



**ENVIRONMENT DIRECTORATE  
JOINT MEETING OF THE CHEMICALS COMMITTEE AND  
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

Cancels & replaces the same document of 29 October 2001

**INITIATIVES TO SHARE THE BURDEN OF THE TESTING AND ASSESSMENT OF ENDOCRINE  
DISRUPTING CHEMICALS**

**32nd Joint Meeting 13-15 June 2001, to be held at the Château de la Muette, Paris, beginning at 9h30 on  
13 June.**

*This document describes the outcome of a tripartite exploratory discussion of Japan, the USA and the EC on options for a global strategy on endocrine disrupters assessment and provides recommendations for a practical approach.*

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***ACTION REQUIRED:***

***The Joint Meeting is invited to:***

- i) take note of the discussions of the Tripartite Meeting,***
- ii) consider and endorse the recommendations for a possible approach (summarised in paragraphs 24 and 25), amended as appropriate, and***
- iii) allocate resources to manage the work involved, and look into possibilities to find extra budgetary means to support this co-operation***

## INTRODUCTION

1. At the request of Member countries and the international industry OECD initiated in 1997 the Special Activity on Endocrine Disrupters Testing and Assessment with the objectives to provide a set of internationally recognised and harmonised testing guidelines and testing and assessment strategies for regulatory use that would avoid duplication of testing and thus save resources, including animals.

2. Managed by the Endocrine Disrupters Testing and Assessment Task Force (EDTA) and its two Validation Management Groups on mammalian tests (VMG-mammalian) and ecotoxicity tests (VMG-eco) respectively, several comprehensive test validation projects have been initiated and some of these are currently well underway or close to completion. In addition, Test Guidelines 414 and 416 have been updated and were subsequently adopted by Council in January 2001. The validation project of the Uterotrophic Assay for oestrogenic effects assessment is nearing its completion as the 3<sup>rd</sup> Meeting of VMG-mammalian in March 2001 agreed that no more experimental work is needed on this assay. The 2<sup>nd</sup> phase of the validation of the additional parameters added to enhance Test Guideline 407 is expected to be finalised at the end of 2001 and the results of the first phase of the validation of the Hershberger Assay for androgenic effects assessment are showing promising results. Another human health Test Guideline relevant for hazard identification of endocrine disrupters is also close to completion (Test Guideline 426 on Developmental Neurotoxicity).

3. In the area of ecotoxicity testing, two Expert Consultation Meetings have been held to discuss and rank currently available reproductive toxicity tests in fish and to recommend a screening test approach that would be ready for formal validation. In March 2001 the 1<sup>st</sup> Meeting of VMG-eco discussed technical issues and agreed on a work plan for this validation. In addition, the VMG-eco also discussed the outcome of an Expert Consultation Meeting on bird reproduction toxicity testing and recommended next steps towards the development of a bird test, relevant for the hazard identification of endocrine disrupters.

4. As this ongoing co-operative work is starting to bear fruit and the tools for testing and assessment of possible endocrine disrupters are taking shape, the next step in the process is to start using these tools. Considering the:

- vast amount of chemicals currently in use that need to be considered,
- time pressure to identify and assess endocrine disrupting chemicals as expeditiously as possible,
- number of studies necessary for screening and, as appropriate, full hazard assessment, and
- lack of resources needed for this work,

it seems appropriate to find ways for sharing (at least some of) the work internationally.

## THE TRIPARTITE MEETING

5. As a follow-up to bilateral discussions with representatives of the USA, the Secretariat arranged for an informal meeting with delegates from the European Commission, Japan and the USA to explore whether there is sufficient common ground to consider formalised co-operation among Member countries on this issue. The tripartite Meeting was held on Friday 30<sup>th</sup> March in Paris. The meeting agenda and list of participants are attached to this document as Annex 1 and 2 respectively.

6. A thought-starter, titled: "Proposed International Initiative to Develop a Global Strategy on Endocrine Disrupters", drafted by the USA, was used as a background and reference document for the

discussion. This document was considered as very useful as it included not only a concise introduction to the potential areas of co-operation but also provided several practical suggestions of how work could be shared in the areas of grouping of chemicals, pre-screening, screening, testing and assessment. The thought-starter is attached to this document as Annex 3.

7. The Meeting participants briefly summarised endocrine disrupters policies and approaches in each of the three regions/countries and informed the Meeting of current activities.

### **USA**

8. The US delegate explained that in the USA the work is based on the EDSTAC recommendations with emphasis on human health. US policy is to look at the universe of chemicals and apply a conceptual framework of chemicals sorting, pre-screening, screening and testing. Therefore, high priority is given to the development and validation of Tier 1 screens to be included in the framework. In addition to the uterotrophic and Hershberger assays, which are internationally agreed as relevant screens, the USA is working on the development and validation of additional screens to cover modes of action other than receptor-binding. Pesticides are treated somewhat differently as they have extensive data sets, including full 2-generation reproductive toxicity tests as well as chronic cancer/systemic toxicity studies. The work on pesticides is focussed on brief assessments of pesticides that come in for re-registration and, in addition, working backwards on registered pesticides in order to avoid that the process would take too long. The work on endocrine disrupters testing and assessment is facing strong time pressure in order to avoid legal implications involved in not meeting rescheduled deadlines for the assessments.

9. The US delegate further explained that there are two other programmes that in one way or another deal with endocrine disrupters: the HPV Initiative and the Children's Health Programme. For approximately 2000 chemicals the HPV Initiative will provide useful data from the reproductive toxicity screening tests (TG 421, 422) included in SIDS to set priorities for endocrine disrupters testing. This will also hold true for chemicals with a full SIDS package included in the OECD Existing Chemicals Programme. The approximately 70 chemicals currently considered for assessment in the Children's Health Programme have been selected based on potential unusually high exposure in children. Results from assessment of these chemicals would also assist in priority setting of chemicals for endocrine disrupters assessment.

### **European Commission**

10. The EC delegation said that the core of the Community Strategy on EDs was the development of agreed test methods within the framework of the OECD Special Activity on Endocrine Disrupters and further research to understand the problem. Since results on these aspects were expected in the medium term, the strategy also included a number of short-term actions. One of these was to focus on what could be problem chemicals and deal with those either through current instruments (e.g., the classification and labelling Directive) or on a case-by-case basis where appropriate. To date a set of 553 candidate substances had been identified in the BKH-TNO study, on the basis of which a set of priority actions for further evaluation would be proposed. Regarding research, in addition to the normal calls for proposals under the 5<sup>th</sup> Framework Programme for R&D, DG Research was about to publish a dedicated call for proposals on endocrine disrupters in May 2001. Furthermore, the EC is organising a Workshop in June in Sweden that will address, inter-alia, research and development and the process of test method/strategy development.

