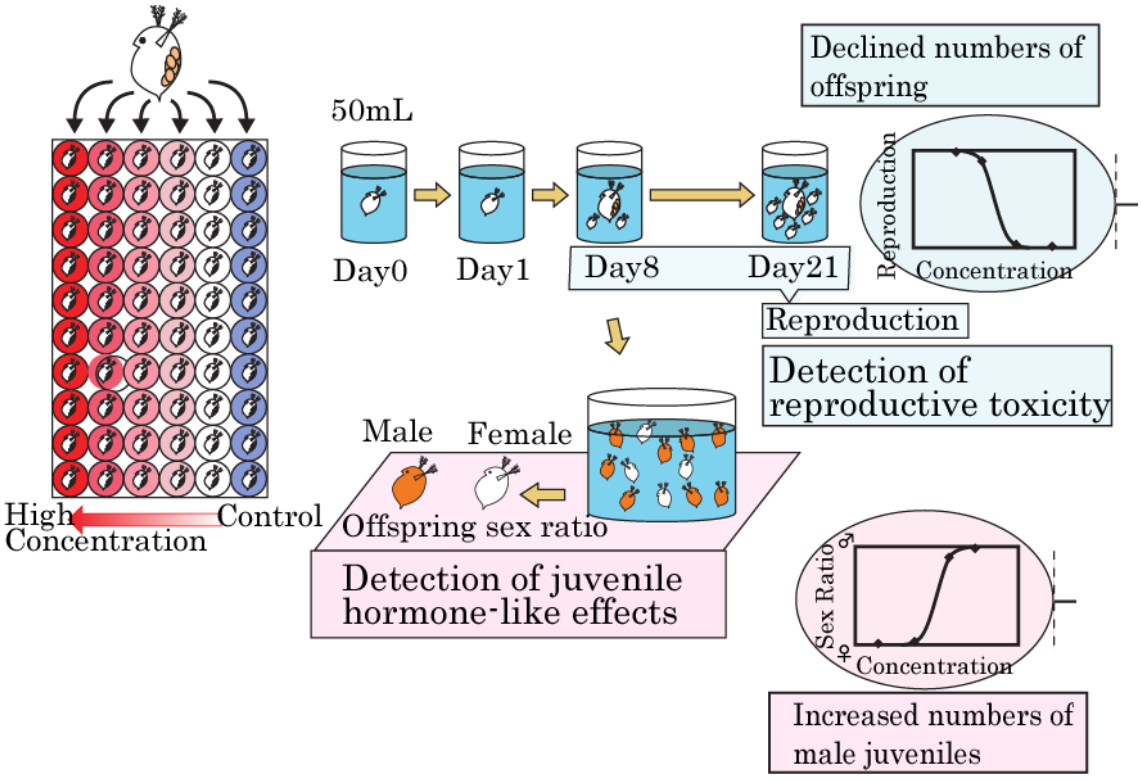

The test design of Larval Amphibian Growth & Development Assay (LAGDA)

## 5. *Daphnia magna* Reproduction Test (OECD TG211)

In *Daphnia magna* Reproduction Test, *Daphnia magna* is used as test animal. A minimum of five chemical concentrations (arranged in a geometric series with a separation factor preferably not exceeding 3.2) and appropriate controls (including solvent controls, if necessary) are used. Ten replicate vessels are used for both the treatment and the control groups (and solvent control, if needed). Water temperature is maintained at 21 (±1) °C and 16 h light: 8 h dark photoperiod is applied. During the exposure period, animals are fed green algae daily and medium water is renewed every other day. At the start of the test, animals less than 24 hours old are exposed. From the next day, mortality and number of molting of parent animals as well as presence of aborted eggs

or dead offspring are recorded. Parent animals grow by molting repeatedly, almost once a day. After 6-8 days from the start of exposure, first brood is produced. Thereafter molting and offspring production are repeated every 2-3 days. The offspring produced should be removed daily from the vessel and counted. If ANNEX 7 is additionally performed to detect juvenile hormone effects, males can be distinguished from females by the length and morphology of the first antennae under a stereomicroscope, and the number of offspring and sex ratio are the endpoints after the termination of exposure.

For endpoints data such as number of living offspring, sex ratio, mortality and number of molting of parent animals, and presence of aborted eggs or dead offspring, NOEC and LOEC or ECx (if necessary to represent efficacy of an effect) values should be calculated evaluating statistically significant differences from control.




