Removal of dioxins and PCBs in fish oils: comparison of CALUX and GC-HRMS results

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Introduction

More and more people want to have a healthier nutrition with high levels of valuable minor compounds like vitamins and anti-oxydants and with a balanced fatty acid composition.

Fish oil has the advantage to contain high concentrations of ω3 fatty acids (EPA, DHA), anti-oxydants and lipophilic vitamins. It has been shown that the intake of these products can reduce the risk of cardiovascular diseases, arterial thrombosis, autoimmune and inflammatory problems.[1,2] Unfortunately, some crude fish oils may also contain contaminants like dioxins and PCBs.

The challenge is to remove these harmful contaminants from fish oils, without affecting its nutritional quality. This project was set up to find the best method to reduce the dioxins and PCBs levels without degradation ofthe nutritional quality of the oil.

The concentration of dioxins and PCBs was calculated using 2 different techniques: GCHRMS (congener specific analyses) and CALUX (global TEQ value).

The oxidative status, the stability of the oil and the levels of the nutritionally valuable compounds were measured as well.

This paper compares results of dioxin and PCB analyses with the classical GC-HRMS technique with the results of the dioxin-like activity obtained by the cheaper CALUX technique.

Materials and Methods

Neutralised and winterised fish oil with a significant content of dioxins and PCBs was obtained from a Scandinavian fish oil processor.

During the first part of the study, 0.1% or 0.5% (w/w) of the following adsorbents was added to the oil:

- 3 types of bleaching earth (polar adsorbents-variable degree of activation),

- 2 other polar adsorbents (silica and filter aid),

- 4 types of activated carbon (apolar adsorbents-different suppliers)

The oil was in contact with the adsorbent during 30 minutes at 70°C and 50 mbar in a rotary evaporator and afterwards, filtered over a paper filter (Whatman N°1) on a Buchner Filter.

The activated carbon provided the best results. It was then tested, in a second part, under different experimental conditions (concentrations of carbon, contact times and temperatures). Experimental design plan was used to find out the optimized conditions.

The third part was dedicated to another approach of dioxins and PCBs removal consisting of a steam-stripping packed column. This technique was complementary with the active carbon adsorbent approach.

CALUX bio-assay

The oil (aliquots of 1 gram) was dissolved in hexane and cleaned through an acid silica column and an activated carbon column. After elution, the acid silica column was discarded. The carbon column was washed with hexane/acetone 9/1 (v/v), the dioxin-like PCB fraction was eluted with hexane/toluene/ethyl acetate 8/1/1 (v/v/v) and afterwards the dioxin fraction was eluted with toluene. Toluene was evaporated and the samples were diluted in hexane.

CALUX analyses were performed using the mouse hepatoma H1L6.1 cell line developed by Xenobiotic Detection System (Durham, US).[3]

The cells were exposed to the purified extracts in 96-well plates during 20 to 24 hours in an incubator at 37°C and 5% CO_2. On each plate, 10 standard solutions of 2,3,7,8-TCDD were added for the calibration curve. After incubation, the cells were lysed and the amount of luciferase produced by the cells was determined by addition of the substrate luciferine. The light emission was measured with a luminometer. This value was reported on the TCDD calibration curve and translated in bio-assay TEQ value.

GC-HRMS

The GC-HRMS analyses were performed by the Center of Analysis of Residues in Traces, Université de Liège. The procedure has been described elsewhere[4]. Briefly, 2g of fish oil was loaded on an automated Power-Prep system (Fluid Management System, inc., Waltham, MA, USA). The sample was then processed through a set of disposable columns: a high capacity acid silica column, a small multi-layer silica column, a basic alumina column and a PX-21 carbon column. The final extract was concentrated to 10µL in nonane prior to GC/HRMS injection.