11. A progress report on the "Community Strategy for Endocrine Disrupters" published in December 1999<sup>1</sup> is due to be adopted by the Commission in May this year. This progress report will present priority actions for Europe, including the need to obtain more information on chemicals with insufficient or no data. It is not intended to duplicate ongoing work on chemicals that already fall under existing legislation, but rather to invite Member States to take existing ED evidence into account in the risk assessment process and to speed up such risk assessments. The progress report identifies 5 groupings of substances: i) those with evidence of endocrine disruption or potential ED which have been or are being addressed under existing legislation; ii) those with evidence of endocrine disruption or potential ED which are not addressed under existing legislation; iii) those generally considered as non-endocrine disrupters; iv) those with not enough information; and v) the universe of chemicals minus the 553 candidate substances. The EC's first focus is on actions for those chemicals in the second and fourth groupings.

12. The progress report on endocrine disrupters coincides with the publication of the EC's White Paper on a Strategy for Future Chemicals Policy<sup>2</sup>. It is likely that many ED candidate substances would fall under the authorisation procedure proposed in the REACH system of the future chemicals policy. The need for particular research efforts on endocrine disruption is highlighted in the White Paper. In the interim, the current legal instruments will be used to implement the EC policy on endocrine disruptors and will provide a bridge to the new system for chemicals control. In the interim, under the auspices of the work on existing chemicals, a number of actions are underway to improve the operation of the existing system e.g. i) identification and assessment of persistent, bioaccumulative and toxic chemicals (PBT's); ii) determination of assessment and/or test needs for these substances; iii) possible inclusion (depending on the timeframe of the implementation of the new strategy) in the EC Existing Substances Programme substances selected based on endocrine disrupter considerations.

### Japan

13. Delegates from Japan explained that the Ministries with an interest in endocrine disrupters testing and assessment independently develop their policies and strategies. An Inter-Ministry Collaboration Body, initially comprising nine Ministries (presently six), co-operates in evaluating scientific evidence of endocrine disruption both on human health and environmental wildlife. Among the Ministries, the Ministry of Economy, Trade and Industry (METI), the Ministry of Environment (MOE), the Ministry of Agriculture, Fisheries and Foods (MAFF), and the Ministry of Health, Labour and Welfare (MHLW) have been specifically sharing their responsibilities on the endocrine disrupter issue and, therefore, mutually exchange information on a detailed level. A series of 5 papers on current activities in Japan were presented and introduced by the delegates from METI, MHLW and MOE. These papers (attached as Annex 4 to this document) were titled:

- Development of a Global Strategy on Endocrine Disrupters: Information Meeting for a Future Strategy - Current Progress in Collaborating Studies of Japanese Ministries (Japanese Position Paper);
- Assessment of Suspected Chemicals in METI;
- Introduction to an Interim Report from the Study Group on Health Effects of Endocrine Disrupting Chemicals, Environmental Health Bureau, MHLW;
- Current State of Toxicity Assessment by the Ministry of Environment;
- The Present Status of Japanese High Throughput Screening System to Detect Hormonal Activity of Chemicals.

<sup>1</sup> Community Strategy for Endocrine Disrupters. Communication from the Commission to the Council and the European Parliament, Brussels, 17.12.1999; COM(1999) 706 final.

<sup>2</sup> White Paper: Strategy for a Future Chemicals Policy, Brussels, 27.02.2001; COM(2001) 88 final.

14. Delegates explained that emphasis in Japan is on the identification of endocrine disrupters by considering the universe of chemicals. Therefore the development and validation of high throughput pre-screens (HTPS) is considered a good way of pre-screening chemicals. An HTPS for oestrogenic activity has already been developed and tested and will be used to screen 1000 chemicals shortly. Another HTPS for androgenic activity is currently being developed. The work in MOE is focussed on the assessment of a series of 67 defined chemicals "suspected of having an endocrine disrupting effect". Tests considered include screening and testing in mammals and ecosystems. METI has selected a subset of 15 chemicals from the 67 chemicals identified by MOE for assessment. The focus of the MHLW is on a variety of issues including: i) contribution to the OECD work on test method development and validation, ii) development of definitive studies, iii) data collection on exposure, iv) scientific research, including the assumed very low dose effects, and v) the HTPS. The delegate from MHLW further informed the meeting that with respect to pesticides opinions differ among the respective ministries involved: whereas MAFF considers all registered pesticide ingredients as sufficiently assessed, including their potential endocrine disrupting effects, other ministries may wish to include specific pesticides in their assessments.

## **EXPLORING OPTIONS FOR INTERNATIONAL CO-OPERATION**

### **Grouping of Chemicals**

15. It appeared that an area where international co-operation would be very useful is in grouping chemicals that, for one reason or another, have triggered a regulatory interest in a critical assessment of their endocrine disrupting potential. The Meeting agreed that development of a common list of chemicals would not be a useful exercise as the criteria for selection could differ from country to country or even within one country between various Ministries. More importantly, a common list would distract the focus from the objective to assess as many relevant chemicals as possible, to a discussion of what should and what should not be on the list.

16. Instead, each country and/or region could develop its own grouping(s) of chemicals and share these series with other countries. In order for such information exchange to be useful, it seems essential that the criteria on the basis of which particular chemicals have been grouped should be provided. In addition, planned actions, including details of scheduled screening/testing would also be very useful information. A co-ordinated database of all chemical groupings, together with criteria and updates of scheduled work would avoid duplication of testing and assessment.

### **Tools for Screening and Testing**

17. The Meeting agreed that co-operation in the OECD work on test method development and validation is a major priority in all three countries/regions. Progress with the validation of the Uterotrophic Assay has been substantial and the VMG earlier in the week agreed that no more experimental work was needed. The validation of the Hershberger Assay and the Enhanced TG 407 are well underway.

18. The Meeting participants shared the opinion that the development of the HTPS in Japan has provided a pre-screening tool that could be very useful for other countries as well. In particular for the USA where the EPA faces the challenge of assessing large numbers of chemicals the Japanese HTPS would be a very welcome instrument for the selection of high priority chemicals. In the USA an Endocrine Disrupter Binding Assay has been developed and the screening of approximately 300 chemicals has been scheduled. Participants agreed that it could be very useful to compare the outcome of this project with the results of the Japanese HTPS. The development of other pre-screening tools such as the DNA micro-array assay and QSAR models are considered as equally relevant. It appeared that Japan, the EC and the USA are all working on QSAR models and existing overlap in this area, in particular between the EC and US activities, could not be excluded.

19. In the area of screening test development, the USA has started initiatives to further develop and validate at the national level a number of screens to cover mechanisms other than receptor-mediated effects. Tests currently considered are those recommended by EDSTAC and include the prepubertal assay. In Japan work is underway to enhance more definitive testing methods such as the mammalian two-generation reproduction test and reproduction tests in birds and fish.

20. The Meeting agreed that ways should be found to share results from all these activities in a structured way. They were also of the opinion that agreement on an internationally recognised battery of screens for chemicals with no data would be a major step forward in the global assessment of endocrine disrupters. Taking into account the vast amount of work involved in validation, the Meeting was of the opinion that decentralising the validation work with several countries/regions taking the lead in validation of particular screens and tests would be the way forward. International acceptance of the results and recommendations of these regionally managed activities would be a condition *sine qua non* for the success of sharing this burden.