The test design of invertebrate testing (*Daphnia magna* Reproduction Test)

## Appendix 6 Overview of Detection of Chemicals Requested for Environmental Study (FY 2010-FY 2015)

| Year Requested (FY) | Chemicals (CAS No.) | Analytical Method Development | Survey Category of Environmental Study | Detection Limit (ng/L) * | Detected Range (ng/L) and Frequency (sites) * |
|---|---|---|---|---|---|
| 2010 | 4-*tert*-Octylphenol (140-66-9) | done | Detailed FY 2012 | 0.36 | nd-31 19/24 |
| | 2,4-Di-*tert*-butylphenol (96-76-4) | done | Initial FY 2012 | 57 | nd 0/14 |
| | 4-(2-Phenylisopropyl)-phenol (599-64-4) | done | Detailed FY 2014 | 2.5 | nd-94 10/20 |
| | *o*-Tolidine (119-93-7) | done | Initial FY 2012 | 1.6 | nd 0/14 |
| | Bisphenol A (80-05-7) | done | Detailed FY 2014 | 1.7 | nd-280 18/20 |
| | Benzophenone (119-61-9) | done | Initial FY 2012 | 4.3 | nd-38 7/25 |
| 2011 | Equilin (474-86-2) | done | Initial FY 2013 | 0.17 | nd 0/16 |
| | Chlormadinone (1961-77-9) | done | Initial FY 2013 | 0.038 | nd 0/18 |
| | Chlormadinone acetate (302-22-7) | | | 0.033 | nd-0.76 13/18 |
| | Propyl Paraben (94-13-3) | done | Initial FY 2012 | 14 | nd-16 1/16 |
| | 4-Methylbenzylidene camphor (36861-47-9) | done | Initial FY 2013 | 440 | nd 0/17 |
| 2013 | Epofenonane (57342-02-6) | done | — | — | — |
| | Oxamyl (23135-22-0) | done | — | — | — |
| | Chlorpyrifos (2921-88-2) | done | — | — | — |
| | Dimethoate (60-51-5) | done | — | — | — |
| | 4-Nonylphenol (branched) (25154-52-3) | done | Detailed FY 2014 | 18 | nd-320 ng/L 16/30 |
| 2015 | Estrone (53-16-7) | — | — | — | — |
| | 2-Ethoxyethanol (110-80-5) | — | — | — | — |
| | 4-Vinyl-1-cyclohexene (100-40-3) | — | — | — | — |
| | Phenytoin (57-41-0) | — | — | — | — |
| | Triphenyl phosphate (115-86-6) | — | — | — | — |

*: in surface water
nd: below detection limit
—: underway

## Appendix 7 Progress of Reliability Evaluation and Tests (FY)

| No. | Chemicals | CAS | Reliability Evaluation | Tier 1 | | | Tier 2 |
|---|---|---|---|---|---|---|---|
| | | | | *in vitro* | *in vivo* | Assessment | *in vivo* |
| 1 | Acrylamide | 79-06-1 | 2010 | 2012 | | | |
| 2 | Acrylic acid | 79-10-7 | 2010 | | | | |
| 3 | Acrylonitrile | 107-13-1 | 2015 | | | | |
| 4 | Acrolein | 107-02-8 | 2011 | | | | |
| 5 | Adipic acid | 124-04-9 | 2009 | | | | |
| 6 | Acetaldehyde | 75-07-0 | 2013 | | | | |
| 7 | Atrazine | 1912-24-9 | 2011 | 2013<br>2015 | | | |
| 8 | Alachlor | 15972-60-8 | 2010 | 2012 | | | |
| 9 | EPN | 2104-64-5 | 2009 | | | | |
| 10 | Estrone | 53-16-7 | 2008 | 2011 | 2011 | | |
| 11 | 2-Ethylhexanoic acid | 149-57-5 | 2015 | | | | |
| 12 | Ethylbenzene | 100-41-4 | 2014 | | | | |
| 13 | Ethylene oxide | 75-21-8 | 2015 | | | | |
| 14 | Ethylene glycol monoethyl ether | 110-80-5 | | | | | |
| 15 | Ethylene glycol monomethyl ether | 109-86-4 | | | | | |
| 16 | Ethylenediaminetetraacetic acid | 60-00-4 | 2015 | | | | |
| 17 | Epichlorohydrin | 106-89-8 | 2012 | | | | |
| 18 | Vinyl chloride monomer | 75-01-4 | 2012 | | | | |
| 19 | Methyl chloride | 74-87-3 | 2015 | | | | |
| 20 | Octabromodiphenyl ether | 32536-52-0 | 2015 | | | | |
| 21 | 4-*tert*-Octylphenol | 140-66-9 | 2014 | 2008<br>2015 | 2014 | | |
| 22 | Hydronium perchlorate | 7601-90-3 | 2013 | 2014 | | | |
| 23 | Carbaryl | 63-25-2 | 2009 | 2011<br>2015 | | | |
| 24 | Carbendazim | 10605-21-7 | 2014 | 2015 | | | |
| 25 | Carbofuran | 1563-66-2 | 2009 | 2011, 2015 | | | |
| 26 | Xylenes | *o*-Xylene (95-47-6)<br>*m*-Xylene (108-38-3)<br>*p*-Xylene (106-42-3) | 2012 | | | | |
| 27 | Glyphosate | 1071-83-6 | 2013 | | | | |
| 28 | Cresols | *o*-Cresol (95-48-7)<br>*m*-Cresol (108-39-4)<br>*p*-Cresol (106-44-5) | 2012 | | | | |
| 29 | Chlorpyrifos | 2921-88-2 | | | | | |
| 30 | Chlorothalonil (TPN) | 1897-45-6 | 2015 | | | | |
| 31 | Chlorobenzene | 108-90-7 | 2012 | | | | |
| 32 | Chloroform | 67-66-3 | 2012 | | | | |
| 33 | 2-Ethoxyethanol acetate | 111-15-9 | 2014 | | | | |
| 34 | Chlormadinone acetate | 302-22-7 | 2015 | | | | |
| 35 | Cyanazine | 21725-46-2 | 2009 | 2011 | 2011 | | |
| 36 | Diuron | 330-54-1 | 2009 | 2011<br>2015 | | | |
| 37 | Diethylene glycol | 111-46-6 | 2011 | | | | |
| 38 | Carbon tetrachloride | 56-23-5 | 2012 | | | | |
| 39 | Dichlorvos | 62-73-7 | 2009 | 2011 | | | |
| 40 | 3,4-Dichloroaniline | 95-76-1 | 2014 | | | | |
| 41 | 1,2-Dichloroethane | 107-06-2 | 2015 | | | | |
| 42 | 1,1-Dichloroethylene (Vinylidene chloride) | 75-35-4 | 2015 | | | | |
| 43 | Dichloroacetic acid | 79-43-6 | 2014 | | | | |
| 44 | 2,4-Dichlorophenoxyacetic Acid (2,4-D, 2,4-PA) | 94-75-7 | 2010 | 2012 | | | |
| 45 | Dichlorobromomethane | 75-27-4 | 2009 | 2011<br>2015 | | | |
| 46 | *p*-Dichlorobenzene | 106-46-7 | 2008 | 2010 | | | |
| 47 | *o*-Dichlorobenzene | 95-50-1 | 2008 | | | | |
| 48 | Dichloromethane | 75-09-2 | 2012 | | | | |
| 49 | 2,4-Dinitrotoluene | 121-14-2 | 2014 | | | | |

