ANTHROPOGENIC AND NATURAL ORGANOHALOGENATED COMPOUNDS IN FISH OIL DIETARY SUPPLEMENTS FROM VARIOUS COUNTRIES

Covaci A[1], Voorspoels S[1], Wyckmans J[2], Gelbin A[3], Neels H[1]

1- Toxicological Centre, University of Antwerp (UA), Universiteitsplein 1, 2610 Wilrijk, Belgium
2- Minami Nutrition , University Business Centre Antwerp, Drie Eikenstraat 661, 2650 Edegem, Belgium
3- AccuStandard, 125 Market St., New Haven, CT 06513, USA

Introduction

Encapsulated fish oils are sold as dietary supplements for their high content of long chain polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These PUFAs are essential for human diet and are needed for many metabolic functions, including growth, structural maintenance and repair of nervous tissue, cellular membrane phospholipid structure or the regulation of plasma lipid levels[1,2]. They have also several health benefits, such as reducing mortality from heart disease and reduction of symptoms of certain diseases, such as multiple sclerosis, rheumatoid arthritis and osteoporosis[1-3].

However, fish oils represent a large accumulation potential for lipophilic persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs)[4-6]. These persistent pollutants accumulate in lipid compartments of animals and further in the food chain[5,6]. A wide range of toxicological and hormonal effects, including endocrine disruption, are stimulated by these environmental contaminants[6]. Additionally, recent studies have described the presence of naturally occurring halogenated compounds, including methoxy-PBDEs (MeO-PBDEs) in the marine environment[7-10], including in fish from which such oils are extracted.

Since fish oil dietary supplements are increasingly consumed, it is important to monitor closely the levels of organic pollutants which might be present. Since there is little available data for contaminants in fish oils, this study reports the levels of PCBs, PBDEs and MeO-PBDEs in a large number of PUFA-rich dietary fish oil supplements obtained through pharmacies and retail outlets from various countries.

Materials and Methods

Sample description: PUFA-rich fish oil dietary supplements were collected between January-March 2006. Although this is not a comprehensive survey of all brands available, samples were chosen to cover the products available for sale on the Belgian market. Additionally, fish oil dietary supplements bought in The Netherlands, Ireland, United Kingdom and South Africa were also analyzed. In total, sixty-nine (n = 69) fish oil samples from 37 producers were collected in duplicate. Product expiry dates were checked to ensure validity of the shelf-life of the samples during the measurement period. Information on recommended dosage provided on the product labels together with other relevant sample details was recorded.

Sample preparation: Prior to analysis, samples were stored in their original containers at ambient temperature. For samples sold as capsules, the casings were excluded and 10 capsules per product were pooled. The pooled samples were individually stored in hexane-washed glass vials. All samples were analyzed for 10 PBDE congeners (no. 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183) and for two tetrabrominated-MeO-PBDEs (2´-MeO-BDE68 and 6-MeO-BDE47), previously reported as naturally occurring brominated compounds in marine samples[7-10]. Additionally, 20 PCB congeners (no. 28/31, 52, 95, 99, 101, 105, 118, 128, 138/163, 149, 153, 156, 170, 180, 183, 187, 194, and 199) and DDT and metabolites (here expressed as DDTs) were also analyzed in all samples. These analytes were selected on the basis of their previously reported presence in fish oils[5,6]. Analytical procedures have been previously described in detail[5,6] and a brief summary is presented below. An aliquot of 0.20 - 0.25 g oil was solubilised in 3 mL hexane, the mixture was vortexed and internal standards (7.5 ng of PCB 46/143 and 1 ng of BDE77/128) were added. The extract was loaded onto a hexane pre-washed cartridge filled with 8 g acidified silica and was eluted with 15 mL of n-hexane and 10 mL of dichloromethane. The final eluate was concentrated with a rotary evaporator and further under a nitrogen stream to approximately 100 µL.

Analysis of PBDEs and MeO-PBDEs: One µL of the extract was injected in solvent vent mode into a gas chromatograph-mass spectrometer (GC/MS) operated in electron-capture negative ionization (ECNI) mode and equipped with a 14m x 0.18 x 0.20µm, AT-5 capillary column. The bromine ions (*m/z* = 79 and 81) were

acquired in SIM mode. For confirmation of MeO-PBDEs, the extract and the corresponding standards were injected in full scan into a GC/MS operated in electron impact (EI) ionization mode.

Analysis of PCBs and DDTs: One µL of the extract was injected in pulsed cold splitless mode into a gas GC/MS operated in EI ionization mode and equipped with a 30m x 0.25mm x 0.25µm, DB-1 capillary column. Two most abundant ions were monitored for each chlorination level of PCBs or for each DDT analogue in SIM mode.

Quality Assurance and Quality Control. The method quality control was done by regular analysis of procedural blanks, duplicate samples (differences were <5%), certified material CRM 350 (PCBs in mackerel oil) and through successful participation to Quasimeme interlaboratory studies (PBDEs in fish and fish oil)[11,12]. Procedural blanks of PBDEs, PCBs and DDTs were consistent (RSD<12%) and therefore the mean value of each analyte in the procedural blanks was used for subtraction. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the analyte in the procedural blanks, which ensures >99% certainty that the reported value is originating from the sample. Method LOQs ranged between 0.1 and 0.2 ng/g oil for individual PBDE and MeO-PBDE congeners and were 0.3 ng/g oil for individual PCB congeners and DDT analogues. Typical recoveries of analytes and internal standards were between 75 and 105%.