21. The Meeting further recognised that the more definitive tests, in particular multi-generation reproductive toxicity tests in mammals, birds and even fish are so expensive that these tests should all be internationally co-ordinated. This would not mean that these tests should be arranged for and managed internationally. Co-ordination should primarily include: i) an early notice (so other countries considering the test would not start as well), ii) use of an internationally agreed protocol, and iii) being prepared to share the results.

### **Hazard Assessment**

22. One of many issues surrounding the endocrine disrupters concern is the poor understanding of the breadth of the issue. The Meeting was aware that a considerable number of experts claim that the globally observed trends in early onsets of puberty/menarche and certain cancers are related to endocrine disrupters. Furthermore, the reported increase in effects at very low dosages is still a matter of much debate and research. Current test methods are to a large extent only covering receptor-mediated effects and judgements are unclear and certainly not unanimous on issues such as whether or not hormonal effects without obvious toxic effects should be considered as adverse. The Meeting agreed that a further area of co-operation could be to exchange information on scientific meetings and involve Member countries in the organisation and participation in those meetings and forum discussions. The EC participants mentioned in this respect the plans for a European Workshop in June in Sweden to discuss progress in science, test methodologies, international co-operation and monitoring.

23. The Meeting showed some reluctance in going the route of international assessments. Experience with other international assessment programmes has taught that this may be a long and slow process. The delegates felt more comfortable with an approach based on sharing assessments rather than reaching agreement on them. In order to facilitate a common understanding of assessments, the Meeting agreed that the first aim should be to reach agreement on a series of elements to be included in assessment reports. The Meeting further agreed that the development of guidance document(s) focussing on the interpretation of particular findings (e.g., fish histopathology, effects on hormone levels) could be a helpful tool. As the ultimate goal would be to fully share assessment reports, the Meeting recognised that this could only be achieved by a structured international co-operation.

## MEETING RECOMMENDATIONS

24. The Meeting recognised that there was sufficient common ground and enthusiasm as well as a need to establish a formal level of international co-operation in the work on endocrine disrupters testing and assessment and agreed that co-operation should involve all OECD Member countries. In considering current activities in the European Union, Japan and the USA, the Tripartite Meeting recommended that:

### **General:**

- International co-operation on endocrine disrupters testing and assessment is essential and desirable in order to meet within a reasonable time the challenge of having an understanding of the possible hazards for human health and environmental safety of existing and new chemicals which interfere with the endocrine system;
- The OECD was best placed and equipped to take the lead in the international co-operation;
- International co-operation should be established as a structured activity aimed at: i) sharing of information on sorting, pre-screening, screening and testing of chemicals, ii) development of common approaches for screening and testing of chemicals without any useable data, iii) sharing of test results and assessment reports; co-operation should not include reaching agreements on international assessments;
- The EDTA could be the management body to oversee and co-ordinate the work involved in this initiative;
- At least annually EDTA should discuss (probably in a special session) the progress made in co-operation and information exchange, allowing Member countries to provide updates of national activities, discuss details of specific co-operation, and agree on objectives for the next year;

### **Specific:**

- Arrangements should be made for Member countries to share their chemicals grouping activities, involving the establishment of a common data base of chemicals of interest;
- Member countries who are interested in co-operation with Japan in the effort to test large numbers of chemicals in High Throughput Pre Screening Assays should be encouraged to do so and arrangements should be made to avoid that any overlap of work will occur;
- Member countries involved in the development and validation of QSARS should share information of progress and results and make this available to other Member countries through the co-operation initiative;
- Work should start to enable Member countries to share in an easily accessible way details of technical work, including test development and validation and test results;
- Work should start to reach agreement on elements that should be included in national or regional endocrine disrupters assessment reports;

- Arrangements should be made for Member countries to share endocrine disrupter assessment reports for all chemicals which have been assessed in the context of national or regional endocrine disrupters activities or programmes;
- Work should start on the development of generic Guidance Document(s) which are considered helpful for the assessment of endocrine disrupters.

25. The Meeting further recommended that the report of their discussions and exchange of thoughts should be forwarded to the 32<sup>nd</sup> Joint Meeting for its consideration, endorsement of the recommendations mentioned above and, as appropriate, resource (re)allocation.

**ANNEX 1**

**Global Strategy on Endocrine Disrupters  
Initial Tripartite Meeting to Explore Possible Options**

**OECD, Paris, 30<sup>th</sup> March, 2001**

**DRAFT AGENDA**

- 9:30**
1. Opening of the Meeting
  2. Introduction of Participants
  3. Brief Introduction of the "Thought-Starter" by US participants
  4. Information on current plans/proposals/actions in the USA
  5. Information on current plans/proposals/actions in the EU
  6. Information on current plans/proposals/actions in Japan
- 12:00**            **Lunch break**
- 14:00**
7. Discussion of possible joint approaches, taking into account:
    - Existing lists of identified chemicals of no or low concern;
    - Existing lists of identified chemicals of high concern;
    - Known endocrine disrupters that could/should be assessed without any unnecessary delay (and regulated);
    - Current High-Through-Put-Systems (HTPS): do they work?
    - Parallels with the HPV assessment activities, if any;
    - Conceptual framework for testing and assessment;
    - Other aspects.
  8. Next steps:
    - Is there sufficient common understanding and preparedness to co-operate on assessment internationally?
    - What would be the best practical approach, doable steps (not being overambitious) and expected successes;
    - Involvement of other Member countries, non-member countries (e.g., South Africa, Israel), other parties [BIAC, TUAC, IPCS, NGO's (such as animal welfare groups? Environmental groups?)]
    - Work plan and time frame, meeting(s), documents;
    - Any other aspects.
  9. Any other business
- 17:00**            **Meeting adjourns**

ANNEX 2

*Initial Tripartite Exploratory Discussion  
Paris, 30 March 2001*

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### ANNEX 3

## **Proposed International Initiative to Develop a Global Strategy on Endocrine Disruptors**

### **Background**

Japan, the EU and its member states, Canada, the U.S. and a few other countries each have developed or are in the process of developing and executing a strategy for dealing with endocrine disruptors. The U.S. has clearly separated its strategy into two complementary components: a research component focusing on basic science issues and the development of methods, and a screening and testing component to identify and characterize endocrine disruptors. The research efforts of the various countries, including the U.S., are being coordinated through the Global Endocrine Disruptor Research Inventory (GEDRI) under the auspices of the International Programme on Chemical Safety (IPCS). International coordination on developing and validating some test methods is being coordinated by the Organisation for Economic Cooperation and Development (OECD) on behalf of its member countries under the umbrella of its test guidelines program. OECD is also developing a framework for a screening and testing scheme. This framework, to date, incorporates many, but not all, of the elements of the EPA scheme, and some additional elements which are not contained in the EPA scheme. Although there is cooperation on a framework and validation of some test methods, no attempt has yet been made to develop a coordinated international overall strategy for testing, assessment and regulatory decision-making. Such a strategy would include agreement of what substances are priorities for testing and assessment, which countries will take the lead on specific tasks or chemicals to be addressed, and how the results of each country's efforts can be shared to leverage resources. Without such a strategy, there will be duplication of efforts and waste of valuable and scarce resources (and, potentially, conflicting results) on testing and assessment of chemicals.

