3. Measures to protect human health

Large quantities of chemicals are used in Japan. The number of these chemicals is estimated at several tens of thousands. Time, budget, and work force available to take urgent measures to protect human health against a number of chemicals are very limited. Therefore, it is necessary to set up a specific program under which studies and evaluations are efficiently carried out.

In these studies, the target chemicals should be divided into two categories, based on toxicity information: a) chemicals suspected to exert endocrine disrupting activities and b) chemicals with insufficient information to decide whether they are endocrine disruptors. Priority should given to the former chemicals (see (1) below). The latter chemicals should be screened using a high-throughput screening method (robot automated technology) (see (2) below).

Below is a specific program for studies and evaluations. On the basis of existing data on toxicity and screening, chemicals should be prioritized for detailed investigations.

(1) Research on chemicals suspected to be endocrine disruptors

Health effects of chemicals on humans are determined on the basis of the extent of the toxicity of these chemicals and their exposure level. Japan will conduct the following activities to assess the risk of each of the chemicals suspected to have endocrine-disrupting effects.

1. Gathering information

   Japan will gather background information showing that the chemicals are suspected to have endocrine-disrupting actions, together with information on their physiochemical characteristics necessary to conduct the studies and research given in sections 2) through 5).

2. Investigating exposure pathways

   Japan will identify exposure levels and pathways for each chemical included in foods, apparatus and containers for food use, toys, household-use articles, drinking water, and air. We will determine exposure levels and also

investigate dynamics of the chemical in the environment.

3. Studying metabolism

   Japan will consider the toxicity of metabolites as well as the toxicity of the original chemicals because chemicals may be metabolized and change into various metabolites in the human body. We will identify the distribution of chemicals in the human body, and will clarify mechanisms of endocrine-disrupting activities.

4. Studying multiple-generation reproduction

   Japan will conduct two-generation reproduction studies based on information and research, and study results obtained in 1) and 3), to provide valuable information for the risk assessment of chemicals. If necessary, we will conduct a 28-day repeated-dose toxicity study, utero-trophic assay, and Hershberger assay before the performance of the multiple-generation reproductive study.

   The two-generation study is globally considered to be most appropriate for investigating health effects of endocrine disruptors, including those on the fetus. This study observes the effects on adult animals and their two-generation offspring, by administrating chemicals to the animals who are allowed to reproduce.

5. Conducting risk assessments

   Japan will conduct risk assessments based on information and results of research obtained under 1) through 4) above.

(2) Other methods of checking chemicals

The chemicals used in Japan are estimated at several tens of thousands. There is the possibility that these chemicals may exert endocrine disrupting activities in humans and wildlife, now or in the future. Therefore, it is necessary to examine chemicals without sufficient observations to see whether there is the possibility. Examinations should be conducted using test systems devised for exclusively that purpose (Plural test systems using animals are considered to be necessary).

However, these tests are very time-consuming and costly. It is almost impossible to test all target chemicals simultaneously. Therefore, for efficiency and economy, priority should be set based on certain indications. There is a common idea among

developed countries: it is necessary to develop the framework of such an approach in international harmonization, given that a great number of chemicals are distributed worldwide. Currently, Japan, the United States and European countries are working together to promote examinations through organizations such as the WHO and the OECD.

1. High throughput pre-screening (HTPS)

   The high throughput pre-screening system uses robotic technology to obtain information on bioactivity for several chemicals in a short time, by conducting comparably simple tests. The information obtained is used for setting priorities for target chemicals for the next-tier study systems. In practice, the device operates a reaction system using cultured-cell strains integrated with a hormone receptor and its response gene, and detects the presence or absence of actions of estrogens, androgens, and thyroid hormones on each receptor, as gene-transcription activity.

2. Priority setting

   Priorities should be set so that substances exhibiting overt or almost overt health risks are selected for next-stage study systems, based on conventional epidemiological and experimental studies. However, few substances exhibit overt health risks. Most of the substances lack useful data on their biological effects. Therefore, priorities should be set, based on results of HTPS.

3. Conduct of studies

   a) Screening

   Screening is designed to provide necessary data to sort out those substances which may have endocrine disrupting actions. If needed, based on HTPS results, further studies should be conducted, including 28-day repeated dose toxicity studies, utero-trophic assays, and Hershberger assays.

   b) Detailed tests

   Detailed tests identify whether substances are endocrine disruptors and characterize the endocrine-disrupting actions of these substances in humans, including a dose-response relationship. The tests not only identify these substances, but also determine whether their activities are direct or indirect. In addition, they yield data useful for conducting risk assessments on humans.

Additional tests may be required for definitive risk assessments.