Results and Discussion

The results obtained for the dioxin fraction by CALUX and those for the 17 PCDD/Fs by GC-HRMS show the same trend. The filter aid (treatments 2 and 3), silica (treatments 4 and 5) and bleaching earth (treatments 6 to 11) don't lead to a decrease in dioxins concentration. Only the activated carbon allows a reduction of the dioxin content of the oil (treatments 12 to 19). n°1= oil without treatment (see Figure 1)

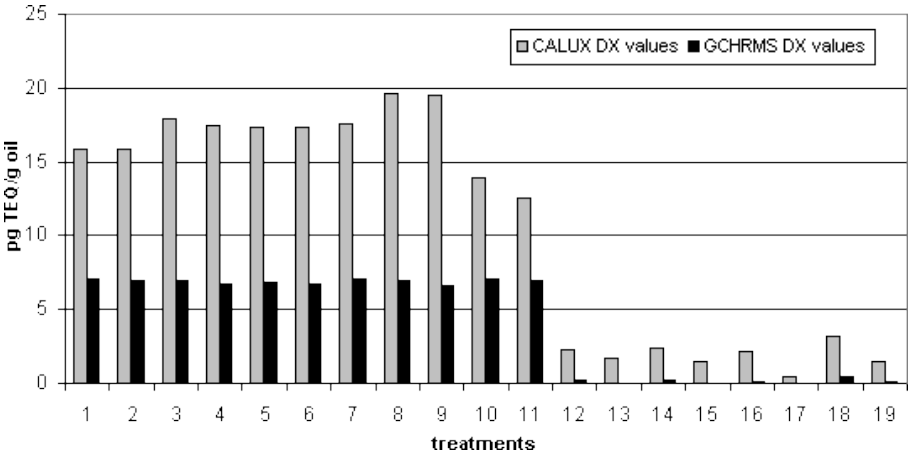

Figure 1: CALUX and GC-HRMS results for the dioxin fraction.

The GC-HRMS analysis allows to determine which congener is more sensible to the treatment. With activated carbon, almost all PCDD/Fs and non-ortho PCBs are removed, but removal efficiencies for mono-ortho PCBs are

much lower. (Figure 2)

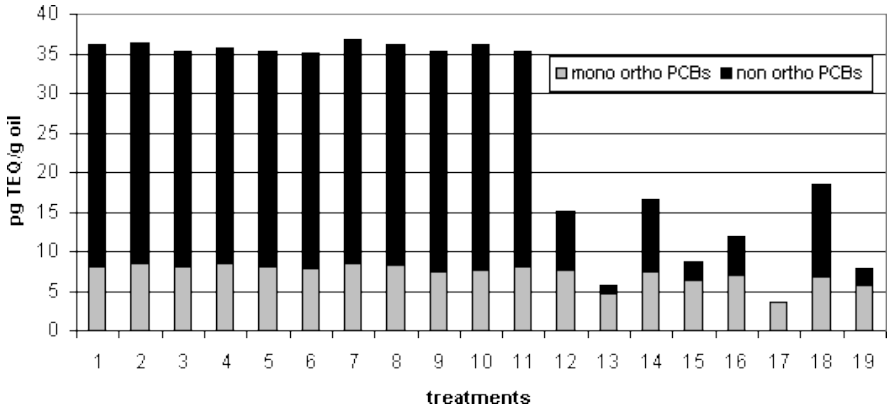

Figure 2: Results obtained by GC-HRMS for the mono-ortho and non-ortho PCBs

The comparison of results obtained for the dioxin fraction by CALUX and GC-HRMS is given in Figure 3 and for the PCB fraction in Figure 4.

As can be seen in Figure 3, the values for the dioxin fraction obtained with CALUX are higher than values obtained by GC-HRMS. All the values are above the bisecting line. These results show that the oil probably contains other compounds than the 17 PCDD/F congeners.Indeed, the GC-HRMS results represent the TEQ value for the 17 PCDD/F congeners.

