APPLICATION OF THE CALUXTM ASSAY TO THE ANALYSIS OF DXNs IN A COMPOSITE FROM SUSHI SAMPLES AND ESTIMATION OF DXN INTAKE FROM THE SUSHI ITEMS

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Introduction

The CALUX assay, which reasonably and rapidly analyzes the amounts of dioxin-toxicity (CALUX TEQ), has been widely used as a method for screenings. The assay has been applied to environmental samples such as water, atmospheric air and soils, biological samples such as milk, blood and fat, and dietary samples such as fish and shellfish[3,4]. In this study we have compared the CALUX assay with high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS), in the analysis of DXN in sushi samples and estimated the DXN intake from the sushi meal. According to the total dietary study on the DXN carried out in the past 3 years in Japan, the DXN intake from fish and shellfish accounts for 74.4% of the total dietary DXN intake[1,2]. As a sushi meal consists of various types of fish, shellfish and rice, we estimated the DXN intake from one meal of sushi using the DXN concentrations obtained by the HRGC/HRMS analysis.

Materials and Methods

We analyzed fifteen sushi samples purchased in the Tokyo and Kanagawa prefectures from October 2002 to October 2003. There were twelve mixed-sushi samples consisting of several types of fish and shellfish and three tuna sushi samples consisting of tuna. All the sushi samples were divided into the sushi items and rice alone. Each sample excluding the rice-parts was weighed, mixed and

ground by a food processor to prepare the composite samples, and then kept in a freezer at –80 until the analysis. Table 1 shows the weights, types of the items and weights of the rice among the sushi samples.

The methods for the CALUX assay and HRGC/HRMS analysis were previously reported[5,6].

The HRGC/HRMS analysis was performed in a single determination and the CALUX assay was performed in triplicate.

Table 1 Component of sushi samples — weight of items (g)

Sample No.	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10	No.11	No.12	No.13	No.14	No.15
Tuna	30.0	32.2	19.2	25.4	11.5	16.4		130.9	17.2	28.6	22.4	117.6	119.1	19.9	35.2
Yellow tail		13.9							12.9	15.3				19.7	
Conger		7.5	53.0	10.9	6.6	7.4	14.1				11.8				29.0
Japanese jack mackerel							9.4				12.6				
Salmon	24.5	16.3	6.4	9.9	7.4	13.4									
Mackerel	10.3														
Sea bream							10.9								
Olive flounder							8.8								12.6
Gizzard shad									12.8						
Alfonsino											11.0				
Squid	25.5	10.0	12.2	8.3	8.6	7.2	10.9			11.1	7.2				
Octopus						14.2			10.9						
Salmon caviar	15.0										12.6			10.0	20.1
Prawn boiled j	19.3	6.9		9.2	6.8	7.3				11.8	18.4			16.3	16.0
Prawn fresh j							11.2								
Mantis shrimp							7.7								
Crab										11.3					
Sea urchin											10.8			9.8	23.0
Scallop		9.0	15.5	11.6					14.4	14.1					
Gaper		8.2				4.8									
Ark shell															14.6
Pen shell											11.2				
Disk abalone														9.4	
Dried herring roe														11.1	
Tuna roll	3.0			8.4							26.6			27.6	18.0
Cuttlefish roll							13.5								
Avocado roll															21.0
Egg	65.0	30.4	24.3	14.0		8.8	65.2		39.9	48.9	33.8			45.2	
Rice	122.8	236.0	145.7	226.6	163.5	162.1	213.3	144.9	158.0	185.1	222.0	101.9	132.2	180.3	128.1

Results and Discussion

Correlation between the CALUX assay and HRGC/HRMS analysis

The TEQ concentrations of DXN in fifteen samples were determined by both the CALUX assay and the HRGC/HRMS analysis. Table 2 shows the DXN concentrations in the samples obtained by the two methods. The DXN concentrations with the CALUX assay were 0.69 to 2.71 (mean 1.23) pg CALUX-TEQ/g and those obtained by the HRGC/HRMS analysis were 0.26 to 7.23 pgTEQ/g. In the CALUX assay, we assumed the DXN concentrations in the

samples having no detectable DXN to be 0.08 pgCALUXTEQ/g, which was half of the detection limits.

The correlation coefficients between the two methods were 0.918 for the PCDDs/PCDFs, 0.798 for the Co-PCBs and 0.767 in the total DXN concentration (Fig. 1). There appeared to be a good correlation between the CALUX assay and the HRGC/HRMS analysis and it showed that the CALUX assay would be a useful application for the rapid screening of DXN concentrations in one meal of sushi. The No.7 sample indicated the highest discrepancy between both methods and it could be because the sample consisted mainly of eggs and was without tuna.