International activities could be visualized in reference to the conceptual framework for the U.S. EPA's Endocrine Disruptor Screening Program (EDSP). That framework envisions sorting the universe of chemicals into four categories: 1) chemicals with no/low concern including polymers and other chemicals exempted from further testing or assessment, 2) chemicals with little or no information that need screening level data to make a preliminary determination of hormonal activity, 3) chemicals with substantial information that they can/do interact with the endocrine system and which need further testing to characterize the effects of the interaction, and 4) chemicals with sufficient data to permit a hazard assessment. The U.S. priority-setting efforts are focused currently on commercial (industrial) chemicals (many of which are also inert ingredients in pesticide formulations) and pesticide active ingredients. The commercial chemicals fall primarily into category 2, that is, they are candidates for screening to identify their potential for interacting with the endocrine system. Chemicals which are positive in the Tier 1 screens will need to undergo further testing and may ultimately need hazard assessment and risk management. The EU and Japan appear to be focusing primarily on commercial chemicals that fit into either categories 3 or 4, that is, suspected or known endocrine disruptors. This focus is similar to that of the U.S. with regard to pesticide active ingredients. In one sense the U.S. and the EU and Japanese strategies seem complementary.

The EU strategy focuses on the development of a priority list of suspected/known endocrine disrupting chemicals for further assessment. The priority list will be used 1) to identify substances for "priority" testing, 2) to identify substances for risk management which can be or are being addressed under existing laws, 3) to identify gaps in knowledge on dose/response sources/pathways and epidemiological studies of cause/effect to guide further research and monitoring efforts, and 4) to identify specific cases of consumer use of special concern, e.g., use in children's products. In a draft report, the European Parliament seems poised to recommend that the EU develop a screening and testing strategy in addition to the focus on assessment and research described above. This could open the door for increased cooperation with the U.S.

In Japan several agencies are involved in efforts related to endocrine disruptors. Japan's Ministry of Health and Welfare has been active in the development of a high throughput pre-screen assay system using an ER reporter gene assay system. They also have developed a docking model for receptor binding which may be useful for predicting ER binding. The Environment Agency has developed a strategy of research focused on a list of suspected/known endocrine disrupting chemicals that they are prioritizing for further investigation including elucidation of environmental loading, human body burdens and exposure pathways, environmental monitoring, and field investigations.

## **Potential Areas of Cooperation**

### Sorting Chemicals and Establishment of Priority Lists

Efforts could be made to harmonize priority lists of chemicals. Chemicals need to be reviewed and classified/sorted according to their data needs and then prioritized for follow-up action. Following the U.S. EPA's scheme, at least four lists could be developed and agreed upon internationally. They are: 1) a list of chemicals of no/ low concern, 2) a list of chemicals needing screening level information and 3) a list of suspected endocrine disruptors needing further testing, and 4) a list of known endocrine disruptors needing hazard or risk assessment. In some cases the chemicals on list 4 would need additional exposure studies to permit an assessment of risk. International efforts could develop criteria for these lists and agreement on chemicals to coordinate international efforts. The EU is charged with and has already initiated efforts to develop a list of 506 substances of known/suspected to be endocrine disruptors (essentially a combination of lists 3 and 4 in the U.S. scheme). Japan has developed a smaller list of EDs which it has prioritized on the basis of exposure and persistence. While the development of a single set of international priority lists (screening, testing, assessment) is highly desirable, the more modest goal of lists of chemicals of common interest formed from the integration of national priority lists may be adequate to ensure international coordination. Areas of potential cooperation are:

- Reviewing existing information and sorting chemicals into various lists.
- Agreeing on the priority of chemicals on each list as a precursor to the development of a cooperative action plan for testing and assessment.

### Pre-screening

In the absence of any hazard information related to endocrine disruption potential on the vast majority of chemicals, there is a need to develop some such information quickly and cheaply for a large number of chemicals to assist in the setting of priorities for further screening. Pre-screening data could be obtained using *in vitro* assay systems. At present two approaches appear promising. A high throughput pre-screen (HTPS) *in vitro* assay system could be developed that could test every chemical of interest. A non-automated version of the assays could be used to test smaller numbers of chemicals. A more indirect approach would rely on computational models (QSARs) based on *in vitro* results. Several hundred to a thousand chemicals would be used to develop and validate the QSARs which would then predict the binding potential of untested compounds. The U.S. is currently investigating and attempting to validate several QSAR models for the purpose of pre-screening chemicals.

Some areas in which international cooperation would be beneficial are:

- Assisting the Japanese in the validation of the ER HTPS by generating data on a large number of chemicals in an ER binding assay.
- Developing an AR cell line that could be used in HTPS.

- Developing a set of QSAR models for ER and AR.
- Developing ER and AR binding data for validating the QSAR models.
- Validating the QSAR models for ER and AR
- Obtaining chemicals/ sharing the costs of running a validated HTPS on chemicals of interest.

### Screening

The purpose of screening is to identify compounds that are endocrine active as candidates for further testing in Tier 2. At present, only three screening assays are being standardized and validated for international use. Currently, only the U.S. is implementing a formal screening program to systematically determine the potential of a large number of pesticides, commercial chemicals and environmental contaminants to interact with the endocrine system. The U.S. EPA is developing and validating a battery of assays to be used for this purpose. The proposed battery was selected on the basis of assay complementarity so that all known modes of action of endocrine disruptors for the estrogen, androgen and thyroid hormone systems would be detected. The final battery will be selected based on validation data. It is anticipated that U.S. industry will sponsor screening and testing of their chemicals. The U.S. government plans to sponsor screening of environmental and drinking water contaminants that are not registered pesticides or commercial chemicals. There are several ways to broaden international cooperation in this area.

- Agree on an international battery of screens and develop an international screening program with shared responsibility modeled after the SIDS program.

In the event agreement cannot be made on an international screening approach, it may be possible to establish cooperation in one or more of the following areas:

- Sponsor some validation studies for assays in the EDSP in countries other than the U.S. so that the confidence in the EDSP is more robust than if validation is confined to U.S. laboratories.
- Develop a mechanism for coordinating screening activity and sharing screening level data among countries.

### Testing

The purpose of Tier 2 testing is to determine which chemicals are endocrine disruptors, characterize the adverse effects caused by them and obtain dose response data necessary for a hazard and risk assessment. Some chemicals known to be endocrine active, but inadequately characterized, are ready for Tier 2 testing now. Others won't need Tier 2 testing until they are identified as endocrine active in Tier 1. There seems to be general agreement that a variety of species will have to be tested in full life-cycle studies in order to conduct an environmental risk assessment of an endocrine disrupting chemical. The tests include the following: mammalian 2-generation reproduction, fish life cycle, avian 2-generation reproductive toxicity, amphibian reproduction, and invertebrate multi-generation reproduction. Guidelines for these studies will be proposed as OECD guidelines. Thus there is greater likelihood of cooperation on Tier 2 testing than on Tier 1 screening. Ways in which international cooperation could proceed on Tier 2 testing include:

- Development and validation of OECD test guidelines for Tier 2 tests
- International agreement on which chemicals need testing and which tests (mammalian, avian, fish, etc; full life cycle or special study) need to be conducted.