| No. | Chemicals | CAS | Reliability Evaluation | Tier 1 | | | Tier 2 |
|---|---|---|---|---|---|---|---|
| | | | | *in vitro* | *in vivo* | Assessment | *in vivo* |
| 50 | 2,4-Dinitrophenol | 51-28-5 | 2012 | 2013 | | | |
| 51 | Dinocap | 131-72-6 | 2010 | | | | |
| 52 | Dibromochloromethane | 124-48-1 | 2015 | | | | |
| 53 | Simazine | 122-34-9 | 2012 | 2013 | | | |
| 54 | *N,N'*-Dimethylformamide | 68-12-2 | 2008 | 2010 | | | |
| 55 | Dimethoate | 60-51-5 | | | | | |
| 56 | Ziram | 137-30-4 | 2015 | | | | |
| 57 | Styrene | 100-42-5 | 2015 | | | | |
| 58 | Spinosad | Mixture of Spinosyn A (131929-60-7) and Spinosyn D (131929-63-0) | 2015 | | | | |
| 59 | Diazinon | 333-41-5 | 2009 | 2011<br>2015 | 2015 | | |
| 60 | Thiuram | 137-26-8 | 2012 | | | | |
| 61 | Thiourea | 62-56-6 | 2015 | | | | |
| 62 | Thiobencarb | 28249-77-6 | 2012 | | | | |
| 63 | Linear alkylbenzene sulfonic acids and their salts (C=10-14) | C-10 (31093-47-4 or 1322-98-1)<br>C-11 (27636-75-5)<br>C-12 (25155-30-0)<br>C-13 (26248-24-8)<br>C-14 (28348-61-0)<br>etc. | 2008 | | | | |
| 64 | Decabromodiphenyl ether (PBDE#209) | 1163-19-5 | 2012 | 2013<br>2015 | | | |
| 65 | Tetrachloroethylene | 127-18-4 | 2012 | | | | |
| 66 | Tetrachlorobenzenes | 1,2,4,5-Tetrachloro-benzene (95-94-3)<br>1,2,3,4-Tetrachloro-benzene (634-66-2)<br>1,2,3,5-Tetrachloro-benzene (634-90-2) | 2010 | | | | |
| 67 | Tetrabromobisphenol A | 79-94-7 | 2010 | 2012 | | | |
| 68 | Tebuconazole | 107534-96-3 | 2015 | | | | |
| 69 | Tebufenozide | 112410-23-8 | 2015 | | | | |
| 70 | Triclosan | 3380-34-5 | 2014 | 2015 | | | |
| 71 | Trichlorphene (DEP) | 52-68-6 | | | | | |
| 72 | 1,1,1-Trichloroethane | 71-55-6 | 2012 | | | | |
| 73 | Trichloroethylene | 79-01-6 | 2012 | | | | |
| 74 | Trichloroacetic acid | 76-03-9 | 2014 | 2015 | | | |
| 75 | 1,2,3-Trichloropropane | 96-18-4 | 2012 | | | | |
| 76 | Trichlorobenzenes | 1,2,3-Trichloro-benzene (87-61-6)<br>1,2,4-Trichloro-benzene (120-82-1)<br>1,3,5-Trichloro-benzene(108-70-3) | 2010 | | | | |
| 77 | Trifluralin | 1582-09-8 | 2008 | | | | |
| 78 | 2,4,6-Tribromophenol | 118-79-6 | 2008 | 2010<br>2012 | | | |
| 79 | Toluene | 108-88-3 | 2012 | | | | |
| 80 | 2,4-Diaminotoluene | 95-80-7 | 2008 | 2010 | | | |
| 81 | Naphthalene | 91-20-3 | 2010 | 2012 | | | |
| 82 | 1-Naphthol | 90-15-3 | 2011 | 2012 | 2012 | | |
| 83 | Nitrobenzene | 98-95-3 | 2013 | | | | |
| 84 | Carbon disulfide | 75-15-0 | 2013 | 2014 | | | |
| 85 | 4-Nonylphenol (branched) | 84852-15-3 | 2014 | 2008<br>2015 | 2014 | | 2015 |
| 86 | Bisphenol A | 80-05-7 | 2015 | 2008<br>2015 | 2015 | | |
| 87 | Hydrazine | 302-01-2 | 2008 | 2010 | | | |
| 88 | Propyl 4-hydroxybenzoate | 94-13-3 | 2015 | | | | |

