APPLICATION OF THE CALUX™ ASSAY TO THE ANALYSIS OF DXNs IN FISH (THE FIRST REPORT)

Junko Fujino [1], Tomoaki Tsutsumi[2], Yoshiaki Amakura[2]Masafumi Nakamura [1], Hiroko Kitagawa [1], Tsukasa Yamamoto [1], Kumiko Sasaki[2], Masatake Toyoda [2]

[2]National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501,Japan
[1]Hiyoshi Corporation, 908 Kitanoshocho, Omihachiman, Shiga, 523-8555, Japan

Introduction

Food has been generally recognized as the main source of human intake of polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and dioxin-like coplanar polychlorinated biphenyls (Co-PCBs); more than 90% of the total daily intake of these contaminants generally derives from food[1]. Recently, a total diet study in Japan revealed that about 60% of the dietary intake of these compounds, collectively know as dioxins (DXNs), is likely to come from the intake of fish and shellfish[2]. Therefore, monitoring the levels of DXNs in fish and shellfish would provide important information for risk assessment. Traditionally, DXNs in food and environmental samples have been analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/MS). However, HRGC/MS requires the use of expensive equipment and the sample preparation procedures are often time consuming and costly. Thus, the use of HRGC/MS is not entirely suited to the task of rapid and frequent monitoring of large numbers of samples.

One possible alternative to HRGC/MS is a bioassay for DXNs such as the CALUX™ assay. We have previously applied the CALUX™ assay to dioxin analysis in Japanese fat samples with success[3]. In this study, we describe the application of the CALUX™ assay for the monitoring of DXNs in fish samples

Materials and Methods

Fish samples

Nineteen fish samples (3 yellow tail, 3 mackerel, 2 cod, 4 tuna, 2 salmon. 2 bonito, 2 sea bass and a flatfish) purchased at the market in Japan were analyzed by the CALUX™ assay. A commercially available certified reference sample of carp was also analyzed (Wellington Laboratories, Guelph, Canada).

Sample extraction and clean-up procedures

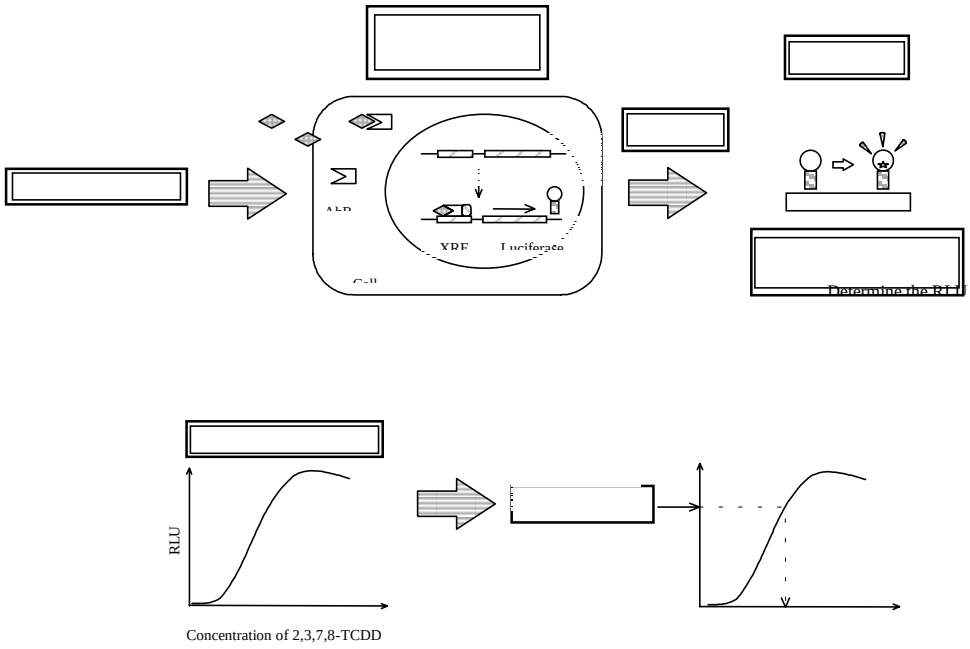
The treatment of samples for the CALUX™ assay was conducted in original way described as follows.

1. Grind sample and aliquot 10 grams of sample.
2. Add 15 milliliters of acetone to sample aliquot.
3. Add 10 milliliters of dichloromethane/hexane (1:2) and mix.
4. Centrifuge the mixture at 500 rpm for five minutes to separate the phases.
5. Apply the dichloromethane/hexane layer to the extraction column.
6. Repeat step 3 through 5 two times.
7. Wash the column with 10 milliliters of dichloromethane/hexane (1:2).
8. Following concentration, the sample extract was cleaned up and separated into a fraction containing PCDDs/PCDFs and a fraction containing Co-PCBs.

