DETERMINATION OF PCDDS/DFS AND DIOXIN-LIKE PCBS IN EGG AND FISH FEED SAMPLES IN TAIWAN BY THE DR CALUX® BIOASSAY

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Abstract:

After an in-depth training and a 3-phase cross-validation by staff of the BioDetection Systems, we obtained the licence for the DR CALUX® bioassay. The control charts were initially set up by using 1 pM and 3 pM 2,3,7,8-TCDD and two certified reference materials for quality assurance and quality control (QA/QC) of performing the DR CALUX® bioassay in our laboratory. Thirty-one egg, six eel-feed, nine seabass-feed, and eight tilapia-feed samples were collected and their fat contents were determined to be 10.3 ± 1.1%, 9.2 ± 1.0%, 11.5 ± 2.5%, and 8.2 ± 1.3%, respectively. By the DR CALUX® bioassay, dioxins and dioxin-like compounds were determined to be 2.0 ± 0.7 pg DR CALUX-TEQ/g fat, 0.4 ± 0.1 ng DR CALUX-TEQ/kg product, 0.9 ± 0.6 ng DR CALUX-TEQ/kg product, and 0.3 ± 0.1 ng DR CALUX-TEQ/kg product, respectively, which were all below the EU-limits for egg and fish feeds.

Introduction

The CALUX bioassay has been used widely as a sensitive method for detection of dioxin and dioxin-like compounds (PCDDs/DFs and dioxin-like PCBs) in various materials.[1-4] We planned to use DR CALUX® bioassay from the BioDetection Systems (BDS, the Netherlands) to screen for the presence of dioxin and dioxin-like compounds in fishery, poultry, livestock products, and various feeds. After an in-depth training and a 3-phase cross-validation by staff of the BioDetection Systems, we obtained the licence for the DR CALUX® bioassay. In this study, we first set up control charts using 1 pM and 3 pM 2,3,7,8-TCDD and two certified reference materials with known amounts of dioxin and dioxin-like compounds. The control charts were and will be used for quality assurance and quality control (QA/QC) of performing the DR CALUX® bioassay in our laboratory. In the end, we determined the contents of dioxin and dioxin-like compounds in 31 egg and 23 fish feed samples using the DR CALUX® bioassay.

Material and Methods

Sample collection

During 2006-2008, 23 fish feed samples were collected from six eel, nine seabass, and eight tilapia aquaculture farms, and 31 egg samples were collected from chicken farms, supermarkets, and vendors. All samples were

homogenized immediately after collection and stored at -20℃ until use. Two certified reference materials (CRMs), one fish oil sample and one feed sample, were purchased from the BioDetection Systems.

Extraction of dioxin and dioxin-like compounds and determination of fat content

Extraction of dioxins and dioxin-like compounds was carried out according to the manuals of the DR CALUX® bioassay.[4] Approximately 9 gram of homogenized fish feed sample or 12 gram of homogenized egg sample was thawed, mixed with a mixture of water and isopropanol, and extracted with a mixture of hexane and diethyl ether (97:3, V/V) for three times. The organic phases were pooled and evaporated by nitrogen gas. Evaporation was interrupted periodically to weight the extract and stopping evaporation as the weight reached to a constant value, which was the fat content of the sample. The exract then passed through a sulfuric acid activated silica column and was eluted with a mixture of hexane and diethyl ether (97:3, V/V). The eluent, which contained dioxins and dioxin-like compounds, was collected, evaporated to dryness, and re-dissolved in 25 μL dimethyl sulfoxide (DMSO) for the DR CALUX® bioassay.

Determination of the amount of dioxins and dioxin-like compounds by the DR CALUX® bioassay

DR CALUX® bioassay was used according to the instruction manual of DR CALUX® bioassay [4]. Rat hepatoma H4IIE cell line containing an AhR-DRE-regulated luciferase gene-carrying plasmid was used for the assay. Briefly, cells were cultivated under controlled conditions until confluent growth. Cells were collected and seeded into in wells of a 96-well microtiter plate. The plate was incubated at 37 ℃ under 5% CO_2 for 24 hrs. Then, sample extract in 25 μL DMSO was added into the wells containing the cells. The microtiter plate was incubated at 37℃ under 5% CO_2 for 22 to 23 hrs. After the incubation, cultural medium was removed from the well and lysis buffer was added to lyse the cells. To measure the luciferase activity of the lysed cells, lucriferase substrate was added to the wells and luminescence was quantified with a luminometer. In the same microtiter plate, 2,3,7,8-TCDD of 0, 0.3, 1, 3, 10, 30, 100, 300 pM in DMSO was included for generation of standard calibration curve, with which the amount of dioxins and dioxin-like compounds in the extracts could be determined by intrapolation. One of the two certified reference materials was also included in the same microtiter plates. Results from these two materials and 1 pM and 3 pM 2,3,7,8-TCDD were used for generation of control charts. One of the two certified reference materials was also included in the same microtiter plates. Results from these two reference materials and 1 pM and 3 pM 2,3,7,8-TCDD were used for generation of control charts according the control chart set up manual from the BioDetection Systems. The amount of dioxin and dioxin-like compounds in fish oil and egg samples were expressed as pg DR CALUX TEQ/g fat, whereas those in feed samples were expressed as ng DR CALUX TEQ/kg product.

