

CALUX Assay as Screening Method of Human Specimens for Dioxin Contamination

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Introduction

There are growing concerns regarding human health effects of dioxins and dioxin-like polychlorinated biphenyls (coPCBs) to groups at higher risk for exposure as well as the general population. Additionally, in utero exposure to the foetus may cause developmental malformations as well as alteration of immune, nerve and endocrine systems. We need to establish prospective cohort studies to evaluate risks of low-dose exposures over an extended period of time. For these studies it is necessary to facilitate development of simpler, quicker, cheaper and more precise measurement of dioxin body burden.

To assess concentrations of dioxins and dioxin-like chemicals in human biological samples, blood may be the most practical specimen for epidemiological study. However, the current gold standard method of detection using high-resolution gas chromatography/mass spectrometer (HRGC/MS) requires collecting 50 ml to 100 ml of blood to accurately detect all the congeners of dioxins and coPCB for WHO-TEQ calculation. The sampling volume is too much for ordinary volunteers in epidemiological studies. In occupational setting, welders, workers around coke ovens, kilns and demolition site of municipal incinerators suffer from higher exposure to dioxins. But occupational monitoring of dioxin exposure is not feasible considering the sample volume needed for HRGC/MS.

Additionally, the measurement by HRGC/MS is expensive and requires several weeks. These conditions make it difficult to conduct large-scale epidemiological studies in the general population and workers at higher risk. In this paper, we demonstrate validation data of a less expensive and quicker bioassay, CALUX (Chemically-Activated Luciferase eXpression) cell assay, for application to human epidemiological studies. The CALUX assay uses a genetically modified mammalian cell line that contains the firefly luciferase gene as a reporter for dioxin exposure.

Materials and Methods

To validate CALUX assay, results for test samples measure by CALUX cell bioassay at Hiyoshi Corporation were compared in a double blind study to HRGC/MS results in adipose tissue and blood measured at Shimazu Techno-Research (Kyoto, Japan), and milk specimens measured at Otsuka Assay Research Laboratory (Tokushima, Japan). Test samples included 21 fat tissue samples collected from autopsy cases in the Tokyo Medical Examiner's Office, for which we obtained informed consent of the family of the deceased. In addition, we collected 70 ml blood samples from volunteers in the medical school. The blood samples were divided into 20 ml and 50 ml aliquots, used for CALUX assay and for HRGC-MS measurement, respectively. Seventy-nine samples of human breast-milk were donated voluntarily from mothers in Shenyang and Dialen in China.

Results and Discussion

The mean concentration of polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in 21 human fat tissues by HRGC/MS was 37 pg-TEQ/g fat, (median: 33 pg-TEQ/g fat, range 16.7-72 pg-TEQ/g fat). The mean concentration of PCDD+PCDF+coPCB was 42.5 pg TEQ/g fat (median: 44 pg-TEQ/g fat, range: 18.5 - 83.8 pg-TEQ/g fat). When the results measured by both HRGC-MS and CALUX bioassay were compared, the correlation coefficient was as high as 0.8675 (Figure1). In addition, we measured dioxin concentrations in 21 blood samples from volunteers by both methods. Most of the samples had low dioxin concentrations. Correlation coefficient for PCDD+PCDF+co-PCB per gram fat was 0.4758. However, when the results for samples with greater than 20 pg/g fat were compared, coefficients on PCDD+PCDF per gram wet weight and per gram fat were 0.7445 and 0.6417, respectively. When we limit applications of CALUX assay for blood specimens to evaluate PCDD+PCDF fraction and set detection limit of 20 pg/g fat, the correlation coefficient of PCDD+PCDF per gram wet weight and per gram fat is 0.9161 (Figure2) and 0.8297

respectively, and practically sensitive enough for screening moderately high exposure groups for dioxins (Table 1). The correlation coefficient for PCDD+PCDF+co-PCB per gram fat of breast milk was 0.9581 (Figure 3).

These results suggest that the CALUX assay is applicable as an economical and quick method for fat tissue or fat rich samples such as breast milk. CALUX bioassay is also sensitive enough to use as a screening method for blood samples in 10 ml of sample volume. These results indicate that the CALUX bioassay can be utilized in epidemiological studies of dioxin burden among workers at risk for higher exposure and can be applicable to fat tissues and fat rich samples like milk from the general populations.