| No. | Chemicals | CAS | Reliability Evaluation | Tier 1 | | | Tier 2 |
|---|---|---|---|---|---|---|---|
| | | | | *in vitro* | *in vivo* | Assessment | *in vivo* |
| | (Propyl Paraben) | | | | | | |
| 89 | Methyl 4-hydroxybenzoate | 99-76-3 | 2012 | 2013 | 2014 | | |
| 90 | Hydroquinone | 123-31-9 | 2012 | | | | |
| 91 | 4-Vinyl-1-cyclohexene | 100-40-3 | | | | | |
| 92 | Fipronil | 120068-37-3 | 2014 | 2015 | | | |
| 93 | Phenanthrene | 85-01-8 | 2009 | | | | |
| 94 | Phenytoin | 57-41-0 | 2009 | 2011 | 2011 | | |
| 95 | Fenitrothion | 122-14-5 | 2009 | 2011<br>2015 | | | |
| 96 | Phenol | 108-95-2 | 2012 | 2013<br>2015 | | | |
| 97 | Phenobarbital | 50-06-6 | 2009 | 2012 | | | |
| 98 | Fenthion | 55-38-9 | 2008 | 2010<br>2011 | | | |
| 99 | Fenvalerate | 51630-58-1 | 2013 | 2014 | 2015 | | |
| 100 | Butachlor | 23184-66-9 | 2015 | | | | |
| 101 | 1-Butanol | 71-36-3 | 2009 | | | | |
| 102 | Diisobutyl phthalate | 84-69-5 | 2014 | 2015 | | | |
| 103 | Dimethyl phthalate | 131-11-3 | 2010 | | | | |
| 104 | 2,6-Di-*tert*-butyl-4-methylphenol (BHT) | 128-37-0 | 2011 | 2012 | | | |
| 105 | 2-Butoxyethanol (Ethylene glycol monobutyl ether) | 111-76-2 | 2015 | | | | |
| 106 | Fluoranthene | 206-44-0 | 2015 | | | | |
| 107 | Flutamide | 13311-84-7 | 2013 | 2014 | | | |
| 108 | Procymidone | 32809-16-8 | 2015 | | | | |
| 109 | 2-Propanol | 67-63-0 | 2011 | | | | |
| 110 | Propiconazole | 60207-90-1 | 2015 | | | | |
| 111 | 2-Bromopropane | 75-26-3 | 2015 | | | | |
| 112 | 1-Bromopropane | 106-94-5 | 2015 | | | | |
| 113 | 1,2,5,6,9,10-Hexabromo-cyclododecanes | 3194-55-6<br>etc. | later excluded | | | | |
| 114 | Benomyl | 17804-35-2 | 2014 | 2015 | | | |
| 115 | Pentadecafluorooctanoic acid | 335-67-1 | 2009 | 2011<br>2015 | | | |
| 116 | Perfluorododecanoic acid | 307-55-1 | 2015 | | | | |
| 117 | Benzyl alcohol | 100-51-6 | 2009 | | | | |
| 118 | Benzene | 71-43-2 | 2012 | | | | |
| 119 | 4-*tert*-Amylphenol | 80-46-6 | 2011 | 2012<br>2015 | 2012 | | |
| 120 | Formaldehyde | 50-00-0 | 2012 | | | | |
| 121 | Manzeb (Mancozeb) | 8018-01-7 | | | | | |
| 122 | Maneb | 12427-38-2 | | | | | |
| 123 | Methomyl | 16752-77-5 | 2011 | 2012 | | | |
| 124 | Methyl methacrylate | 80-62-6 | 2009 | | | | |
| 125 | Methyl *tert*-butyl ether | 1634-04-4 | 2015 | | | | |
| 126 | 2-Methylpropan-2-ol (*tert*-Butyl Alcohol) | 75-65-0 | 2015 | | | | |
| 127 | Metolachlor | 51218-45-2 | 2015 | | | | |
| 128 | Mercaptoacetic acid | 68-11-1 | 2010 | | | | |
| 129 | Molinate | 2212-67-1 | 2010 | 2012 | | | |
| 130 | Linuron | 330-55-2 | | | | | |
| 131 | Tricresyl phosphate | 1330-78-5 | 2013 | 2014 | | | |
| 132 | Triphenyl phosphate | 115-86-6 | 2010 | 2012<br>2013 | 2012 | | |