Results and Discussion

Control charts set up for laboratory QA/QC

Four control charts were set up using 1 pM and 3 pM 2,3,7,8-TCDD and the two certified reference materials. The results of 1 pM and 3 pM 2,3,7,8-TCDD are showed in Figure 1. The mean value for 1 pM 2,3,7,8-TCDD was 0.9 ± 0.1 pM, whereas those for 3 pM 2,3,7,8-TCDD was 3.1 ± 0.1 pM.

The control charts of the two certified reference materials are shown in Figure 2. The average amount of one certified reference material, the feed sample, was 1.0 ± 0.2 ng DR CALUX TEQ/kg product (CV= 20%), whereas those for the other certified reference material, the fish oil sample, was 3.7 ± 1.0 pg DR CALUX TEQ/g fat (CV=27%). The 63 values of these two certified reference materials are all within the mean value ± 3SD. The results indicating the DR CALUX performing system is under controlled and kept at relatively stable condition.

According to the certificates, the two certified reference materials, feed and fish oil samples, contained 1.5 ng PCDD/PCDF/PCB TEQ/kg product and 2.2 pg PCDD/PCDF/PCB TEQ/g fat, respectively, by the HRGC/MS analysis. However, they were determined by the DR CALUX® bioassay to contain 1.4 ± 0.63 (uncertainty) ng DR CALUX TEQ/kg product and 3.7 ± 2.1 (uncertainty) pg DR CALUX TEQ/g fat, respectively. Our determination value (1.0 ± 0.2 ng DR CALUX TEQ/kg product) of the feed reference sample by the DR CALUX® bioassay was lower than the expected value (1.4 ng DR CALUX TEQ/kg product), but was within the range, whereas our determination value (3.7 ± 1.0 pg DR CALUX TEQ/g fat) of the fish oil reference sample was almost identical to the expected value (3.7 DR CALUX TEQ /g fat).

Bioassay results of egg and fish feed samples

The fat contents and the dioxin and dioxin-compound contents of 31 egg and 23 fish feed samples were determined and the results are shown in Table 1. For the egg samples, the fat content ranged from 7.8% to 12.2% and the average dioxin and dioxin-compound amount was 2.0 ± 0.7 pg CALUX-TEQ/g fat. The dioxin and dioxin-compound contents of all of the 31 egg samples were lower than the EU limit for egg, i.e., 6 pg WHO-PCDD/F-PCB-TEQ/g fat. [4,5]

For the 23 fish feed samples, the fat contents ranged from 5.9% to 14.5%. The average contents of dioxin and dioxin-like compounds of feed samples from six eel, nine seabass, and eight tilapia aquaculture farms were 0.4 ± 0.1, 0.9 ± 0.6, 0.3 ± 0.1 ng CALUX-TEQ/kg product, respectively (Table 1). Of the three groups of fish feed samples, the average content of dioxin and dioxin-like compound of seabass-feed samples was higher than those of the two other groups of fish feed samples. But the dioxin and dioxin-like compound contents of all of the fish feed samples were below the EU limit for fish feed, i.e., 2.25 ng WHO-PCDD/F-PCB-TEQ/kg product. [4,6]

In the future, we plan to use DR CALUX® bioassay and the HRGC/MS assay to determine the contents of dioxin and dioxin-like compounds in feeds, fishery, livestock and poultry products. The results from the two methods will be compared in order to evaluate the application of DR CALUX® bioassay for analysis of these materials in Taiwan.