- Dividing up the chemicals/testing responsibility among countries.
- Sharing test data.

#### Assessment

Although data on chemicals will have to be reviewed for adequacy to permit sorting/classification (i.e., assignment to lists), the chemicals with generally adequate data will need a more critical review and hazard assessment. This is a resource intensive effort that should be coordinated internationally. International cooperation could proceed by:

- Development of hazard assessment guidelines indicating what effects should be evaluated and how they should be scored in conducting a hazard assessment.
- Agreeing on the depth and format of hazard assessment reports.
- Dividing the chemicals to be assessed among countries and assigning responsibility for conducting the assessments.
- Sharing of assessment reports.

#### **Options for International Engagement**

- OECD
- US/EU/Japan Trilateral
- EU/US, US/Japan Bilaterals
- NAFTA/EU/Japan Trilateral
- IPCS for hazard assessment harmonization for human health; OECD for harmonization of ecotox

#### **Next Steps**

- Identify interests of countries
- Set up international meeting

**ANNEX 4.1****OECD****Development of a Global Strategy on Endocrine Disruptors:  
Informal Meeting for a Future Strategy  
-Current Progress in Collaborating Studies of Japanese Ministries-****1. Japanese inter-ministry standard policy:**

The body of Japan inter-ministry collaboration, consisting nine (presently six) ministries, started to work for correcting and evaluating the scientific evidences on this issue, because of the shortage of scientific evidences of the effect of endocrine disruptors on human health as well as wild life eco systems. Among the six ministries, four ministries, METI, MOE, MAFF, and MHLW, have been specifically closely collaborating each other, and sharing their responsibilities pertinent to the issue one another. They also have been exchanging their information, as well.

Under mutual collaboration between one ministry to the other, and also under collaborating researches over industries, academia, and governmental laboratories, the documentation of scientific information, international collaborating research in general, and further development of screening and testing methodologies, are shared among the ministries.

However, difference of policies among the ministries are seen, for example, while Japanese MOE alerts the issues listing “67 candidate chemicals (reduced to 65 chemicals at present)” to public, the METI is aware of issuing any possible list in order not to induce public panics or confusions. Since two-generation reprotox study is mandatory for the pesticide evaluation, MAFF is in a position to reserve any possible oversight in safety assessment.

In Japan, any prioritization of possible endocrine disruptors for further evaluations are not issued yet, except some testing trials for establishing the testing system conducted by METI and/or MOE.

**2. Outlined current activities :****2-1. Development of testing and screening methodologies****2.1.1. Development of pre-screening system :**

To prioritize the screening test for a large number of chemicals, a rapid screening system should be facilitated. Therefore two prescreening assay systems have been developed.

**2.1.1.1. High-through put prescreening system.**

HTPS for initial phase screening has been developed by METI and MHLW : the method includes binding assay, reporter gene assay. Over 1000 chemicals are scheduled within two years (500 chemicals are on going).

**2.1.1.2. Development of the 3-D QSAR system for prescreening or prioritization of the screening chemicals :**

Development of the 3-D QSAR in silico system has stated as a special research project for prescreening or prioritization of the screening chemicals predicting a possible hormone mimic activity. Data are to be obtained by the end of 2002, and if possible, informational data will be exchanged between U.S. and Japan.

**2.1.1.3. Bio-field 3-D QSAR in silico :**

Another bio-field 3-D QSAR in silico is also conducted by MHLW for possible prioritization of the pre-screening chemicals.

**2.1.2. Development of screening & testing system:****2.1.2.1. Mammalian studies focusing on human effect:****2.1.2.1.1. Rodent in vivo screening and testing :**

Participating the collaboration studies organized by OECD-VMG for the following three assays:

**a. Rodent uterotrophic assay**

Collaborating pilot study for OECD VMG has been conducted. Further more 25 chemicals are on going.

**b. Rodent Hershberger assay**

Collaborating pilot study for OECD VMG has been conducted. Furthermore, seven chemicals (BBP, 4-nitrotoluene, DEP, 2,4-D, n-Butyl benzene, benzophenone, DCHP conducted by NEDO project are on going.

**c. Repeated dose 28day-oral toxicity study** with evaluation of hormone activities (TG407 enhanced)

Collaborating validation study for OECD VMG has been conducted. (see separate sheet: EE2, Fl, L-thyroxine, Genistein, p,p'-DDE, CGS18320B).

Based on the above validation study, further study is scheduled.

2.1.2.1.2. One generation reprotoxicology study in rodents:

To define a possible definitive testing system, following one gen-studies will be conducted for ten out of twelve selected chemicals, in addition to the above OECD validation 2.1.2.1.1.testings.

2.1.2.1.3. Two generation reprotoxicology study in rodents:

Considering revision of the current guideline, two generation reprotox study has been conducted for seven chemicals by using a protocol in which hormonal effects can be evaluated.

To investigate possible enhancement, two generation reprotox study has been conducted for p-p' DDT and methoxychlor.

2.1.2.2. Wild life effect:

2.1.2.2.1. Fish:

*Medaka* to be developed for screening and testing system: establishment of FLF/d-rR medaka, developing a vitellogenin assay system, conducting a partial life cycle study, full cycle study, etc.

In vitro study: estrogen receptor binding assay/reporter assay (Er •lpha)

The study is conducted for 8 substances and the results are currently being evaluated.

2.1.2.2.2. Birds:

(a) Screening

VLDL assay, vitellogenin assay

The study is conducted for 8 substances and the results are currently being evaluated.

(b) Development of testing

In order to develop a method of Two-Generation Reproductive Toxicity Study, basic research of (1) sex differentiation and growth, (2) fertility, (3) development, and (4) central nervous system function/behavior to be conducted.

2.1.3 **DNA micro-array**

Along with developing a DNA chip that conveniently spots approximately 400 genes thought to be associated with endocrine disruption, expose (1) cultivated human and mouse cells and (2) adult and prenatal mice to EE and 7 substances and analyze the gene manifestation pattern of extracted mRNA using a DNA chip that spots approximately 8,000 genes for which the base sequence has been confirmed.

Research of cDNA micro array for establishing possible testing frame work has been started.

2.1.4. **Seeking a definitive testing**

Research fund to seek a possible definitive testing is released from MHLW from 2001 – 2004.

To define a possible definitive testing system, following one gen-studies will be conducted for ten out of twelve selected chemicals, in addition to the above OECD validation 2.1.2.1.1.testings.

**ANNEX 4.2****Assessment of Suspected Chemicals in METI**

METI constituted the subcommittee for endocrine disrupter chemicals in the chemical product council in July 1997 and collects and assesses lots of scientific data of those chemicals actively. The subcommittee is promoting the assessment of the suspected endocrine disrupter chemicals which have to be collected scientific data on a priority basis, and development of new test methods.

