Endocrine Disrupters Research in National Institute for Environmental Studies

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On behalf of the National Institute for Environmental Studies, I would like to take this final opportunity at this symposium being held at Tsukuba to speak briefly about research conducted at the Endocrine Disrupter Research Laboratory that was constructed in April this year.

Research here is conducted by about twenty full-time researchers. In addition to this, about fifty researchers, including temporary researchers and supporting staffs, are involved in research of endocrine disrupters including dioxins. On behalf of the organization, I would like to provide a brief description of the research being conducted here.

The overall scheme of research is as follows: “Development of methods of measurement” is written on the far left. To find out what is happening here, tools are polished. Using these tools, we attempt to get an understanding of the current status of environmental pollution and to aid in assessment of it.

Research to discover the impact of endocrine disrupters in the environment is also conducted here. We furthermore tie all this research together and further develop techniques and technologies for combating such effects. These four types of research form the backbone of our research activities.

This is the Endocrine Disrupter Research Laboratory that was completed at the end of March. With 5200 square meters of space, it is quite an impressive building. Several types of facilities are contained here and they are interoperable with other types of research. One of its functions is to be used for research of endocrine disrupters. At the same time, we try to function as a center of information concerning endocrine disrupters, and are attempting to more fully equip our information department.

Now I’d like to talk a little about what our research involves. Because research of endocrine disrupters is quite involved, experiments involving dioxin are conducted at facilities used for research of other specified chemical substances as well as at the Endocrine Disrupter Research Laboratory. And experiments involving birds are conducted at the same time at facilities used for animal experiments.

Primarily I talk about research concerning methods of measuring chemical substances. Here observation is conducted based upon analysis by gas chromatography and mass spectroscopy, or GCMS. We are testing a method of detecting new pollutants using the negative ion / chemical ionization method. At the same time, we are involved in identifying substances using liquid chromatography and mass spectroscopy, as well as the magnetic resonance method, with its extremely high resolution.

Here is an example of results of analysis. One of the substances that has a female hormone effect has a large impact which comes from a natural source: the human body. This is the female hormone, estradiol. The figure shows the results of a study we conducted of distribution in river areas.

High resolution GCMS is a particularly necessary tool for analyzing extremely minute quantities of substances requiring high sensitivity such as dioxin. Dioxin is analyzed in this special area.

The situation cannot be grasped only by scientific measurement using analytic devices, so the experiment also involves using various biological test methods. Here for example we are using a type of test that uses a binding assay with hormone receptors called “estrogen receptors” as an index. Research is also conducted using for example an enzyme immuno-assay or a two-hybrid gene assay.

As a type of detection of the impact of freshwater organisms, we primarily use medaka, but we also use several other types of organisms. For example, we use paratya compressa improvisa and insects such as culex pipens molestus Forskal, as well as a fish called “Beta”.

Here is an example of a study conducted in the field to see what kind of phenomena occur. There have been reports for example of male sinotaia gradarata histrica turning into females at Kasumigaura. We are therefore going there to get a grasp of the situation. This sort of sampling can only be conducted at an extremely limited number of places such as Kasumigaura where the natural lakeshore and seashore are gradually eroding. From the standpoint of conservation of nature, I think that problems include not only chemical substances, but the way levees are constructed as well. In either case, using sinotaia gradarata histrica, we are conducting a wide-ranging study that encompasses the morphology and appearance of gonads, proportion of males to females, and habitat of the specimens.

Here is the impact on marine organisms. One of our researchers, Dr. Horiguchi, has been involved primarily in research involving reishia clavigera (kuster), but is now gradually expanding the study to include conches. With the phenomenon of imposex in the type of conch called reishia clavigera (kuster), if the condition becomes extremely severe, the eggs contained within rot and the organism becomes incapable of laying eggs. This phenomenon is observed in an extremely wide range of the world of conches.

On the right we have members of the abalone family that have the highest commercial value among conches. Abalone are not equipped with a penis, but among the eggs of female abalone, we have sperm dyed black as you see here, so you can see that sperm is formed. We are now studying if such phenomena are related to the recent decrease in a haul of abalone in Japan. We know that this phenomenon is mainly caused by toxic substances used to protect the hulls of ships called “organotins.” Measures to address this problem are currently being implemented, but problems remain, including what to do about the organotins that have settled on the seabed. Organotins not only affect conches; they affect fish as well. Joint research in this area is now being conducted together with researchers of other universities.

