CALUX® AND HRGC/HRMS DETERMINATION OF DIOXINS IN FISH OIL AND ANIMAL OIL USED FOR FEED INGREDIENTS

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Introduction

Animal originated foodstuffs contribute more than 80% of the human exposure to dioxins and POPs, while animal contamination mainly comes from feeds. The mixed animal feeds consisted with various animal and plant derived components, including fish and animal oils. Among them, the highest dioxin contamination was found in fish oils compared to other animal originated matrixes.[1,2] To control dioxins in animal feed, it is important to routing analyze of dioxins in vast number of samples from various animal feed components to avoid producing feeds with elevated dioxin levels. The regular method for dioxin analysis would be costly and time consuming to cover up large number of samples. Therefore it is an important issue to validate rapid, cheap and reliable method to screen high dioxin contaminated feed ingredients.

The CALUX® bioassay has been widely employed as a rapid analysis of dioxin-like toxicity in environmental and biological samples.[3,4] Recently, Japan Ministry of Environment has been acknowledged to employ bio-analitycal methods to screen dioxins in the environmental samples. In this study we carry out CALUX and HRGC/HRMS analysis of 17 fish oil and 12 animal oil samples which have been used as animal feed ingredients during year 2001-2003.

Materials and Methods

Approximately 1g of oil sample was loaded into 20g of 45% H_2SO_4 silica gel filled glass column and eluate the dioxin like compounds with 25ml of hexane. The eluate further passed through a XCARB column. After XCARB cloumn washed with 10ml hexane, coplanar PCBs were separated by 15ml of hexane:toluene:ethylether (8:1:1 v/v). The PCDDs/DFs were separated with 20ml of toluene. The recovery of dioxins on the XCARB column was >70%.[4] Fractionated extracts were evaporated and redissolved in DMSO. Standard solution (2,3,7,8-TCDD) and extracts in DMSO were futher diluted in culture medium.[5]

H1L6.1c2 cells ($1.5x10^5$/well) were cultured in 96 well plates. After 24h incubation, cells were exposed in quadruplicate to extracts and duplicate for the standards, followed by 20-24 hours of incubation, the cells were lysed. A luciferine containing solution was added and the luciferase activity was measured using a luminometer. Avarage RLUs (relative light units) were calculated for extracts and standards. Blank valuse were sustracted. TEQs for each sample was calculated according to standard curves.[5]

Quantitative analysis for dioxins in oil samples was carried out according to the guildlines for dioxin analysis in feed described by FFIS, Japan.[5] Samples were extracted by KOH digession followed by treatment with acid-silica gel cleanup. PCBs and PCDDs/DFs fractionated by an active carbon column. The quantification was carried out by a HRGC/HRMS with recovery rate of 42 – 91% and lower limit of quantification (LOQs) for dioxins within 0.05 – 1.0 pg/g.[1,6]

Results and Discussion

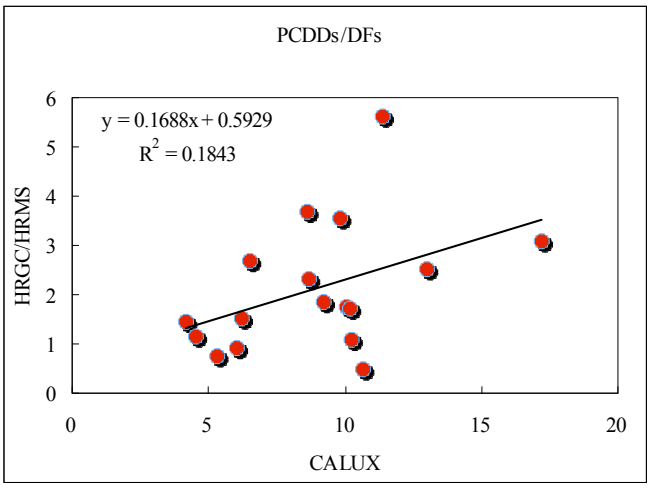

Figure 1. Comparison of PCDDs/DFs-TEQs derived from HRGC/HRMS and CALUX assay (pg/g wet wt) for fish oils

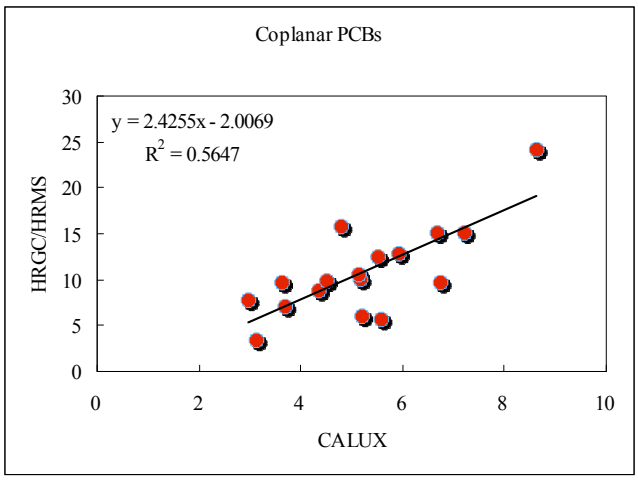

Figure 2. Comparison of coplanar-TEQs derived from HRGC/HRMS and CALUX assay (pg/g wet wt) for fish oils