Nine chemicals have already been assessed with existing and new scientific knowledge and literatures and Uterotrophic assay, Hershberger assay and 2-Generation reprotoxicity study are conducted for seven chemicals. Six chemicals are under discussion.

## Progress of Assessment

Chemicals	Present assessment	Further work (•)
Octachlorostyrene	There is not enough data to evaluate the hormone-like activity. Exposure is very low because of by-product	No needed
Styrene dimer, Styrene trimer	There is no hormone-like activity.	No needed
n-butylbenzene	There is not enough data to evaluate the hormone-like activity.	Needed
Dicyclohexyl phthalate	There is not enough data to evaluate the hormone-like activity.	Needed
Benzophenone	There is not enough data to evaluate the hormone-like activity.	Needed
Polybrominated Biphenyls	There is not enough data to evaluate the hormone-like activity. (No production, no import, and no detection from environment in Japan.)	No needed
2,4-Dichlorophenol	There is not enough data to evaluate the hormone-like activity.	Needed
Diethyl phthalate	There is not enough data to evaluate the hormone-like activity.	Needed
Butyl benzyl phthalate	There is not enough data to evaluate the hormone-like activity.	Needed
4-Nitro-toluene	On going (There is not enough data to evaluate the hormone-like activity.)	On going
Diethylhexyl Adipate	On going	--- (depends on assessment)
Di-n-butyl phthalate	On going	--- (depends on assessment)
Diethylhexyl phthalate	On going	--- (depends on assessment)
Nonyl phenol	On going	--- (depends on assessment)
Bisphenol A	On going	--- (depends on assessment)

•Uterotrophic assay, Hershberger assay and 2-Generation reprotoxicity study are carried out when the chemical is needed further work.

### Annex 4.3

#### Introduction to an Interim Report from the Study Group on Health Effects of Endocrine Disrupting Chemicals

#### **Environmental Health Bureau, Ministry of Health and Welfare, Japan**

## 1. Background

The Study Group on Health Effects of Endocrine Disrupting chemicals was established in April 1998 under the Director General of the Environmental Health Bureau of the Ministry of Health and Welfare. The purpose of the Study Group is to identify the issue of endocrine disrupting chemicals (hereinafter referred to endocrine disruptors) and to discuss approaches toward this issue.

The Study Group has held six sessions to date. During these sessions, the Group reviewed effects on human health. Also, the Group invited experts from foreign countries and international organizations such as WHO and OECD, and introduced approaches of these organizations and foreign governments toward these chemicals. The Study Group compiled and published an interim report in the session held on November 9<sup>th</sup>, 1998 on the basis of findings and understanding obtained through the previous sessions.

## 2. Outline of the interim report

The report consists of four sections and an appendix. The four sections are individually titled "Introduction," "Examining Endocrine Disruptors," "Strategy for Resolving the Problems of Endocrine Disruptors," and "conclusion."

### (1) Introduction

This section describes the background of problems of endocrine disruptors and what are points of these problems. The section also outlines the circumstances under which the report was compiled.

The descriptions include:

Indications that certain types of chemicals may have endocrine disrupting actions at trace levels and may badly affect human health.

Needs to take action, based on a tiered program, because many problems remain to be resolved scientifically.

### (2) Examining Endocrine Disruptors

This section gives basic information required to understand human health effects of endocrine disruptors, such as hormonal actions in the human body and the definition of endocrine disruptors by the International Programme on Chemical Safety/the World Health Organization (IPCS/WHO). The section also discussed expected action mechanisms of endocrine disruptors defined here and gives information on human health effects, based on domestic and international documents and reports.

The IPCS defines and endocrine disruptor as "an exogenous substance or mixture that alters function(s) of the endocrine system and causes adverse health effects in an intact organism, or its progeny, or (sub) populations."

The information on human health effects given here includes effects on the female reproductive system and mammary glands such as uterine cancer, endometriosis, and breast cancer; effects on the male reproductive system such as decreased sperm counts, prostate cancer, testicular cancer, and hypospadias;

and effects on the thyroid system.

Based on currently available findings and documents, the following evaluations are given:

- 1) There have been no reports on definite causal relationships between endocrine disruptors and health effects on humans, excluding instances caused by diethylstilbestrol (DES) which was administered for possible pharmacological actions on the endocrine system.
- 2) It is possible to take temporary measures for exposure levels in everyday life, if appropriate risk assessment and exposure evaluation are conducted. However, in order to resolve endocrine disruptor problems, it is imperative that the assumptions given below be established through assessments and evaluation.
  - No hormonal control can be easily disrupted, although the fetal period is thought to be vulnerable.
  - No unexpected synergistic effects are caused by multiple chemicals.
  - No unknown modes of activities exist at low dose levels.

### (3) Strategy for Resolving Problems of Endocrine Disruptors

This section shows approaches for endocrine disruptors which are being conducted at home and abroad, and discussed basic policies to resolve problems and research/studies to be performed to help secure human health.

The basic policies include concepts of chemical safety, the development of the information management/provision system, the promotion of international cooperation, and the promotion of comprehensive research. Target problems for research are divided into two groups: problems which require research to obtain needed data; problems which are expected to be resolved with the promotion of state-of-the-art science.

### (4) Conclusion

As a conclusion of the report, this section indicates three matters we should keep in mind, when placing in practice measures required to resolve endocrine disruptor problems.

The matters are:

- 1) There are various questions to be deliberated in endocrine disruptor problems.
- 2) Endocrine disruptors are an issue to be handled globally.
- 3) Endocrine disruptor problems may extend to multiple generations.

### (5) Appendix

This section reports the results of studies which were conducted on three chemicals used as ingredients of plastic containers for food use, and indicates plans for future research/studies. Based on available study findings, the Study Group has so far determined that it is not necessary to take immediate action including 'prohibition of use,' but that it is necessary to continuously conduct more research/studies.

The three chemicals are:

- 1) Polycarbonate (bisphenole A)
- 2) Polystyrene (styrene monomer, dimmer, and trimmer)
- 3) Polyvinyl chloride (phthalates)

## **Current Situation On The Issue Of Endocrine Disruptors And Future Approach**

### 1. Current situation

There have been no new findings which require the alteration of the following conclusion given in the Interim Report published in November 1998.

*Thus far there have been no reports on definite causal relations between health effects on humans and endocrine disruptors excluding instances caused by diethylstilbestrol (DES) which have been used for possible pharmacological actions on the endocrine system.*

### 2. Future program

1. To choose target substances for HTPS (High Throughput Pre-Screening).
  2. To the QECO screening methods.
  3. To develop full testing methods to identify/ confirm endocrine disruptors.
  4. To establish sampling test methods.
  5. To clarify inverted U effects (presence or absence of low-dose activity, effects).
  6. To collect and analyze data on exposure epidemiology
- . To promote risk communication.

### 3. Implementation of the Programme

The Ministry of Health, Labour and Welfare will set up the five working groups (see below) to forward the above programme.