## Appendix 8 Overview of Public Seminars on Endocrine Disrupting Effects of Chemical Substances (EXTEND2010) Part 1

| Date | Venue | Brief Summary | Total Number of Participants |
|---|---|---|---|
| FY 2010<br><br>15 December 2010 (Wednesday) | Large Conference Room, Sanjo Conference Hall, University of Tokyo | A Public Seminar was held to provide information to experts and general public the results achieved in ExTEND2005 on researches and studies on endocrine disrupting effects of chemicals substances, and to publicize EXTEND2010. | 114 |
| FY 2011<br><br>3 December 2011 (Saturday) | International Conference Hall, Plaza Heisei, 3rd Floor, Tokyo International Exchange Center | The history of research on endocrine disrupting effects of chemicals substances, current status of actions in EXTEND2010, and most recent study achievements under EXTEND2010 were presented. Overseas information was also provided by foreign invited speakers. | 137 |
| FY 2012<br><br>17 December 2012 (Monday) | The Hamarikyu Asahi Hall, Small Hall | Current status of actions in EXTEND2010, progress of related studies, and overseas activities were introduced with a focus on “how to assess the endocrine disrupting effects on animals?” | 75 |
| FY 2013<br><br>13 December. 2013 (Friday) | International Conference Hall, Plaza Heisei, 3rd Floor, Tokyo International Exchange Center | Experts from U.K. gave presentations on the current status of actions in Europe and achievements in Japan-UK joint research regarding endocrine disrupting effects of chemicals substances. Achievements in related studies in Japan were also reported. | 92 |
| FY 2014<br><br>15 January 2015 (Thursday) | Small Hall , The Hamarikyu Asahi Hall | Invited speakers from U.S. and France gave presentations on current status of actions in Europe and United States regarding endocrine disrupting effects of chemicals substances. Current status of test method developments and other actions in Japan were also reported. | 96 |
| FY 2015<br><br>20 August 2015 (Thursday) | International Conference Hall, Plaza Heisei, 3rd Floor, Tokyo International Exchange Center | Two invited speakers from U.S. gave presentations on current status of actions regarding endocrine disrupting effects of chemicals substances from the expert viewpoint. Actions in Europe and Japan were also reported. | 120 |

## Appendix 8 Overview of Public Seminars on Endocrine Disrupting Effects of Chemical Substances (EXTEND2010) Part 2

| Date | Program |
|---|---|
| FY 2010<br><br>15 December 2010 (Wednesday)<br>10:00-16:40 | 10:00 Opening address (Ministry of the Environment, Japan)<br>10:10 Part I Actions in ExTEND2005<br>• Overview of researches and studies under ExTEND2005 (Ministry of the Environment, Japan)<br>• Presentation of results<br>- Collection and analysis of basic information related to abnormal sex differentiation of wild Medaka.<br>Satoshi Hamaguchi (Niigata University)<br>- Studies on mechanism of endocrine disrupting effects of chemicals on reproductive endocrine system in Medaka.<br>Yoshitaka Nagahama (National Institute for Basic Biology, National Institutes of Natural Sciences)<br>- A mechanism of nuclear hormone receptor-mediated transcriptional regulation by environmental chemicals<br>Toshiharu Iwasaki (Gunma University)<br>- Analysis of genetic factors of experimental animals influencing toxicological test results in mammals<br>Hiroaki Aoyama (Institute of Environmental Toxicology)<br>- Environmental contamination and fate of estrogenic compounds from municipal effluents<br>Hiroaki Tanaka (Kyoto University)<br>13:30 Part II Further direction of EXTEND2010 and actions to endocrine disrupting effects<br>• Further actions to endocrine disrupting effects of chemical substances EXTEND2010- (Ministry of the Environment, Japan)<br>• Achievements and problems in developing biological test methods<br>Norihisa Tatarazako (National Institute for Environmental Studies)<br>• Trends of researches and studies on endocrine disrupting effects on animals and its desirable directions of further actions.<br>Taisen Iguchi (National Institute for Basic Biology, National Institutes of Natural Sciences)<br>• Trends of researches and studies on endocrine disrupting effects on humans and its desirable directions of further actions.<br>Chiharu Tohyama (University of Tokyo)<br>15:25 Panel Discussion<br>Coordinator: Masaru Kitano (Meiji University)<br>Panelists: Yoshiko Arita (SHUFUREN)<br>Taisen Iguchi (National Institute for Basic Biology, National Institutes of Natural Sciences)<br>Fumiaki Shono (Japan Chemical Industry Association)<br>Norihisa Tatarazako (National Institute for Environmental Studies)<br>Chiharu Tohyama (University of Tokyo)<br>Teruyoshi Hayamizu (Ministry of the Environment, Japan)<br>16:40 Closing address (Ministry of the Environment, Japan) |
| FY 2011<br><br>3 December 2011 (Saturday)<br>10:00-17:00 | 10:00 Opening address (Ministry of the Environment, Japan)<br>10:10 History and current status of the issues surrounding endocrines disrupting effects of chemical substances.<br>• Looking back the issues regarding endocrine disrupting effect. Hiroaki Aoyama (Institute of Environmental Toxicology)<br>• What do endocrine systems and endocrine disrupting effects refer? Norihisa Tatarazako (National Institute for Environmental Studies)<br>• Current status of MOE's actions (Ministry of the Environment, Japan) |