One of the endocrine disrupters that has attracted the most attention is dioxin. The group studying dioxin discovered that the health of fetuses was susceptible to the minutest quantities of dioxin. Experiments are being conducted to monitor the health of adults whose mothers had been exposed to minute quantities of dioxin while the subjects were still in the fetal stage.

In these experiments, pregnant rats are administered doses of dioxin – here you see the swollen belly. When the baby is born, it is raised to adulthood and then observed to see what effects the substance has on their health. Here we have the distance from the anus to the sexual organs. There is a close

correlation between this distance and sexual development into males and females; this is the technique introduced by Dr. Aoyama a little while ago. After an extremely small dose of dioxin is administered, the difference becomes prominent when the subjects mature. The asterisk indicates where a statistical significance can be seen.

We know that endocrine disrupters affect not only the reproductive system, but the immune system and cranial nervous system as well. We are currently expanding our research of endocrine disrupters to include effect on the brain and nervous system.

One part of this research involves observing how various pollutants impede function of the hippocampus, which has an important role in memory. We have introduced a magnetic resonance imaging method, or MRI, in the laboratory to observe the workings of the human brain. We are using the equipment to detect whether contamination by chemical substances affects brain morphology and function. It is expected to play an important role in our research.

We are studying the effect of such substances on behavior using several types of organisms. Here for instance we have a quail. We are observing these animals to see if minute quantities of endocrine disrupters cause feminization of the brain of quail. Here we have a mouse, and this is a fish called “Beta” – they’re easily riled. We’re studying the effect of endocrine disrupters on behavior of such fish.

This part concerns data. We have prepared several data systems, and are attempting to create a tool to help us find out the endocrine disrupter and its effect and causal relationship of them. We feed information concerning the status of contamination of several endocrine disrupters and information concerning various effects that may be related to those substance put into the geographical data system and use it to analyze the whole data.

This shows how a female hormone, called “estradiol” shows up in rivers and the degree to which estradiol released into rivers has an impact on the environment. Estradiol is contained in the urine of females and is carried into the environment together with sewage. Population pressure places the largest stress on the environment. If we connect the estradiol observed in environmental monitoring and superimpose it on a map, it would appear as you see here.

Here is another example. It has been pointed out that endocrine disrupters may cause a difference in the male and female sexual organs. This map shows the distribution of excessive proportions of males and females in Japan.

This is a nationwide comparison of the Prime Minister’s Office reports from 1990 to 1995. The places where an inordinate number of females were born are indicated in red and the places where an inordinate number of males were born are indicated in blue. Of course, we have no idea what this means by itself, but we are trying to analyze the data by superimposing the status of contamination by endocrine disrupters and various types of data that was superimposed on the map a little while ago on the same data. Incidentally, in the case of the Kanto area, it is high in the Shibuya ward of Tokyo, but we do not as of yet understand the significance of this. New tools for analysis are however being developed.

Research of methods to combat or prevent these problems is progressing. A particularly large effort is being made to purify endocrine disrupters through biotechnologies that utilize plants and

microorganisms. Here is one example of this. If you take tobacco bio-cells and then introduce a probable endocrine disrupter called “bisphenol A,” the cells immediately take in the bisphenol A till no more remains. A separate substance other than bisphenol A is simultaneously produced in the cells. When a bioassay of this substance is conducted, we find that this substance group loses its female hormone effect. In this sense, bisphenol A has been deactivated. We are trying to find out if various other plants have this endocrine disrupter purifying effect. We are currently testing various plants.

Finally I would like to talk about how such various types of information are provided to others. We are currently preparing a database, and soon various types of data will be available to people outside our organization. Concerning information on substances, we are currently preparing databases containing information on substances, effects of those substances and methods of analysis. We are also considering allowing people outside our organization to access the information.

We are also preparing an environmental information database. We are planning to make it available by superimposing the environmental data upon geographical data. We are accumulating data and plan to expand specimen banking, which has been partiality done in some places up to now. We therefore plan to collect samples gathered from all over the world in one place and use them as a sort of time capsule in the future.

Various types of research are currently being conducted in Japan. Research of endocrine disrupters in Japan is rapidly growing, and has now grown to a global level. Along with making inventory of such researches, we are considering a system that facilitates access from all over the world.

As we prepare a natural system of analyzing environmental data including this, we hope to build a foundation to support risk management for all endocrine disrupters. Although not yet complete, I would like to help provide safety and freedom from anxiety about endocrine disrupter by providing the people of Japan and the world community with information about these substances.

Although my presentation was not as well structured as I would have liked, I hope you got an idea of our research activities and the direction. Thank you for your attention.