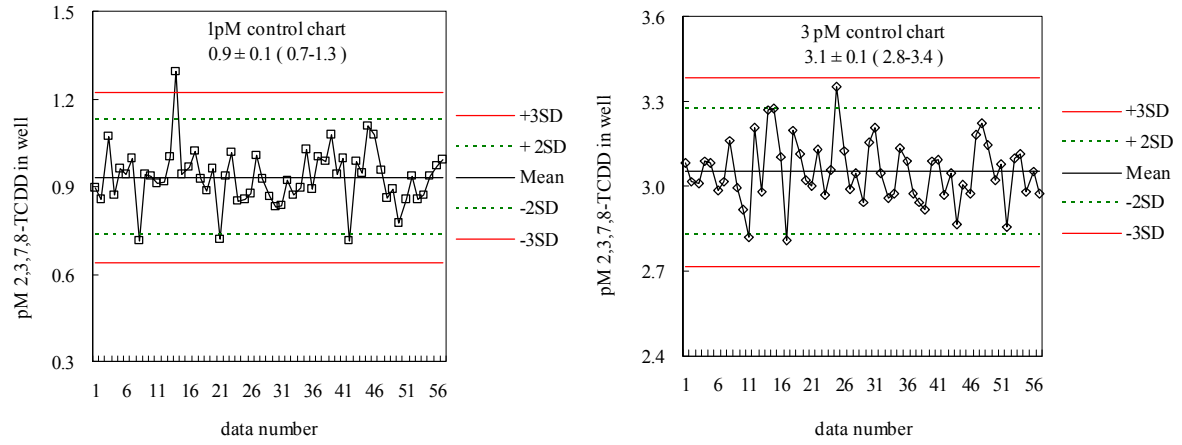

Figure 1. The control charts of 1 pM (left) and 3 pM (right) 2,3,7,8-TCDD are being used to monitor the performance of DR CALUX .

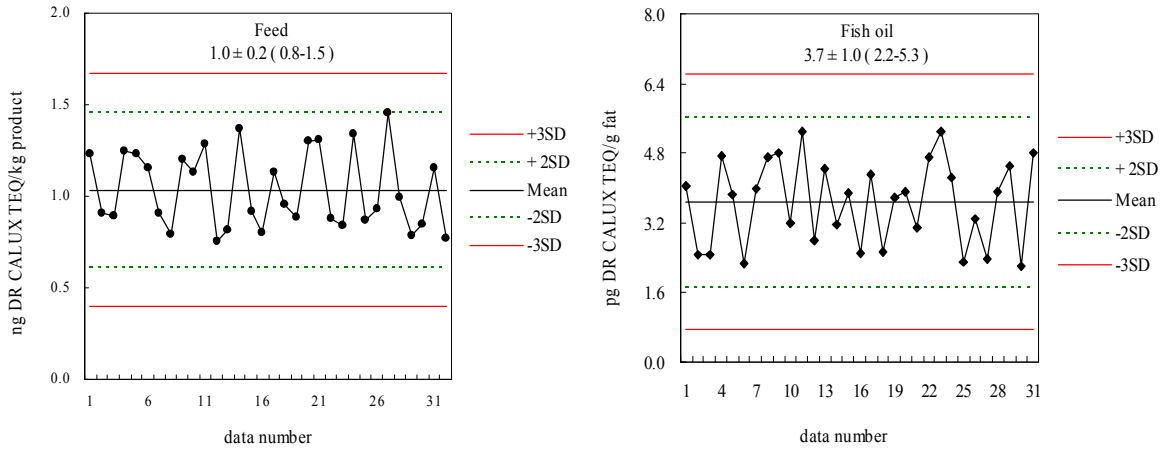

Figure 2. Control charts of the two certified reference materials, one feed and one fish oil samples. The analysis results of these two certified reference materials are all within the mean value ± 3SD.

Table 1. Fat contents and the DR CALUX® analysis results of dioxins and dioxin-like compounds in egg and fish feed samples

Sample	Number of sample	Fat content (%)	Dioxin and dioxin-like compounds (pg DR CALUX TEQ /g fat)[2] or (ng DR CALUX TEQ /kg product)[2]
Eggs	31	10.3 ± 1.1 (7.8~12.2)[1]	2.0 ± 0.7 (1.3~4.8)[1]
Fish feeds	23		
eel-feed	6	9.2 ± 1.0 (7.9~10.4)	0.4 ± 0.1 (0.2~0.5)
seabass-feed	9	11.5 ± 2.5 (5.9~14.5)	0.9 ± 0.6 (0.3-1.7)
tilapia-feed	8	8.2 ± 1.3 (6.1~10.2)	0.3 ± 0.1 (0.2~0.5)

[1]Numbers represent mean ± SD with the ranges shown in parentheses.

[2]Unit for egg is (pg DR CALUX TEQ /g fat), for feed is (ng DR CALUX TEQ /kg product).

Acknowledgements

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