| | |
|---|---|
| | 13:30 Results of researches and studies under EXTEND2010<br>• Overview of research and study projects under EXTEND2010 (Ministry of the Environment, Japan)<br>13:40 Presentations of research and study achievements<br>- Survey on the declining population of Dragonfly *Sympetrum frequens* and elucidation of its causes.<br>Tetsuyuki Ueda (Ishikawa Prefectural University)<br>- Studies on toxicogenomics of marine invertebrate Ascidiacea<br>Kaoru Azumi (Hokkaido University)<br>- Contamination of wildlife by bioaccumulative potential endocrine disrupting chemicals: Status of contamination by emerging POPs in Japan and Asia<br>Shinsuke Tanabe (Ehime University)<br>15:30 Presentations from overseas<br>- The U.S. EPA's Endocrine Disruptor Screening Program (EDSP)<br>Dr. Leslie Touart (Office of Science Coordination and Policy, U.S. Environmental Protection Agency)<br>- Environmental Risk Assessment & Endocrine Disrupter Research – A European Update<br>Professor Thomas Hutchinson (Centre for Environment, Fisheries and Aquaculture Science, U.K.)<br>16:50 Closing address (Ministry of the Environment, Japan) |
| FY 2012<br><br>17 December 2012 (Monday)<br>13:30-17:00 | 13:30 Opening address (Ministry of the Environment, Japan)<br>13:40 Current status of MOE's actions to endocrine disrupting effects of chemicals substances (Ministry of the Environment, Japan)<br>14:25 Can we measure endocrine disrupting effects using frog, medaka, or *Daphnia*?<br>Norihisa Tatarazako (National Institute for Environmental Studies)<br>15:25 Biological effects caused by endocrine disrupting chemicals in Japanese coastal areas: research on coastal marine organisms<br>Kiyoshi Soyano (Nagasaki University Graduate School)<br>16:10 16 Years after outbreak of the endocrine disruptor issue: current status of actions overseas including Europe, the United States, and Australia<br>Taisen Iguchi (National Institutes of Natural Sciences)<br>16:55 Closing address (Ministry of the Environment, Japan) |
| FY 2013<br><br>13 December 2013 (Friday)<br>13:00-16:30 | 13:00 Opening address (Ministry of the Environment, Japan)<br>13:10 Present State of the Ministry of the Environment's Program on Endocrine Disruption (Ministry of the Environment, Japan)<br>13:30 Dealing with Potential Endocrine Disrupting Chemicals - the development of regulatory approaches in Europe<br>Dr. Mike Roberts (Department for Environment, Food and Rural Affairs, UK)<br>14:10 Understanding the Importance of Endocrine Disruption in Fish<br>Prof. Charles Tyler (University of Exeter, UK)<br>15:10 Mechanism of Endocrine Disruption in *Daphnia magna*<br>Taisen Iguchi (National Institutes of Natural Sciences, Japan)<br>15:50 Identification of trophic factors involved in hyperactive disorders and its disruption by environmental chemicals<br>Noriyuki Koibuchi (Department of Integrative Physiology, Gunma University)<br>16:30 Closing address (Ministry of the Environment, Japan) |
| FY 2014<br><br>15 January 2015 (Thursday)<br>13:00-16:30 | 13:00 Opening address (Ministry of the Environment, Japan)<br>13:10 Fresh News about Endocrine Disruption Issues in Europe<br>Dr. Dominique Gombert (French Agency for Food, Environmental and Occupational Health & Safety)<br>14:00 US EPA Endocrine Disruptor Screening Program: Use of Computational Approaches in Prioritization and Screening<br>Dr. Scott Lynn (Environmental Protection Agency, United States)<br>15:10 Update on Japanese program on endocrine disruption<br>Takuma Kato (Environmental Health and Safety Division, Ministry of the Environment, Japan) |

