CALUX ASSAY IS APPLICABLE TO HUMAN EPIDEMIOLOGICAL STUDIES.

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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 fetus may cause alteration of development of immune and nerve systems as well as endocrine systems. We need to establish prospective cohort studies to evaluate risks of low-dose exposures over an extended period. For these studies it is necessary to carry out large-scale measurement of dioxin exposures.

However, current methods of detection using high resolution gas chromatography/mass spectrometer (HRGC/MS) require more than 50 ml of blood. This sampling volume is too much for ordinary volunteers in epidemiological studies. Additionally, the measurement is expensive and requires several weeks. These conditions make it difficult to conduct large-scale epidemiological studies. In this paper, we have tried to validate a less expensive and quicker bioassay, CALUX assay, for application to human epidemiological studies. CALUX assay uses a genetically modified mammalian cell line to contain the firefly luciferase gene as a reporter for dioxin exposure.

Material and Methods.

To validate CALUX assay, results for test samples were compared in a double blind study to HRGC/MS results from at a laboratory certified for dioxin measurement. 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.

Result and Discussion

The mean concentration polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in 21 human fat tissue 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 for these samples from both GC-MS and the CALUX bioassay were compared, the correlation coefficient was as high as 0.8675 (Figure1). In addition, we measured dioxin concentrations in 17 blood samples from volunteers by both methods. Most of the samples had low dioxin concentrations. Correlation coefficient for PCDD+PCDF per wet weight and per gram fat was 0.604, and 0.543, respectively. 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 0.763 (Fig.2), and 0.702, respectively. The correlation coefficient for PCDD+PCDF+coPCB per gram wet weigh of blood was 0.746. These results suggest that CALUX assay is applicable as an economical and quick method for fat tissue or fat rich samples such as breast milk. However, at low level concentration of dioxins such as in serum and blood the measurement was difficult These results indicate that the CALUX bioassay for dioxins can be utilized in epidemiological studies of dioxin burden among workers at risk for exposure and can be applicable to fat tissues and fat rich samples for individuals from the general population.

References

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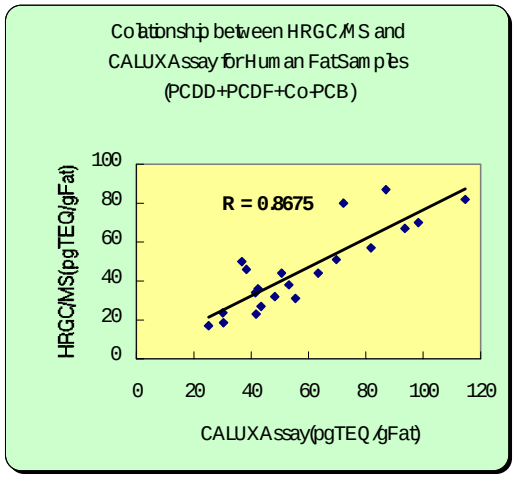

FIGURE 1

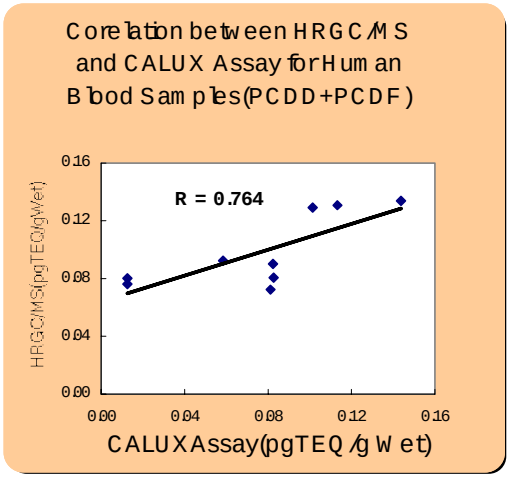

FIGURE 2