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A Trend of Hazardous Substance Detection in the Environment

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6.1 Introduction

Environmental pollution manifesting in the form of serious diseases such as the Minamata disease in the 1950s through the 1970s was caused by limited kinds of pollutants including locally accumulated organic mercury. Today, we are facing threats from environmental pollution caused by a variety of hazardous substances that are present in a minimal-quantity but over a vast extent, (for example, endocrine disrupters including nonylphenol and dioxins).

Today, physical and chemical measurement techniques are employed as the mainstream of measuring technology applied to such trace hazardous substances, but this does not necessarily mean that they are satisfactory as environmental measurement techniques. In recent years, environmental measurement techniques using biological means have drawn greater attention as a means to supplement the former, and various R&D efforts have been started.

In this article, the recent trend of measuring technology for trace hazardous substances using physical, chemical and biological techniques will be reviewed.

6.2 Physical and chemical techniques

Environmental measurement using physical and chemical techniques includes gas chromatography /mass spectrograph (GC/MS) measurement and high-performance liquid chromatography/mass spectrograph (HPLC/MS) measurement. The problems currently experienced in conducting environmental measurement with GC/MS are depicted below.

6.2.1 GC/MS

GC/MS has become one of the most powerful tools for the analysis of complex organic and biochemical mixtures. GC/MS consists of two components; (i) gas chromatography (GC), which single out chemical substances and (ii) mass spectrometry (MS), which quantitatively identifies each chemical substance.

By the way, what is the required level of accuracy for GC/MS in measuring trace hazardous substances? For dioxins, as an example, which are known for their high toxicity levels, the Japan Industrial Standards (JIS) adopted the lower limit of detection in 1999 as follows.

According to JIS, the lower limit of detection is 0.1 pg (pico gram = one trillionth of a gram) for the tetra-chlorinated and the penta-chlorinated, 0.2 pg for the hexa-chlorinated and the hepta-chlorinated, 0.5 pg for the octa-chlorinated and 0.2 pg for coplanar PCB [1]. Therefore GC/MS must have a very high accuracy to detect very small amounts of hazardous chemicals.

Incidentally, the presence of these substances in the environment is normally expressed by the concentration level (expressed by the mass of such a substance contained in 1g of water. Concentration of chemical substances in the environment is expressed in terms of ppm (parts per million), ppb (part per billion) and ppt (parts per trillion).

6.2.2 Problems of measurement with GC/MS

To measure traces of hazardous substances by GC/MS, sample pretreatment such as singling out an object substance from samples (extraction, refining, etc.) is necessary, making the process more complicated, expensive and time consuming, than that of normal substances.

A measurement of dioxins requires an accuracy level in the order of ppt. An expensive high-accuracy equipment (which costs 100 million Yen or so) and as well as time-consuming pretreatment are necessary. Even when a measurement is requested from an outside specialist organ, it costs 200,000 Yen and takes 1 to 2 weeks per sample. Although on-the-spot measurement is desirable to prevent contamination at places other than sampling by object substances of measurement, realization of on-the-spot measurement is difficult due to the involvement of large-scale equipment.

Due to these problems, it is also difficult to conduct measurement for a long period and frequently in a factory or at a number of points scattered over an extensive area.

6.3 Biological techniques

In recent years, environmental detection using biological means has begun to draw our attention. These techniques can be classified as

1) techniques using individual organisms;
2) techniques using organic substances;
3) techniques using gene recombined organisms.

6.3.1 Techniques using individual organisms

In this technique, an/a group of animals or plants is/are used. The toxicity degree of the applied chemical substances can be measured from the changes of the animal(s) or plant(s).

For example, groups of mice or Japanese killifish (medaka) were fed with different concentration levels of endocrine. An individual organ, such as a spermary or ovary, of the mice was investigated. This can bee used to explain a breeding ability of mice in the environment contaminated with endocrine for several months.

In terms of quantitative measurement, such techniques have limitations in providing accurate information. Moreover, time duration ranging from several weeks to several years is needed in some cases. However, these techniques offer a relatively easier means to investigate the influence of chemical substances on living bodies, which cannot be performed by physical or chemical techniques. In this sense, such biological techniques offer means suitable for determination of toxicity for environmental measurement.

6.3.2 Techniques using organic substances

Here what we call organic substances are receptors and antibodies produced in living bodies. These techniques employ a system utilizing such organic substances, or a biosensor, for measurement.

Biosensors using receptors or antibodies are introduced below.

(1) Biosensors utilizing receptors

Dr. Masaharu Murata of the Engineering Study Course of the Post Graduate School of Kyushu University has developed a new small biosensor that can response to endocrine disrupters within 1 minute.

This sensor has female hormone (estrogen) receptors held on an electrode. When the endocrine disrupter, which is similar to the female hormone, is present. The receptors change their three-dimensional structure significantly. This leads to a change in the conductance of the receptors, allowing us to determine the concentration of the endocrine disrupter accurately. Using this principle, any endocrine disrupters can be measured, if the appropriate receptors are applied.