| | |
|---|---|
| | 15:25 Current Progress of Testing Methods for Assessment of Potential Endocrine Disrupting Chemicals in Japan<br>Taisen Iguchi (National Institutes of Natural Sciences, Japan)<br>16:00 Development of Screening System for Thyroid Hormone Disrupting Substances Using Xenopus Metamorphosis Assay<br>Akihiko Kashiwagi (Graduate School of Science, Hiroshima University, Japan)<br>16:30 Closing address (Ministry of the Environment, Japan) |
| FY 2015<br><br>20 August 2015 (Thursday)<br>13:00-16:45 | 13:00 Opening address (Ministry of the Environment, Japan)<br>13:05 DOHaD: A Good Start Lasts a Lifetime<br>Dr. Linda Birnbaum (National Institute of Environmental Health Sciences, United States)<br>13:55 Overview of Some Key Past and Current U.S. EPA Studies on Endocrine Disrupting Chemicals (EDCs)<br>Dr. James M. Lazorchak (Environmental Protection Agency, United States)<br>15:05 Introduction of the Concept of "Signal Toxicity" for the Strategic Planning of Research on Endocrine Disrupting Chemicals Issues and related "Low-Dose, Early Exposure-Late Effects"-type Toxicity.<br>Jun Kanno (National Institute of Health Sciences, Japan)<br>15:55 EU Conference on Endocrine Disruptors Criteria for Identification and Related Impacts, Brussels, 1st June 2015<br>Yukio Kawashima (Japan NUS Co., Ltd.)<br>16:25 Present State of the Ministry of the Environment's Program on Endocrine Disruption<br>Takuma Kato (Ministry of the Environment, Japan)<br>16:40 Closing address (Ministry of the Environment, Japan) |

## Appendix 9 OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012)

<table>
<tr><td colspan="3">Mammalian and Non-Mammalian Toxicology</td></tr>
<tr><td>Level 1<br>Existing Data and Non-Test Information</td><td colspan="2">• Physical & chemical properties, e.g., MW reactivity, volatility, biodegradability<br>• All available (eco)toxicological data from standardized or non-standardized tests.<br>• Read across, chemical categories, QSARs and other in silico predictions, and ADME model predictions</td></tr>
<tr><td>Level 2<br>In vitro assays providing data about selected endocrine mechanism(s) / pathways(s) (Mammalian and non-mammalian methods)</td><td colspan="2">• Estrogen or androgen receptor binding affinity (OECD TG493)<br>• Estrogen receptor transactivation (OECD TG455)<br>• Androgen or thyroid transactivation (OECD TG458)<br>• Steroidogenesis in vitro (OECD TG456)<br>• Other assays as appropriate</td></tr>
<tr><td rowspan="2">Level 3<br>In vivo assays providing data about selected endocrine mechanism(s) / pathway(s)</td><td>Mammalian Toxicology</td><td>Non-Mammalian Toxicology</td></tr>
<tr><td>• Uterotrophic assay (OECD TG440)<br>• Hershberger assay (OECD TG441)</td><td>• Fish short term reproduction assay (FSTRA) (OECD TG229)<br>• 21-Days fish screening assay (OECD TG230)<br>• Androgenized female stickleback screen (GD140)<br>• Xenopus embryo thyroid signaling assay<br>• Amphibian metamorphosis assay (AMA) (OECD TG231)</td></tr>
<tr><td>Level 4<br>In vivo assays providing data on adverse effects on endocrine relevant endpoints</td><td>• Repeated dose 28-day study (OECD TG407)<br>• Repeated dose 90-day study (OECD TG408)<br>• 1-Generation reproduction toxicity study (OECD TG415)<br>• Male pubertal assay (GD 150, Chapter C4.3)<br>• Female pubertal assay (GD 150, Chapter C4.4)<br>• Intact adult male endocrine screening assay (GD 150, Chapter Annex 2.5)<br>• Prenatal development toxicity study (OECD TG414 if enhanced)<br>• Chronic toxicity and carcinogenicity studies (OECD TG451-3)</td><td>• Fish sexual development test (OECD TG234)<br>• Fish reproduction Partial Lifecycle Test<br>• Larval amphibian growth and development assay (LAGDA) (OECD TG241)<br>• Avian reproduction assay (OECD TG206)<br>• Daphnia reproduction test (with male induction) (OECD TG211)<br>• Mollusc partial lifecycle assays (OECD TG242 and TG243)<br>• Chironomid toxicity test (TG218 and TG219)<br>• Earthworm reproduction test (OECD TG222)<br>• Enchytraeid reproduction test (OECD TG220)</td></tr>
</table>

| | | |
|---|---|---|
| | • Reproductive screening test (OECD TG421)<br>• Combined 28-day/reproductive screening assay (OECD TG422)<br>• Developmental neurotoxicity (OECD TG426) | • Sediment water Lumbriculus toxicity test using spiked sediment (OECD TG225)<br>• Predatory mite reproduction test in soil (OECD TG226)<br>• Collembolan reproduction test in soil (OECD TG232) |
| Level 5<br>*In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism | • Extended one-generation reproductive toxicity study (OECD TG443)<br>• 2-Generation reproduction toxicity study (OECD TG416 most recent update) | • Medaka extended one-generation reproduction test (MEOGRT) (OECD TG240)<br>• Sediment water chironomid life cycle toxicity test (OECD TG233)<br>• Daphnia multi-generation assay<br>• Mollusc full lifecycle assays |