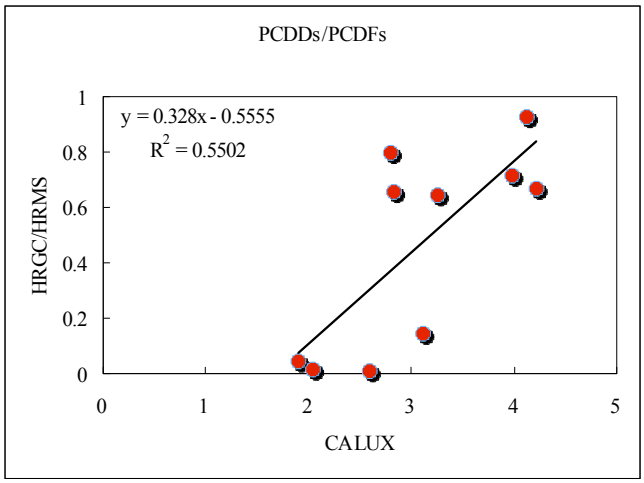

Figure 3. Comparison of PCDDs/DFs-TEQs derived from HRGC/HRMS and CALUX assay (pg/g wet wt) for animal oils

HRGC/HRMS determined TEQ levels for PCDDs/DFs and coplanar PCBs in fish oil were ranged from 0.46 to 5.6 pg/g wet wt and 3.3 to 24 pg/g wet wt, while for animal oil were ranged from 0.014 to 0.923 and 0.012 to 0.49 pg/g wet wt respectively. The data suggested that the composition of dioxins were highly varied with the origin of the matrix. The coplanar PCBs and PCDDs/DFs ratio to total TEQ was approximately 7:1 and 1:1 for fish oil and animal oil, respectively. CALUX derived TEQs for PCDDs/DFs and coplanar PCBs were ranged from 4.22 to 17.3 pg/ g and 3.13 to 8.64 pg/g wet wt, respectively for fish oil (Figs 1, 2). All the values were few folds higher than the limit of quantification (LOQ: 1.94 pg/g). Therefore 1 g of fish oil can easily be used for CALUX assay. For the animal oil, CALUX derived TEQs for PCDDs/DFs and was ranged from LOQ (1.96 pg/g)to 4.13 pg/g wet wt (Fig 3). Only 2 samples of animal oil had PCDDs/DFs TEQs less than the LOQ. However 10 out of 12 animal oil samples had TEQs levels less than LOQ (1.85 pg/g wet wt) for coplanar PCBs. This suggested that 1 g of animal oil with less coplanar-PCBs (TEQs <0.5 pg/g) is not suitable for CALUX assay; hence sample amount should be increased.

The correlation between CALUX-TEQ and HRGC/HRMS-TEQ for fish oil (n=17) was 0.42, o.75 and 0.55 for PCDDs/DFs, co-PCBs and for PCDDs/DFs + co-PCBs, respectively. Moreover bioassay derived TEQs for PCDDs/DFs were atleast 5.8 times higher, while for coplanar PCBs were atleast 0.42 times less than GCMS determined levels. For animal oil correlation could only calculated for PCDDs/DFs fraction with the value of 0.71, by means of bioassay derived TEQs for PCDDs/DFs were atleast 3 times higher than that of GCMS values. However, for fish oils, when removing samples which had higher TEQs (GCMS TEQs > 18 pg/g wet wt, n=4; and one outlier), the correlation was better such as 0.81, 0.61 and 0.76 for PCDDs/DFs, co-PCBs and for PCDDs/DFs + co-PCBs, respectively. Any compound capable of binding and activating AhR and DREs may interfere with the induction of CYP1A enzymes during the bioassay. Hence, higher

bioassay-TEQs for PCDDs/DFs fraction can be expected.[7] CALUX assay derived TEF for coplanar PCB congeres were relatively lower than WHO-TEF due to low response.[8] In addition, fish oil contained very high levels of ortho-PCBs which might interfere in an inhibitory manner in the bioassay response to coplanar PCBs in samples. Hence, bioassay derived TEQs for coplanar PCBs expected to be less than the values obtained for GCMS.

In the conlusion, the CALUX assay could be a useful tool, allowing the screening of relatively large number of feed ingredients such as fish oil with 1 g of sample weight. However, for the fish oils with high dioxin levels, it is important to develop a better clean up with compounds response to cell line other than dioxins are removed or destroyed, and/or a diluted samples during the assay.[9,10]

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