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APPLICATION OF THE CALUX™ ASSAY TO THE ANALYSIS OF DXNs IN A COMPOSITE FROM SUSHI SAMPLES (THE THIRD REPORT)

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Introduction

The main source of dioxins (DXNs) such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like coplanar polychlorinated biphenyls (Co-PCBs) is food. From the results of a total diet study in Japan, the intake of fish and shellfish was the main contribution to the dietary exposure of DXNs [1,2]. For the risk management of DXN intake from fish, frequent examination of DXN levels in fish is needed. In DXN2002, we reported that the CALUX™ (Chemically Activated Luciferase Expression) assay [3] is a useful method for the screening and monitoring of DXNs in fish samples [4], with good correlation between high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) and CALUX™ (r = 0.820). Japanese people like to eat *sushi*, a special dish composed of boiled and cooled rice with vinegar, and filets of many kinds of raw or cooked fish and shellfish, such as tuna, conger eel, salmon, squid, octopus and others. Our purpose was to determine the applicability of the CALUX™ assay to the rapid screening of DXN concentrations in one meal of *sushi*. The concentration data by CALUX™ assay in the composite mixture prepared from packed *sushi* samples were compared to those by the HRGC/HRMS method as a reference method.

Materials and Methods

Sushi samples

Twelve different packs of *sushi* samples containing 7~11 pieces were purchased at a *sushi* restaurant, take-out shop or supermarket in Japan, and all parts (fish, shellfish, etc.) without rice in one pack were weighed, mixed and ground by a food processor. The 12 prepared composite samples included everything but the rice were frozen at −80ºC until analysis. Samples No.8 and 12 contained only tuna; all other samples were composed of several kinds of fish and shellfish.

CALUX™ assay [4]

Fifteen ml of acetone and 10ml of dichloromethane/n-hexane (1:2) were added to 10 grams of the ground composite and then mixed. The mixture was centrifuged at 500rpm for five minutes. The

dichloromethane/hexane layer was applied to the extraction column, which consisted of celite and anhydrous sodium sulfate. The extraction procedures were repeated two more times. The column was washed with 10ml of dichloromethane/n-hexane (1:2) and the eluate was separated into a fraction containing PCDDs/DFs and a fraction containing Co-PCBs using an activated carbon (XCARB) column.

The assay was carried out as previously described[4]. Briefly, a patented recombinant mouse cell line that contains the luciferase reporter gene under the control of dioxin responsive elements were grown in 96-well view plates and exposed to *sushi* composite sample extracts or 2,3,7,8-TCDD standards using DMSO as the vehicle. The plates were incubated at 37ºC and 5% CO_2 for 20-24hrs to produce the optimal expression of luciferase activity. Following incubation, the medium was removed and the cells were lysed. Luciferase activity was determined using a luminometer (Lucy 1, Anthos Corp.). The luciferase activity was reported as relative light units (RLU), and expressed as pgTEQ. Results that were less than the limits of detection (LOD = 0.16pgTEQ/g) were reported as one-half the LOD.

HRGC/HRMS analysis

The extraction and cleanup of the *sushi* composite samples for HRGC/HRMS followed previously published protocols (Japan guideline method for foods and measurements relating to DXNs by Japan Ministry of Health, Labor and Welfare)[1] with a minor modification. Briefly, 100 grams of ground composite spiked with ^{13}C-labelled surrogate dioxin and Co-PCB standards. Samples were alkaline digested, and then extracted with an organic solvent. Extracts were subjected to a sequential cleanup using several column chromatography steps. The analysis of the 17 active PCDDs/DFs and 12 Co-PCBs (4 non-ortho and 8 mono-ortho PCBs) by HRGC/HRMS was carried out using SIM with a Micromass Autospec Ultima HRMS equipped with a Hewlett-Packard 6890 GC. The TEQs for PCDDs/DFs and Co-PCBs were calculated using WHO-TEF values (1998)[5].

Results and Discussion

Table 1 shows the data for the concentration of the total DXNs in the composite from *sushi* samples analyzed by the CALUX™ assay. Column A shows the mean of three replicated examinations. Column B shows the mean of two data sets selected by excluding the most deviated data among those from the three trials, because in a few samples, a slightly higher variation (more than 30%) was obtained. The mean concentrations in three trials ranged from 0.69 to 2.04pgTEQ/g depending on the kinds of fish and shellfish and other foods used to prepare the *sushi*, and the mean concentration of the 12 samples was 1.13pgTEQ/g. The mean concentrations in Column B ranged from 0.78 to 1.99pgTEQ/g and, the mean concentration of the 12 samples was 1.14pgTEQ/g, which suggests that similar results were obtained using both methods. On the basis of the fat content, these values were 9.03 to 60.2pgTEQ/g fat and the mean was 20.8pgTEQ/g fat. The DXN concentrations of the 12 composite were almost in the same range as the concentration of 19 fish samples previously determined by the CALUX™ assay[4]. Samples numbered 7 (coastal fish and shellfish) and 8 (tuna) contained rather high DXNs TEQ/g fat. In the CALUX™ assay, the mean recoveries of the DXNs and Co-PCB (#126) spiked in the No.4 sample by two trials were 70.5% and 67.2%, respectively.