#### Working Groups:

1. on testing scheme (Item Nos. 1 -3)
2. for establishing sampling/testing methods (Item No.4)
3. for evaluating the low-dose issue (Item No.5)
4. for investigating exposure/epidemiology (Item No 6)
5. on risk communication (Item No.7)

### 4 Miscellaneous

#### 1. Schedule

Each working Group will compile a report in the summer of 2001.

#### 2. International and inter-ministerial cooperation

More importance should be put on cooperation with international organizations, other countries, other Japanese ministries for the efficient conduct of the program.

#### ANNEX 4.4

### **Current State of Toxicity Assessment by Ministry of the Environment**

\*Toincjty assessment of 12 substances began in the year 2000 (over 40 substances to be assessed in 3 years)

Breakdown of 12 substances

Tributyltin. 4 octyl phenol. nonyl phenol, n-dibutyl phthalate, octa-chlorostyrene. benzophenone, dicyclohexyl phthalate, 2-diethylhexyl phthalate. butylbenzyl phthalate, diethyl phthalate. 2-diethylhexyl adipate. triphenyltin

\*The Mimstiy of the Environment has listed 65 substances as “substances suspected of having an endocrine disrupting effect.”

The ministry has determined that at the present it would be impossible to realistically estimate risk for styrene dimer/tnmer and n-butylbenzene. so they have been deleted from the list.

#### I. Manunals

##### 1. In vivo study

‘(1) Uterotrophic assay

Tests have currently been conducted for 8 of the 12 substances (tributyltin. 4 octyl phenol. nonyl phenol, n-dibutyl phthalate. octa-chlorostyrene. benzophenone, dicyclohexyl phthalate, 2-diethylhexyl phthalate). The results are currently being evaluated.

(2) Hershberger assay Scheduled to start in the year 2001.

(3) Revision OECDTG407 Repeated Dose 28-day Oral Toxicity Study

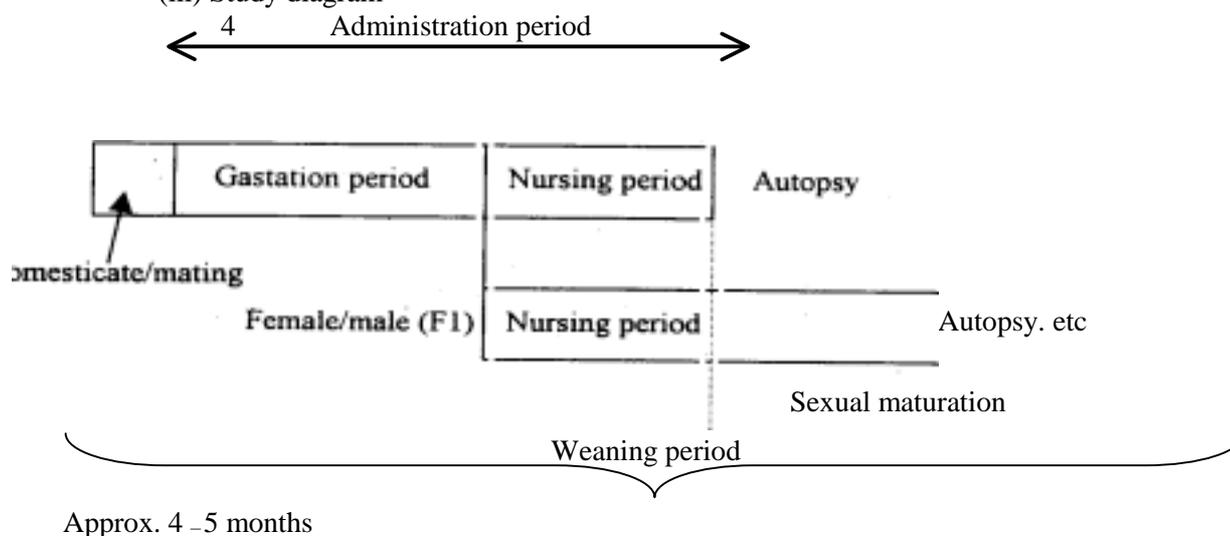
Based on the results that the Ministry of Economy, Trade and Industiy, Ministry of Health, Labor and Welfare, Ministry of Agriculture, Forestry and Fisheries validate test substances by request of the Ministry of Economy, the study is scheduled to be conducted by cooperating and dividing with the Ministry of Economy. Trade and Industry.

(4) One-Generation Study in Rodents

(i ) The pilot study of the One-Generation Study is now being conducted concerning 10 of the 12 substances, with.the exception of alkyl phenols.

(ii) Two of the substances (4 octyl phenol, nonyl phenol) are suspected of having an estrogen effect, so a pilot study using EE, which is a positive control substance, is now being conducted.

(iii) Study diagram



(5) DNA micro-array

Along with developing a DNA chip that conveniently spots approximately 400 genes thought to be associated with endocrine disruption, expose (1) cultivated human and mouse cells and (2) adult and prenatal mice to EE and 7 substances (tributyltin, 4 octyl phenol, nonyl phenol, n-dibutyl phthalate, octa-chlorostyrene, benzophenone, dicyclohexyl phthalate) and analyze the gene manifestation pattern of extracted mRNA using a DNA chip that spots approximately 8,000 genes for which the base sequence has been confirmed.

## II. Ecosystem

### 1. Fish (*medaka* used in all cases)

#### (I) Screening

(i) Development of FLF/d-rR *medaka*

Early life stage study using *medaka* (FLF/d-rR) for which sex can be genetically distinguished in a simple manner is being developed.

(ii) Development and conducting of vitellogenin assay

The study is conducted for 8 substances (tributyltin, 4 octyl phenol, nonyl phenol, n-dibutyl phthalate, octa-chlorostyrene, benzophenone, dicyclohexyl phthalate, 2-diethylhexyl phthalate) and the results are currently being evaluated.

(iii) Reproductive study

Now being studied using positive control substances.

(iv) Partial life cycle study

The study is conducted for 8 substances (tributyltin, 4 octyl phenol, nonyl phenol, n-dibutyl phthalate, octa-chlorost Tene, benzophenone, dicyclohexyl phthalate, 2-diethylhexyl phthalate) and the results are currently being evaluated.

(2) Testing

Full life cycle study

Now being studied using positive control substances.

(3) In vitro study

(i ) Estrogen receptor binding assay

The study is conducted for 8 substances (tributyltin, 4 octyl phenol, nonyl phenol, n-dibutyl phthalate, octa-chlorostyrene, benzophenone, dicyclohexyl phthalate, 2-diethylhexyl phthalate) and the results are currently being evaluated.

(ii) Reporter gene assay (ER cL) Currently being developed.