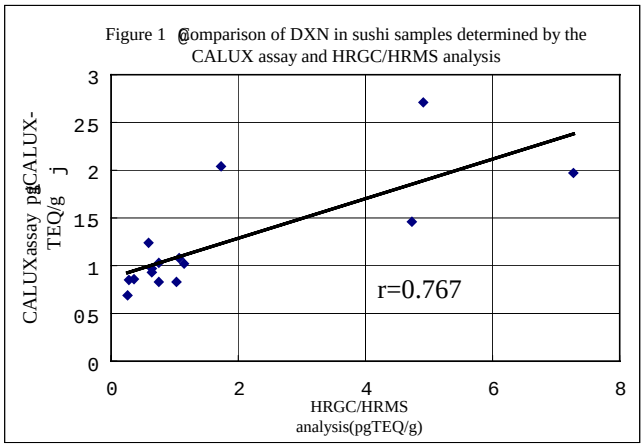

Figure 1 Comparison of DXN in sushi samples determined by the CALUX assay and HRGC/HRMS analysis

Estimation of DXN intake from the sushi items

Table 2 and Fig.2 show the DXN intake from each meal of sushi calculated from the DXN concentration obtained by the HRGC/HRMS analysis. The DXN intake from each meal was 0.2 to 18.9 (mean 5.0) pgTEQ/kgbw assuming that the average body-weight among Japanese was 50kg (Table 2). The PCDDs/PCDFs and Co-PCBs accounted for 16.4% and 83.6% respectively of the total intake (Fig.2). The DXN intake from four samples, No.7 (5.25 pgTEQ/kgbw), No.8 (18.9 pgTEQ/kgbw), No.13 (11.2 pgTEQ/kgbw) and No.15 (18.6 pgTEQ/kgbw), exceeded 4pgTEQ/kgbw, which is TDI in Japan (Table 2). Concentrations of No.8 and No.13 appeared to be higher since those samples had tuna only in the meals. No.7 and No.15 contained a higher DXN as well since they consisted of some fish and shellfish fished in the Tokyo bay-area. The investigation which has been conducted by the Fisheries Agency since 1999 revealed that DXN concentrations tended to be high among fatty tunas and adult-yellowtails, as well as fish and shellfish fished in urban areas such as Tokyo bay and Osaka bay.

Figure 2 Dioxin intake from one meal of sushi

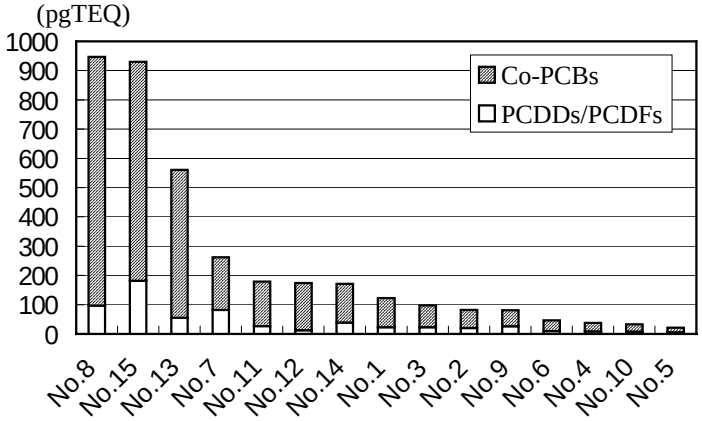

Table 2 Dioxin concentrations in sushi samples determined by the two methods and DXN intake from sushi meal

Sample No.	HRGC/HRMS analysis (pgTEQ/g) n=1	Amount of sushi [1] (g)	Intake (pgTEQ/meal)	Intake [2] (pgTEQ/kgbw)
No.1	0.64	192.6	123.3	2.5
No.2	0.64	134.4	86.0	1.7
No.3	0.75	130.6	98.0	2.0
No.4	0.36	97.7	35.2	0.7
No.5	0.28	40.9	11.5	0.2
No.6	0.59	79.5	46.9	0.9
No.7	1.73	151.7	262.4	5.2
No.8	7.23	130.9	946.4	18.9
No.9	0.75	108.1	81.1	1.6
No.10	0.26	141.1	36.7	0.7
No.11	1.07	178.4	190.9	3.8
No.12	1.15	117.6	135.2	2.7
No.13	4.71	119.1	561.0	11.2
No.14	1.01	169.0	170.7	3.4
No.15	4.91	189.5	930.4	18.6
		Average intake	247.7	5.0

[1] without rice

[2] Body weight 50kg

It would not matter even if the DXN intake exceeded the TDI by taking one meal of sushi, because the TDI is defined by continual intakes over a lifetime. The DXN intake from mixed-sushi Samples No.1 to 6, 9 to 11, and 14, which contained various kinds of fish fished in various areas was 1.8 pgTEQ/kgbw in the mean, and it was not particularly high compared with the mean of DXN daily-intake, 1.5 pgTEQ/kg, based on a total diet study in Japan in 2002. Even if we replace one meal with mixed sushi, the DXN daily-intake will not exceed the TDI.

The DXN concentrations differ depending on the types of sushi items and the sea-regions of the fishes, and the DXN absorption may decrease due to other food components such as dietary fibers, which are ingested simultaneously with DXN; therefore, a balanced dietary habit is desirable.

Acknowledgments

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