The linear correlation equation obtained is y=2.06x+1.7 with a determination coefficient R^2=0.9468. The intercept value of 1.7 shows that when the GC-HRMS values equal to 0, CALUX detects compounds. This can be explained by other compounds than the 17 congeners and for the very low concentrations, by the lower bound value (0) assigned to congeners under the LOQ.

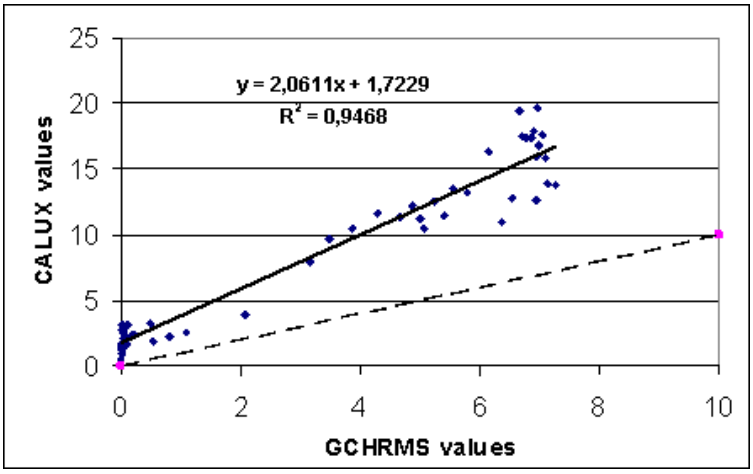

Figure 3: Comparison of CALUX results for the dioxin fraction and the GC-HRMS results for the 17 PCDD/Fs (- - - bisecting line, ___ linear correlation) (pg TEQ/g oil)

The figure 4 shows that for the PCB fraction, the CALUX results are under the bisecting line. The linear correlation equation obtained is y=0.06+1.0 with a determination coefficient R^2=0.71.

The much lower results obtained by CALUX can be explained by lower REP values[5], antagonistic effects and losses.

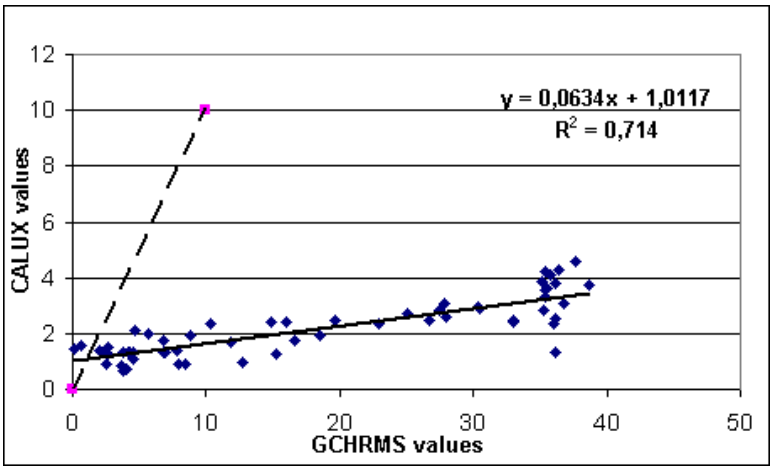

Figure 4: Comparison of CALUX results for the PCB fraction and the GC-HRMS results for the 12 WHO PCBs (- - - bisecting line, __ linear correlation) (pg TEQ/g oil)

Conclusions

There is a good correlation between the CALUX and GC-HRMS results for the dioxin fraction. The CALUX results can be used to detect a decrease of dioxin's concentration in fish oil due to different treatments. However, the CALUX results for the PCB fraction are much lower than the GC-HRMS results. The changes of concentrations of PCBs can not significantly be determined by CALUX.

The CALUX results can point out the presence of other compounds that react with the Ah receptor but these compounds have to be identified by GC-HRMS.

The GC-HRMS has the advantages to show the profile of the different PCDD/F and PCB congeners and to give the concentration of each congener present. By this way, it indicates the rate of elimination of each congener.

Acknowledgements

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