

APPLICATION OF SIMPLIFIED ANALYTICAL METHODS (FOR DIOXIN TESTING) THAT COMPLY WITH JAPANESE REGULATIONS

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1. Introduction

Japan enacted the Law Concerning Special Measures against Dioxins (Law No. 105 of 1999, promulgated on July 16, 1999) to prevent and eliminate serious impacts on the environment and human health caused by dioxins (polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and co-planar polychlorinated biphenyls (co-PCB)). In response to the Stockholm Convention on persistent organic pollutants, which entered into force in May 2004, Japan promotes the reduction of emissions of these substances by the use of the best available techniques and environmental practices.

In order to promote effective and efficient monitoring, Japan has also decided to introduce low-cost, rapid, and simplified analytical methods based on the characteristic of the substances concerned, and has been promoting the development of such methods.

This paper outlines how these simplified analytical methods are applied under Japanese regulations.

2. Application of bioanalytical method for emission gas and ash from waste incinerators

The Ministry of the Environment established an expert group (chaired by Masatoshi MORITA, National Institute for Environmental Studies) in 2003 to examine, from the technical perspective, the potential to use the bioassay method. This method has been attracting attention as a simplified analytical method—due to its advantages in terms of cost and time required for measurement. The expert group invited submissions of technologies to be examined, then conducted tests using dioxin standard mixtures and interviews of applicants. The expert group found that some of the technologies were adequate for monitoring at facilities where emission standards permitted high concentrations.

Based on these findings, it was decided to attempt the gradual introduction of simplified analytical methods in Japan in applications where the measurement technologies were adequately developed. To begin with, it was decided that these methods should be used to measure the pollution caused by dioxins in emission gases, incineration ash (fly ash and bottom ash) from waste incinerators. (One reason for this decision was that although the government required operators of waste incinerators having a capacity of more than 50kg/hour to measure their emission gases not less than once a year, small operators in particular

Analysis - Biological methods

were failing to do so due to the high cost of implementation.) The expert group conducted a technical evaluation and examination based on reproducibility of the correlation with a measurement method that utilizes a high resolution gas chromatograph-mass spectrometer system (HRGC/HRMS) for dioxins, which was the official method in 2005. As a result, in September 2005, the Ministry amended the existing law to allow the use of the following simplified analytical methods based on bioassays, as methods for measuring dioxins in emission gases from incinerators having an incineration capacity of more than 50 kg/hour and less than 2,000 kg/hour, and for verifying incineration ash (fly ash and bottom ash) from all waste incinerators (Table 1):

- Arylhydrocarbon Receptor (AhR)/ Reporter gene bioassay (3 methods)
- Enzyme-linked immunosorbent assay (1 method)

It should be noted that government bodies may not use these methods where high accuracy is necessary for verifying violations of emission standards.

3. Application of simplified analytical methods for sediment and soil

In March 2005 the expert group invited submissions of simplified analytical methods for it to consider, with a view to evaluate and examine technical aspects relating to their possible application to measure various types of soil and sediment contamination. Based on the results of this review and additional investigation, it was decided that the expert group would examine measuring methods for sediment and soil, with the goal of having the government make its recommendations in 2009.

In particular, the group is considering the potential to use these measurement methods as screening methods for HRGC/HRMS (the officially-sanctioned methods), for the purpose of assessing the scope of countermeasures needed to deal with contaminated soil; verifying the presence of pollution when land is bought and sold and helping to determine its source; and determining appropriate responses to accidents.

4. Quality control and assurance in bioanalytical methods

It is important to note that bioassays often do not produce a linear response to dioxin concentrations, a point that differs from HRGC/HRMS methods. Thus, under the bioassay method, the approaches to deal with detection limits and quantification limits differ from the HRGC/HRMS method. In addition, with the bioassay method, confirmation of the recovery rate is difficult because it is difficult to add sampling spiking, clean-up spiking and syringe spiking to internal samples. To address the difficulties of using the bioassay method, in March 2006 the government issued a guide for quality control and assurance when using bioanalytical methods for emission gas, incineration ash (fly ash and bottom ash) from waste incinerators.

5. Conclusin

More progress can be expected in the future due to research and development into technologies for simplified analytical methods, and it is certainly desirable that such methods find more uses in various areas of measurement. In the future, we will examine simplified analytical methods other than those introduced previously (e.g., quadrupole mass spectrometer system), and methods for media other than emission gas, incineration ash (fly ash and bottom ash), sediment, and soil.

Table 1. Official bioanalytical methods for dioxins in japan

Method		CALUX® Assay ¹	P450HRGS (P450Human Reporter Gene System) ²	AhR luciferase assay ³	Dio-Quicker ⁴
Principle		AhR/Reporter gene assay using recombinant (modified) H1L6.1c2 cells	AhR/Reporter gene assay using recombinant (modified) 101L cells	AhR/Reporter gene assay using recombinant (modified) HeB5 cells	Enzyme immunoassay using antibodies that identify PeCDFs/HxCDFs, that are generated from the hybridoma of a mouse origin
Pre-treatment	Extraction	Soxhlet extraction (with toluene, more than 16 hours) or Liquid-Liquid extraction (with methylene chloride, 3 times)			
	Cleanup	Sulfuric acid-impregnated silica gel and Activated carbon column	Sulfuric acid-impregnated silica gel and Activated carbon column	Multilayered silica gel column	Multilayered silica gel and Activated carbon column
Quantification		Determine the sample concentration from the standard curve developed using the standard samples. The corresponding toxicity equivalence is then determined by multiplying this value by the specific conversion coefficient of each medium.			
Analytical time (excluding of the pre-treatment method)		2 - 3 days	About 2 days	3 - 5 days	About 4 hours
Developers		Xenobiotic Detection Systems International, Inc.	Columbia Analytical Services	Sumitomo Chemical Co., Ltd.	TAKUMA Co., Ltd.
Vendors		Hiyoshi Co., Ltd.	Nihon Environmental Services Co., Ltd.	Sumika Chemical Analysis Service, Ltd.	Kankyo Sol-tech Co., Ltd.

1 <http://www.dioxins.com/pages/licensespage.shtml> , <http://www.calux.jp/>2 <http://www.caslab.com/>, <http://www.n-kankyo.com/gyoumu/epa.html>3 http://www.sumitomo-chem.co.jp/japanese/csr/pdf/2004/csr_report2004.pdf ,
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