References

1. Kayama F, Hamamatsu A, Sagisaka K, Brown D, Clark G, Suzuki T (2000), The 3rd Annual Meeting of Japan Society of Endocrine Disruptors Research.
2. Brown D, Kishimoto Y, Ikeno O, Chu M, Nomura J, Murakami T, and Murata H. (2000) Organohologen Compounds 45: 200-203

FIGURE 1

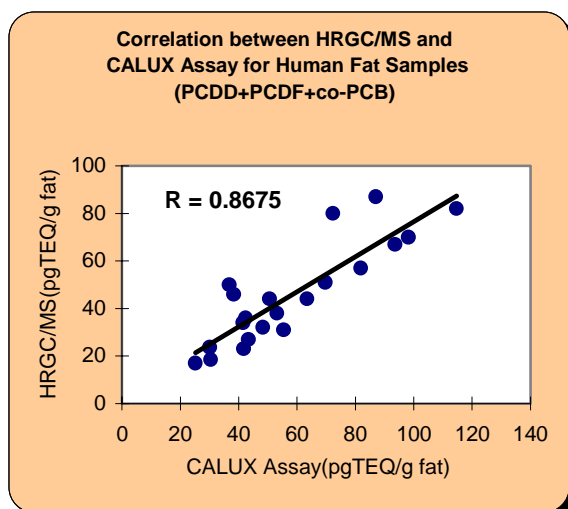


FIGURE 2

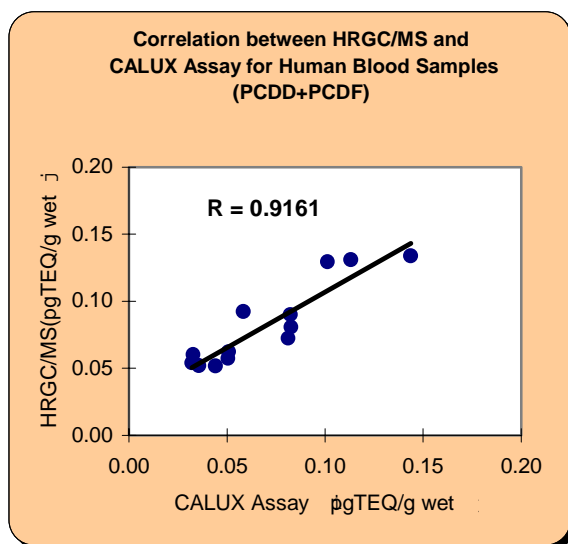


Figure 3

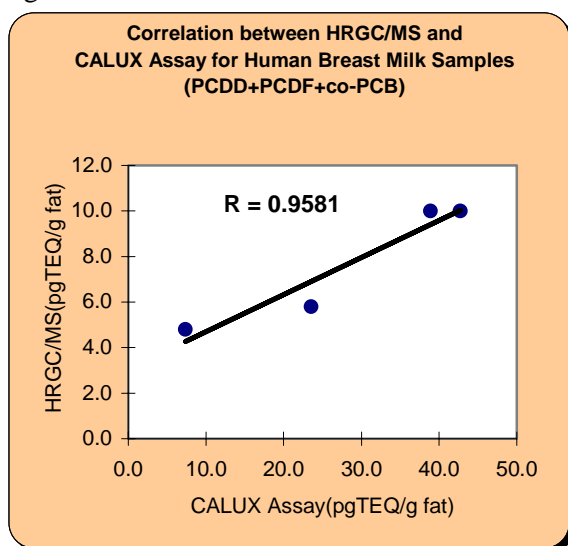


Table 1

Correlation coefficients, (R), between HRGC/MS and CALUX Assay

		pg/g wet weight	pg/g fat
PCDD+PCDF+co-PCB			
All sample	(n=21)	***	0.4758
Samples > 20pgTEQ/gFat	(n=14)	0.8218	0.7305
PCDD+PCDF			
All sample	(n=20)	0.7445	0.6417
Samples > 20pgTEQ/gFat	(n=13)	0.9161	0.8297