(2) Biosensor utilizing antigen-antibody reaction

Dr. Naoya Omura of the Abiko Central Research Institute of the Electric Power Industry has developed a simple biosensor utilizing antigen-antibody reaction that can measure several kinds of female hormones, PCB, etc., present in an extremely small quantity with quite high sensitivity.

Object antigens for measurement are adsorbed on the surfaces of plastic balls of a diameter measuring around 100μm and held in a measuring device. Then sample fluid mixed with antibodies marked with fluorescent coloring matter and a sample is flown through the measuring device. If antigens are not included in the sample fluid, all antibodies marked with fluorescent coloring matter are coupled with antigens on the plastic balls and captured. So, the plastic balls will emit strong light, when light is irradiated onto them.

On the other hand, if the sample fluid contains antigens, antibodies marked with fluorescent coloring matter are coupled with antigens present in the sample fluid. In this situation, less antibodies are coupled with antigens on the plastic balls, making the intensity of light weaker. From the difference of light intensity measured between when antigens are present in sample fluid and when they are not, the quantity of antigens that are present in the sample fluid can be measured. In the case of estradiol (E2), a kind of female hormone, the sensor can detect it at a concentration level as low as 1 ppt, within 10 minutes.

The Institute is planning to develop a portable type instrument with a size of 30 cm × 30 cm × 30 cm within this year.

6.3.3 *Techniques using gene recombinant organisms*

These techniques use gene recombinant organisms to show the influence of hazardous substances. These organisms are called reporters. The reporter organisms include enzymes that change color or emit light when exposed to specific hormones or substances.

The following is an example of a reporter plant study.

Associate professor Kenichi Yamazaki of the Graduate School of Environmental Earth Science of Hokkaido University and his colleagues are creating a gene-recombinant plant that can detect endocrine disrupters in water or soil.

This plant has the genes representing a female hormone receptor and a gene representing a fluorescent protein substituting the specific protein manifestation of which is induced by the said female hormone through gene manipulation. As a result, when the plant takes in an endocrine disrupter that activates the female hormone, the fluorescent protein is produced within the plant body.

Through measurement of the intensity of green fluorescence emission during the irradiation of blue light onto the plant, quantitative measurement of the fluorescent protein in the plant body can be made relatively easily. When the quantity of an endocrine disrupter increases, the quantity of fluorescent protein also increases, thus, in their opinion, making estimation of the quantity possible through measurement of the intensity of fluorescence emission from the plant body.

6.4 Direction of environmental measurement

The characteristics of physical and chemical techniques and the three types of biological techniques are compared in Chart 1. The quantity acting on organisms means the quantity of the substance coupled with specific parts (such as receptors and antibodies) of organisms. This parameter is essential since the hazardous substances present in the environment do not show any influence unless they really act on the organisms. Time, cost, and restriction of the place for measurement are considered as the ease and convenience of measurement.

The physical and chemical techniques can

Chart 1: Comparison of environmental measurement techniques

Environmental measurement techniques	Measurement of the quantity of a substance present in the environment	Measurement of the quantity acting on organisms	Ease and convenience of measurement
Physical and chemical techniques	◎ (possible up to ppq)	×	△
Techniques using individual organisms	△	×	△
Techniques using organic substances	○ (possible up to ppt)	◎	◎
Techniques using gene recombinant organisms	△	○	○

◎: Most suitable ○: Suitable △: Suitable to a limited extent ×: Not suitable

Prepared by the Science and Technology Foresight Center accurately measure the amount of hazardous substances present in the sample. In December 2000, one Japanese electronic instrument maker developed the equipment that can measure dioxins as low as the order of ppq (parts per quadrillion).

However, these techniques are not convenient as it is not portable and a sample pretreatment is needed. Moreover, the influence of hazardous substance on the organisms cannot be determined. Therefore the instrument which are smaller in size lower in cost and allows simplified pretreatment are being developed.

Techniques using individual organisms offer an approximate measurement. The influence of hazardous substances can be observed. They, however, lack of the ease and convenience, as they require long-time observation, e.g. breeding, under consistent conditions. At present, the available techniques allow the measurement of a single substance at a time. Efforts are being made to establish a technique able to measure more than one substance at a time.