# Appendix 10 Major Published Papers (FY2010-FY2015)

## 1. Publications from Research for Biological Observation of Wildlife, Fundamental Studies, Feasibility Studies and Other Related Studies


2010

Alam, M.S., Ohsako, S., Matsuwaki, T., Zhu, X.B., Tsunekawa, N., Kanai, Y., Sone, H., Tohyama, C. and Kurohmaru, M. Induction of spermatogenic cell apoptosis in prepubertal rat testes irrespective of testicular steroidogenesis: A possible estrogenic effect of di(*n*-butyl) phthalate. *Reproduction,* 139, 427-437 (2010).

Alam, M.S., Ohsako, S., Tay, T.W., Tsunekawa, N., Kanai, N. and Kurohmaru, M. Di(*n*-butyl) phthalate induces vimentin filaments disruption in rat Sertoli cells: A possible relation with spermatogenic cell apoptosis. *Anat. Histol. Embryol.,* 39, 189-193 (2010).

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## Appendix11 Members of the Task Force and its Sub-Committees

Members of the Task Force on Endocrine Disrupting Effects of Chemical Substances

| Name | Affiliation | FY |
|---|---|---|
| Yoshiko Arita | Environment Section Manager, The Federation of Consumer Organization "SHUFUREN" | 2010- |
| Taisen Iguchi | Professor, Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences | 2010- |
| Masako Ueji | Technical Adviser, Japan Plant Protection Association | 2010- |
| Ryoko Kizawa | Environment Counselor | 2015- |
| Masaru Kitano* | Professor, College of Humanities, Shukutoku University | 2010- |
| Hiroko Kono | Editor, The Yomiuri Shimbun | 2010-2012 |
| Yuko Sakita | Journalist and Environment Counselor | 2010-2014 |
| Hiroshi Satoh | Vice President, National Institute for Environmental Studies | 2010-2011 |
| Fumiaki Shono | Executive Director, Japan Chemical Industry Association | 2010- |
| Hiroaki Shiraishi | Fellow, Research Center for Environmental Risk, National Institute for Environmental Studies | 2010- |
| Shinsuke Tanabe | University Special Professor, Division of Environmental Chemistry and Ecotoxicology, Center for Marine Environmental Studies, Ehime University | 2010- |
| Chiharu Tohyama | Professor Emeritus, University of Tokyo | 2010- |
| Akira Naganuma | Professor, Graduate School of Pharmaceutical Sciences, Tohoku University | 2012- |
| Akiyoshi Nishikawa | Director, Biological Safety Research Center, National Institute of Health Sciences | 2010- |
| Chisato Mori | Professor, Department of Bioenvironmental Medicine, Graduate School of Medicine, Graduate School of Medicine, Chiba University | 2010-2012 |
| Makoto Watanabe | Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba | 2010 |

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Note: Title and affiliation at time of service.

Members of the EXTEND2010 Sub-Committee for Biological Observation of Wildlife

| Name | Affiliation | FY |
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| Kiwao Kadokami | Professor, Faculty of Environmental Engineering, University of Kitakyushu | 2012- |
| Shin-ichiro Kawai | Specially-appointed Professor, Department of Food Design, Faculty of Nutrition, Koshien University | 2012- |
| Koichi Goka | Principal Researcher, Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies | 2012- |
| Noriko Takamura | Director, Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies | 2011 |
| Shinsuke Tanabe† | University Special Professor, Division of Environmental Chemistry and Ecotoxicology, Center for Marine Environmental Studies, Ehime University | 2010- |
| Yoshitaka Tsubaki | Professor emeritus, Center for Ecological Research, Kyoto University | 2010- |
| Takayuki Hanazato | Professor, Research and Education Center for Inlandwater, Institute of Mountain Science, Shinshu University | 2010 |
| Yukio Murata | Senior Officer, World Wildlife Fund for Nature Japan | 2010-2012 |
| Makoto Watanabe* | Professor, Graduate School of Life and Environmental Sciences, University of Tsukuba | 2010 |

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| Name | Affiliation | FY |
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| Yasuyoshi Okuno | Chair, New Chemical Issues Working Group, Japan Chemical Industry Association | 2010-2012 |
| Jiro Koyama | Professor, Education and Research Center for Marine Resources and Environment, Faculty of Fisheries, Kagoshima University | 2010- |
| Hiroshi Satoh* | Hiroshi Satoh Professor, Tohoku University School of Medicine | 2010-2011 |
| Chiharu Tohyama | Professor emeritus, University of Tokyo | 2010- |
| Akira Naganuma† | Professor, Graduate School of Pharmaceutical Sciences, Tohoku University | 2010- |
| Satoshi Hagino | Manager, Environmental Science Department, Environmental Health Science Center, Sumika Technoservice Corporation, Japan | 2010-2011 |
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