CALUX^{TM} *Assay*

The CALUX^{TM} assay uses a patented recombinant mouse cell line that contains the luciferase reporter gene under control of dioxin responsive elements[4]. When these cells are exposed to environmental ligands such as DXNs, luciferase protein is synthesized. The amount of light produced by the luciferase protein is directly related to DXNs-TEQ. The CALUX^{TM} assay method used has been described previously[5]. Briefly, the cells were grown in the 96-well view plates and exposed to fish sample extracts and 2,3,7,8-TCDD standards (250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.9, 0.9, 0.5 ppt), using DMSO as the vehicle (final DMSO concentration 1% in cell culture medium). The plates were incubated at 37^{o} C and 5% CO_2 for 20 hours to produce 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 produced by Anthos Corp.). Luciferase activity was reported as relative light units (RLU).

Figure 1. Method of the CALUX^{TM} analysis of the samples



HRGC/MS analysis

The extraction and cleanup of samples for HRGC/MS followed previously published protocols [6]. The analysis of the 17 active PCDDs/Fs and 12 Co-PCBs (non-ortho and mono-ortho PCBs) were performed by HRGC/MS using a HP6890 plus gas chromatograph coupled to a JMS-700 mass spectrometer (JEOL Ltd., Japan). The TEQ concentrations were calculated using the WHO-TEFs (1997).

Results and Discussion

Table 1 shows the data of the concentration of DXNs in fish analyzed by the CALUX™ assay. The averages of TEQ of salmon, tuna, bonito, candlefish, flatfish, mackerel, sea bass and yellowtail were 1.948, 1.368, 0.496, 1.432, 0.759, 1.110, 1.292 and 3.697 pg-TEQ/g wet weight (wg) respectively. We previously reported the data of the concentration of DXNs in Japanese fishes. From that study the averages of TEQ of flatfishes, mackerels and sea basses were 0.404, 0.992 and 10.397 pg-TEQ/wg respectively[5]. These data indicate that TEQ levels of flatfishes or mackerels analyzed by the CALUX™ assay are approximately equal to the data by HRGC/MS.

Furthermore the samples of commercially available carp whose DXNs concentration was certified were analyzed by the CALUX™ assay and these results were compared with the data analyzed by HRGC/MS (Table 1). PCDDs/Fs-TEQ analyzed by the CALUX™ assay and by HRGC/MS were 23.300 and 19.681 pg-TEQ/wg respectively. Co-PCBs-TEQ analyzed by the CALUX™ assay and by HRGC/MS were 9.345 and 69.404 pg-TEQ/wg respectively. TEQ analyzed by the CALUX™ assay and analyzed by HRGC/MS was 32.645 pg-TEQ/wg and 89.085 pg-TEQ/wg respectively. The CALUX™ assay results and HRGC/MS data were very similar but there are small differences that might be dependent on the characteristic of low sensitivity to Co-PCBs of the CALUX™ assay.

Table 1. Concentration of PCDDs/Fs and Co-PCBs in fish

Sample		pg TEQ/g on wet weight basis		
		PCDD/Fs	Dioxin-like PCBs	PCDD/Fs+dioxin-like PCBs
Salmon	No.1	0.756	0.311	1.067
	No.2	2.056	0.773	2.829
	Mean	1.406	0.542	1.948
Tuna	No.1	ND	ND	ND
	No.2	2.375	0.385	2.760
	No.3	2.000	0.710	2.700
	No.4	ND	ND	ND
	Mean	1.094	0.274	1.368
Bonito	No.1	0.435	0.120	0.555
	No.2	0.240	0.197	0.437
	Mean	0.338	0.159	0.496
Cod	No.1	0.968	0.579	1.547
	No.2	0.693	0.623	1.316
	Mean	0.831	0.601	1.432
Flat fish	No.1	0.524	0.235	0.759
Mackerel	No.1	1.638	0.754	2.392
	No.2	0.498	ND	0.498
	No.3	0.340	0.100	0.440
	Mean	0.825	0.285	1.110
Sea bass	No.1	1.505	0.553	2.058
	No.2	0.526	ND	0.526
	Mean	1.016	0.277	1.292
Yellowtail	No.1	2.511	1.038	3.549
	No.2	3.501	1.646	5.147
	No.3	1.666	0.729	2.395
	Mean	2.559	1.138	3.697
Carp	No.1	23.300 (19.681)	9.345 (69.404)	32.645 (89.085)

The values in parentheses were determined by HRGC/HRMS.

Conclusions

Considering Japanese lifestyle of eating, it should be necessary for its safety to screen more

kinds of fishes and to monitor the DXNs in fish more frequently and continuously. But now in Japan few extensive surveys of the concentrations of DXNs in foods such as fishes were conducted. In this study we applied CALUX™ assay to the analysis of DXNs in fishes. This result confirmed its similarity to HRGC/MS, thereby from the standpoint of its rapidity and costs CALUX™ assay will be useful for monitoring and screening. Belgium government's Scientific Institute of Public Health has already reported DXNs in foods such as a chicken fat sample, porcine fat samples and samples of eggs analyzed by the CALUX™ assay[6]. That report also showed the usefulness of the CALUX™ assay as an alternative method for HRGC/MS to determine TEQ levels in foods. More data of the concentration of DXNs in fish are required to verify the correlation of CALUX™ assay with HRGC/MS method.

References

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