4. Risk assessments

Risk assessments are conducted, based on results obtained under 1) through 3)

5. Considerations

The tests mentioned in section (2) have a little accumulation of data on the reliability of test methods, because these problems are quite new. It is necessary to validate test methods used in each stage of screening and specific studies, such as multi-generation reproduction studies. Also, international coordination should be promoted for the effective and appropriate performance of studies.

(3) Other research

The following research should be performed, in addition to the studies given in sections (1) and (2).

1. Exposure

a) Development of elution tests and/or material tests for foods, food packages/containers, and toys

It is necessary to develop elution tests and material tests for food containers and toys, and residue tests (in foods) for some chemicals suspected of being endocrine disruptors. Because elution volumes vary with solvent, temperature, and time, these evaluation methods should be suited to the actual chemical intake of humans. The methods should be simple and internationally standardized. Also, it will be important to develop standard assays for these chemicals in human blood and abdominal fluid.

b) Investigation of exposure from foods, food packages/containers, and toys

Because there are a wide variety of foods, food containers, and toys, it is essential to investigate exposure to chemicals from these pathways. It is also necessary to investigate such exposures comprehensively, along with those from drinking water and the atmosphere. Then, these results and toxicity study results should be compared. If needed, measures should be taken to

reduce exposure to these individual chemicals. Reports indicate that some endocrine disruptors accumulate in the human body, which can affect health. It is vital to determine the levels of chemicals taken into the body by analyzing biological samples (blood, abdominal fluid, human milk) when conducting risk assessments. In particular, test results from umbilical cord blood are important to understand fetal exposure, and results from human milk to understand infant exposures. In addition, these samples should be preserved for a long period for future studies.

2. Toxicity studies

There are problems to solve on toxicity study methods and the interpretation of the study results. These problems include the three given below. Assessment parameters in individual study methods should be set from a comprehensive aspect. Therefore, it is necessary to encourage research of setting these parameters.

a) Studies on individual chemicals

Inverted U effects (Clarification of action mechanisms)

There are reports that certain types of chemicals exhibit the so-called inverted U effect in animal experiments and *in-vitro* tests.[*87] This effect refers to chemicals which exhibit a positive dose-relationship at a high dose but a negative dose-relationship at a low dose, so that the nature of the response differs for low- and high-doses. The reliability of such reports is still open to question, because these reports are limited to *in-vivo* results, for which reproducibility can not always be confirmed. Conventional toxicity studies and evaluations have been based on the dose-relationship, assuming that there is a threshold. So far, the inverted U effect has not been examined. Therefore, it is necessary to confirm whether the inverted U effect exists. If its existence is confirmed, action mechanisms should be immediately identified.

Fetal and neonatal exposures

There are reports that offspring from animals fed certain types of chemicals during pregnancy had malformations, and some of the offspring developed learning disorders, cancers, immunological hypofunctions, and reproductive disorders, after reaching adulthood. Mechanisms should be clarified in a multi-faceted and comprehensive approach because the mechanisms of the

development of these effects are not clear, and it can take a long time for the effects to be expressed.

Quantitative studies for structure-activity relationship

Research into structure-activity relationships is aimed at identifying the characteristics of a chemical by mathematically analyzing the chemical structure using a computer. This type of research is widely used for selecting chemicals in developing new pharmaceuticals because it has a number of merits, including savings in cost, time, and experimental animals when conducting risk assessments. On the other hand, such research shows reliability and usefulness only for some types of chemicals because 1) it cannot be used for chemicals like steroid hormones, in which a slight change in a side chain causes great changes in bioactivity, and 2) there is limited information on human health-effects. However, if a comprehensive quantitative study for structure-activity relationships were to be conducted, it could become an effective approach for easily evaluating toxicity effects on humans. Such a study would be based on screening test data to be obtained from the research and studies given in (1) above, and detailed study data including chronic toxicity studies, as well as those on the physiochemical properties of existing chemicals.

b) Combined effects of multiple endocrine disruptors

There are reports that separate chemicals did not have any effect on experimental animals in some studies, but multiple chemicals, when combined, did have an effect. In addition to the effects of separate chemicals, there is an emerging concern about combined effects of chemicals to which humans are or may be exposed at rather high levels. John McLachlan (who took an important role in the argument for combined effects) and others had reported that multiple chemicals, when combined, exhibited about 1,000 times higher synergistic effects. They withdrew the report due to lack of reproducibility.[*89] At the moment, the additive effects of endocrine disruptors are indicated but synergistic effects are not confirmed. However, the potential synergistic effects of multiple chemicals may not be ruled out. Therefore, additional studies are needed on such potentiality.

c) Phytoestrogens

Phytoestrogens are consumed through foods in larger amounts than are

synthetic chemicals. These estrogens are reported to have higher estrogenic activity than that of some synthetic chemicals in *in-vitro* tests[*90] and may have health effects on humans. However, there is the interesting fact that although Japanese people consume a large amount of soybeans, which include phytoestrogens such as genistein, the incidence of breast cancer is lower in Japanese women than in Western women. Humans have a long history of consuming phytoestrogens. There is an assumption that humans have developed resistance to them through a feedback action and a metabolizing or eliminating action for certain types of estrogen-like substances. It is necessary to clarify the actions of phytoestrgens the living body, including those between species.