Table 1 Concentration of total DXNs in 12 composite sushi samples

Sample	CALUX assay pgTEQ/g fresh weight A Mean±SD (n=3)	CALUX assay pgTEQ/g fresh weight B Mean(n=2)	CALUX assay pgTEQ/g fat Mean(n=3)	HRGC/HRMS pgTEQ/g fresh weight (n=1)
No.1	0.97±0.28	1.12	17.3	0.64
No.2	0.93±0.23	0.87	13.8	0.64
No.3	1.03±0.28	1.19	9.61	0.75
No.4	0.86±0.12	0.91	12.4	0.36
No.5	0.85±0.17	0.78	37.5	0.28
No.6	1.24±0.42	1.00	18.0	0.59
No.7	2.04±0.75	1.61	33.5	1.73
No.8	1.97±0.03	1.99	60.7	7.30
No.9	0.83±0.16	0.89	12.4	0.75
No.10	0.69±0.28	0.85	15.9	0.26
No.11	1.08±0.35	1.27	11.9	1.07
No.12	1.02±0.32	1.20	9.01	1.15

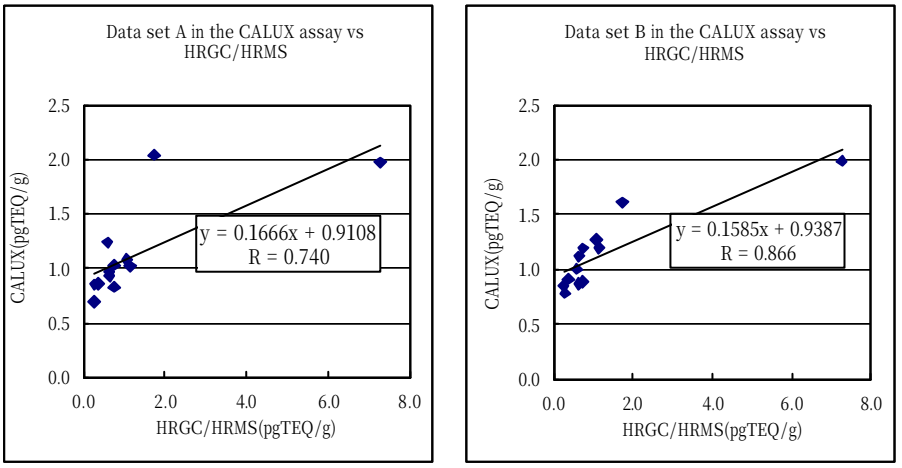

Figure 1 Correlation between CALUX™ assay and HRGC/HRMS (pgTEQ/g)
A: from data shown in Table 1-A ; B: from data shown in Table 1-B

Samples No.8 had the highest concentration (pg TEQ/g) as determined by HRGC/HRMS, and although the CALUX™ assay result was also high, it was much lower than that of HRGC/HRMS. The reason for this difference has not been clarified, but it may be due to a rather high content of dioxin-like Co-PCBs. Excluding sample No.8, the ratio of the CALUX™ assay to HRGC/HRMS ranged between 0.93 and 3.27 (mean 1.63)

in the B data set (sample No.8 ratio = 0.27). These results approximately agreed with those of the previous report that showed the CALUX™ assay had a tendency to overestimate the total TEQ compared with HRGC/HRMS in the fish samples [4]. Similar to the previous report, the lower response of Co-PCBs in the CLUX™ assay than HRGC/HRMS was also obtained in this study.

Figure 1 shows the correlation between data from the CALUX™ assay and those from the HRGC/HRMS analysis using data set A or data set B. A better correlation (r = 0.866) was obtained in the B data set that used the data selected from A. For the rapid analysis, it is recommended that if a replicated analysis by the CALUX assay in one *sushi* composite gave similar results, it is enough to use the mean value without further analysis.

This is a first report for analyzing the DXN levels by the CALUX™ assay in a composite sample from one meal of *sushi* mainly composed of fish and shellfish. It is concluded that although HRGC/HRMS is essential for precise data, the CALUX™ assay is a useful, simple and rapid tool for the purpose of screening many *sushi* composite samples, because this bioassay uses a smaller sample volume of 10 grams, costs less and has a shorter test time of within one week. Further study is under consideration to obtain more precise information on the relationship between both methods.

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