2. Birds (1) Screening (i ) VLDL assay

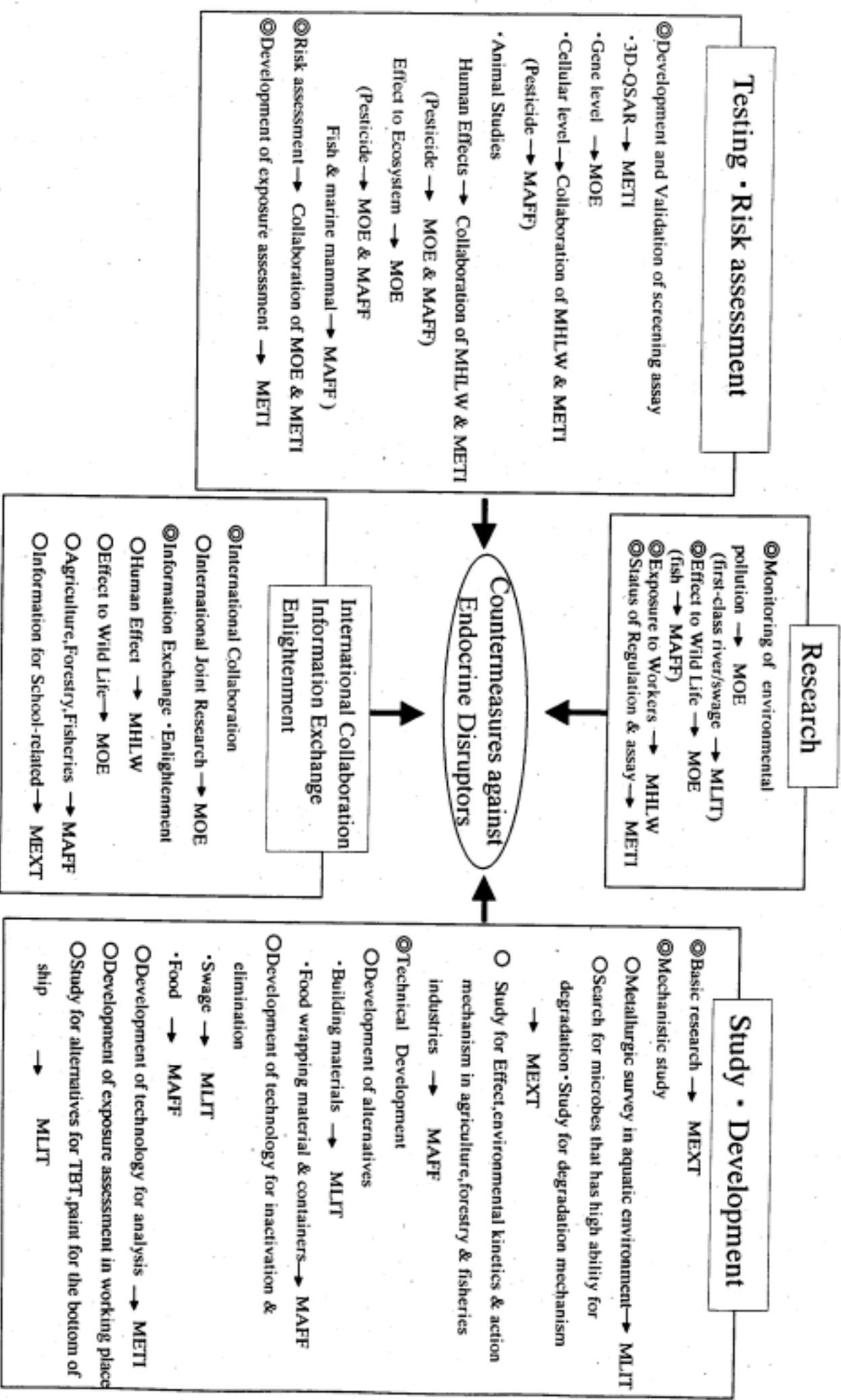
(ii) vitellogenin assay

The study is conducted for 8 substances (tributyltin, 4 octyl phenol, nonyl phenol, n-dibutyl phthalate, octa-chlorostyrene, benzophenone, dicyclohexyl phthalate, 2-diethylhexyl phthalate) and the results are currently~being evaluated.

(2) Development of testing

In order to develop a method of Two-Generation Reproductive Toxicity Study, basic research of (1) sex differentiation and growth, (2) fertility, (3) development, and (4) central nervous system function/behavior to be conducted.

# Main Countermeasures against Endocrine Disruptors (Environmental Hormones) in 2001 annual budget



**ANNEX 4.5****The Present Situation of Japanese High Throughput Screening System to Detect Hormonal Activity of Chemicals**

Chemicals Assessment Center, Chemicals Evaluation and Research Institute, Japan

**Transient transfection system**

Assay system	Receptor origin	Present situation of assay system
ER alpha	Human	<ul style="list-style-type: none"> <li>Rat ER alpha mediated transient transfection assay system is already established.</li> <li>This system can induce over 10-fold transcriptional activity with 1nM of E2 compared with the control (DMSO).</li> <li>Dozens of pilot chemicals were already examined.</li> </ul>
ER alpha	Rat	<ul style="list-style-type: none"> <li>Rat ER alpha mediated transient transfection assay system is already established.</li> <li>This system can induce about 10-fold transcriptional activity against 100pM of E2 compared with the control (DMSO).</li> <li>This system can induce more than 10-fold transcriptional activity against 1nM of E2 compared with the control (DMSO).</li> <li>Dozens of pilot chemicals were already examined.</li> </ul>
ER beta	Human	<ul style="list-style-type: none"> <li>This assay system is under construction.</li> </ul>
ER beta	Rat	<ul style="list-style-type: none"> <li>This assay system is under construction.</li> </ul>
AR	Human	<ul style="list-style-type: none"> <li>Human AR mediated transient transfection assay system is established.</li> <li>The system can induce more than 10-fold transcriptional activity with 10nM of DHT compared with the control (DMSO).</li> <li>Dozens of pilot chemicals were examined.</li> </ul>

**Stable cell line system**

Assay system	Receptor origin	Present situation of assay system
ER alpha	Human	<ul style="list-style-type: none"> <li>Stable cell line is already established.</li> <li>Cell line is stable at least 4 months with over 30 passages.</li> <li>The cell line can continuously induce about 10-fold transcriptional activity with 100pM or 1nM of E2 compared with the control (DMSO).</li> <li>DATA collection of 500 chemicals is now proceeding.</li> </ul>
ER beta	Human	<ul style="list-style-type: none"> <li>Cell line is not established at present.</li> <li>Screening for prospective cell line is now proceeding.</li> </ul>
AR	Human	<ul style="list-style-type: none"> <li>Stable cell line is already established.</li> <li>Cell line is stable at least 1 month with over 10 passages.</li> <li>The cell line can continuously induce about 10-fold transcriptional activity with 10nM of DHT compared with the control (DMSO).</li> <li>DATA collection of 500 chemicals is now proceeding.</li> </ul>
TR alpha	Human	<ul style="list-style-type: none"> <li>Cell line is not established at present.</li> <li>Screening for prospective cell line is now proceeding.</li> </ul>
TR beta	Human	<ul style="list-style-type: none"> <li>Cell line is not established at present.</li> <li>Screening for prospective cell line is now proceeding.</li> </ul>