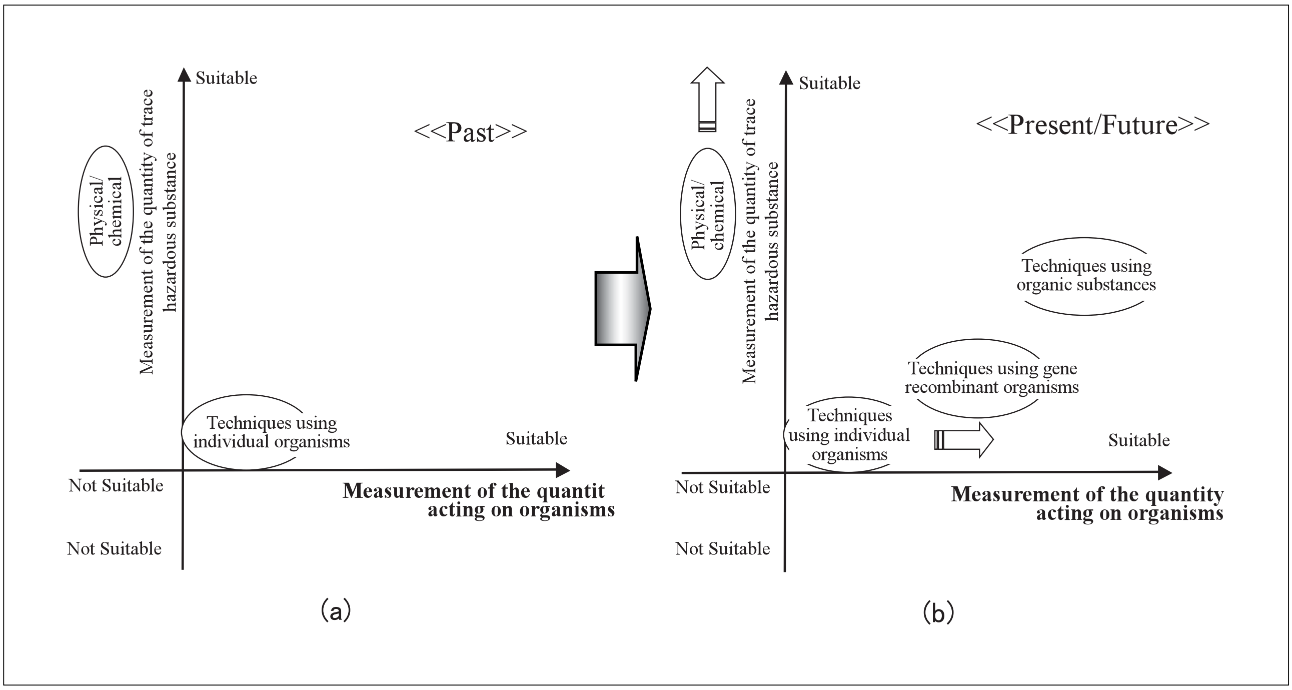
Techniques using organic substances employ a compact sensor for measurement. They offer highly accurate measurement (up to the order of ppt), short time consuming, portability and low cost. On condition that the testing samples are limited and sensitive. Therefore care must be taken during the measurement. Otherwise the measurement result can be estimated in error.

Techniques using gene recombinant organisms are simple and easy. The reporter plants are grown using testing water or soil containing hazardous substance(s). However, organic substances and genes used as samples in this technique are very limited. If these techniques are developed, long-term and fixed-point observation will be possible.

The four environmental measurement techniques are shown in Chart 2, according to their accuracy levels.

As indicated in Chart 2(a), physical and chemical techniques assumed the responsibility of measurement of the mass of a substance and techniques using individual organisms, the quantity acting on organisms at a measurement level indicating an approximate yardstick of its influence in past environmental measurement practices. This basic structure is beginning to change with the enhancement of biological techniques and is now heading for the structure shown in Chart 2(b) as the current and future trend. In other words, the trend toward increasingly enhanced measurement of the quantity acting on organisms can be recognized from these figures. At the same time, the accuracy of physical and chemical techniques is being improved and developed toward the direction

Chart 2: Classification of environmental measurement/assessment techniques (objects of measurement/assessment)



shown by an arrow in the Chart 2(b) is also observed for techniques using individual organisms.

6.5 Conclusion

Biological techniques using organic substances and gene recombinant organisms are only in their infant R&D stages. To establish these techniques and enrich the available options for environmental measurement, efforts should be made in the following areas.

Normally, creation of antibodies takes several months and it is difficult to create antibodies for a substance that has toxicity against an animal body. Accordingly, it is desired to create a system that renders support in distributing or creating antibodies and offers a basis to R&D of organic substances usable for a sensor such as antibodies.

It is also considered to be necessary to promote follow-up tests of newly developed techniques for their verification, and give an official certification when such technique is found appropriate as a measuring method to help such technology spread widely.

The law of "Pollutant Release and Transfer Register (PRTR)" was enforced in March last year, and management of chemical substances was enhanced to prevent problems harmful to environmental preservation before they actually occur. The said law also states clearly that it is the government's responsibility to promote researches in a comprehensive manner to obtain scientific knowledge on the influence of specified chemical substances affecting human health, animals and plants.

In view of such legislation, the ability to perform monitoring of chemical substances including hazardous substances mentioned in this report for their movement in the environment and the assessment of their influence on human bodies and the ecosystem in a more detailed and simpler manner has become necessary.

Therefore, it is desirable to advance R&D of biological environmental measurement technologies to obtain diverse means for measurement, while continuing to improve the physical and chemical techniques, so that they may offer appropriate combinations to realize effective environmental measurement.

Notes:

[1] Taken from JIS K0311, "Method of measurement of dioxins and coplanar PCB in exhaust gases," and JIS K0312, "Method of measurement of dioxins and coplanar PCB in industrial water and plant wastewater."