3. Epidemiological studies

It is a fact that endocrine disruptors occur in the environment and have some effects on people. Also, some populations are anticipated to be occupationally exposed to high levels of endocrine disruptors. Therefore, it is necessary to identify how Japanese people's health can be affected by occupational exposure to endocrine disruptors according to exposure level. To that end, effective research should be conducted on human health effects based on carefully designed epidemiological research.

The effects to be investigated include those on male and female genital organs and reproductive functions, the hormonally mediated cancers, and the hormonal environment of the body. Exposure levels should be exactly determined from biological samples, and factors which may signify a relationship between exposure to endocrine disruptors and health effects should be investigated, including phytoestrogens and endogenous hormones.

Epidemiological studies should be conducted on the general public and on occupational populations, using suitable methods including case-control studies, cohort studies, cross-sectional studies, ecological studies (regional-relationship studies), and random comparative tests. Also, attention should be paid to sampling methods, the number of required subjects, setting of controls, and ethical aspects of the studies.

(4) Development of implementation systems for research

Research required to solve endocrine disruptor problems cover a wide variety of areas. For the smooth conduct of research, a framework should be established.

1. Structuring of a new framework

   Since 1996, the Ministry of Health and Welfare has supported research and studies on endocrine-disruptor problems in a program of "Health Sciences Research" with a view to preventing health hazards. This program has been carried out in cooperation with the industrial and academic sectors. To further promot research, the Ministry needs to strengthen its planning and evaluation systems for research. The Ministry also needs to promote comprehensive research, including voluntary research by industry and the publication of data by other countries and related governmental agencies.

2. Training and securing researchers

   Japan has fewer researchers capable of evaluating chemical safety than do other countries. Researchers are required to acquire a wide range of expertise beyond conventional academic boundaries. Japan needs to develop a training system to secure researchers capable of solving chemical-safety problems.

3. Procurement of instruments for research

   Highly sensitive instruments are required to study endocrine disruptors because these chemicals are considered to have health effects on humans at trace levels. Japan needs to install state-of-the-art analytical instruments in our laboratories.

4. Efficient performance of research

   The number of chemicals distributed in the world is estimated at the tens of thousands. Cost-saving approaches are needed to evaluate the safety of these chemicals. Japan has to promote research under a practical program with a long-term view to protect human health (e.g., the tiered program adopted in the United States). Such research must be performed efficiently in collaboration with the private, academic, and industrial sectors.

(5) Development of an information management and distribution system, based on a database

Currently, various types of research and studies are, and will be, conducted on

endocrine disruptors by the Ministry of Health and Welfare and the other agencies concerned, several industries, and non-governmental organizations, from their own particular view points. This will result in the generation of great amounts of information. Such information should be compiled into a database in order to utilize it effectively for several purposes such as regulation, research, and publication. The Ministry of Health and Welfare is scheduled to build up a database on endocrine disruptors because it already has a database on human health.

(6) Encouragement of international cooperation

Japan needs to work together with major international organizations and foreign countries which handle problems of endocrine disruptors. It should also take a leading part in international cooperation. The following are the activities Japan will carry out:

1. The International Programme on Chemical Safety / The World Health Organization (IPCS/WHO)

- To participate in the risk assessment of endocrine disruptors, results of which will be compiled in two years by the IPCS/WHO.
- To compile and assess results of various studies conducted in Japan, and to contribute to international risk assessments.
- To coordinate databases between the IPCS/WHO and Japan, in order to enable them to be mutually accessible.
- To provide information on the experience obtained in building up a database in Japan.

2. The Organization of Economic Cooperation and Development (OECD)

- To help to set up an assessment system for endocrine disruptors, based on study results obtained in Japan. The establishment of this system is under way in the OECD.
- To participate in the program for drawing up test guidelines for endocrine disruptors.

  In practice, Japan will help to draft revised versions of the 28-day repeated toxicity study, utero-trophic assay, Hershberger assay, and multi-generation reproduction study. These study methods should be reviewed, from the focus

on the assessment of Endocrine disruptors.

- To conduct studies under the revised guidelines.

3. The United States

- To exchange validation results of assessment systems and compare results between Japan and the United States.
- To coordinate target items for research between our countries to improve the accuracy of tests, heighten efficiency, and reduce costs.

The United States is validating an assessment system for endocrine disruptors and compiling the results. The assessment system consists mainly of three tiers: high throughput pre-screening, screening, and detailed testing. This system is similar to that used in Japan.