Statistical analysis. Samples with levels below the LOQ were assigned a value of zero. Concentrations of pollutants in the samples were not normally distributed (Shapiro-Willks test, p>0.05), and therefore, non-parametric statistics have been applied. Outliers were detected with Grubb's test. Spearman-rank correlations were calculated between concentrations of PBDEs and MeO-PBDEs in the samples. A non-parametric Kruskal-Wallis test was used to test for differences between concentrations of pollutants function of their production provenience. All statistical analyses were performed using SPSS v.11 for Windows (SPSS Inc.).

Results and discussion

As expected, PCBs and DDTs were the most abundant anthropogenic contaminants and ranged between <0.3 - 95 ng/g oil and <0.3 - 234 ng/g oil, respectively (Table 1). The congener profile of PCBs was dominated by PCBs 138, 153, 180, 149 and 118, while *p,p´*-DDE was the most abundant DDT analogue. The PBDE levels were lower (range <0.1 - 45 ng/g oil), except for some samples in which PBDEs were present in higher concentrations than PCBs. The congener profile was dominated by BDE 47 in accordance with previously reported PBDE data in fish oils[5,6]. BDE 47 was detected at concentrations comparable to PCB 118.

These results are in good agreement with the levels of congener specific PCBs reported for fish oil dietary supplements from the UK[6] or Danish market[13] and with the levels of PBDEs in fish oils from the UK market[6].

MeO-PBDEs were detected in the vast majority of samples, sometimes at higher levels than PBDEs. Two tetrabrominated MeO-BDEs, namely 2'-MeO-BDE68 and 6-MeO-BDE47, were identified in the GC/ECNI-MS chromatogram based on retention times and confirmed by EI-MS spectrum (right) (Figure 1). Concentrations of tetrabrominated MeO-BDEs were very high in some samples (up to 1670 ng/g oil). However, this is not unusual, since such high concentrations have been previously reported in marine samples[7]. The presence of a methoxy group in the *ortho*-position of both compounds supports their natural origin[8,14]. The elevated concentrations of these compounds found in higher trophic levels of the marine food chain demonstrate their bioaccumulative properties.

Table 1. Median concentrations and range (ng/g oil) of persistent organohalogenated pollutants in fish oil dietary supplements. For samples for which individual measurements of analytes felt under the LOQ, zero was used for data treatment.

	Belgium	**The Netherlands**	**United Kingdom**	**Other Countries***
N	27	17	12	13
Sum DDTs	3.9 (<0.3-153)	2.9 (<0.3-234)	4.4 (<0.3-62)	3.8 (<0.3-21)
Sum PCBs	11.6 (<0.3-57)	13.9 (<0.3-60)	7.6 (<0.3-22)	6.0 (<0.3-95)
Sum tri-hepta PBDEs	0.4 (<0.1-45)	0.5 (<0.1-17)	0.2 (<0.1-2.8)	0.4 (<0.1-7.5)
Sum tetra-MeO-BDEs	5.1 (<0.1-1670)	8.7 (<0.1-22)	13.5 (<0.1-316)	4.1 (<0.1-232)

* - Denmark, South Africa, USA, France and Sweden

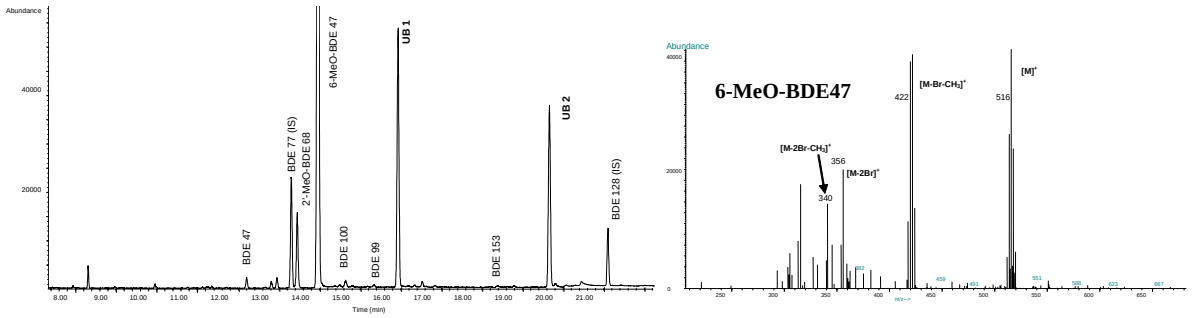

Figure 1. GC/ECNI-MS chromatogram (left, TIC of *m/z* = 79 and 81) of a fish oil extract showing several PBDE and MeO-PBDE congeners (left), together with two unidentified compounds containing bromine (UB 1 and 2, respectively). Mass spectra obtained of 6-MeO-BDE47 with EI-MS in fish oil extract (right).

Two unidentified brominated compounds (UB1 and 2) were also present in most chromatograms, but their retention times did not match the retention times of available penta or hexa-brominated MeO-BDEs. However, they were quantified based on the closest eluting available MeO-BDE congener (6´-MeO-BDE99 for UB 1 and 6-MeO-BDE157 for UB 2). Unfortunately, their concentrations were never high enough to be able to perform a full scan analysis on GC/EI-MS for spectral identification. UB 1 and UB 2 were found always at lower concentrations than the tetrabrominated MeO-BDEs. Although identified in several other studies[10,14], another naturally occurring compound (dimethyl-tetrabromo-dichloro-bipyrrole – DBP-Br_4Cl_2) was not found in the analyzed samples. No other relevant peaks except for the 2 tetrabrominated MeO-BDEs could be seen between BDE 47 and BDE 100, the chromatographic interval where DBP-Br_4Cl_2 was shown to elute on a DB-5 column[14].

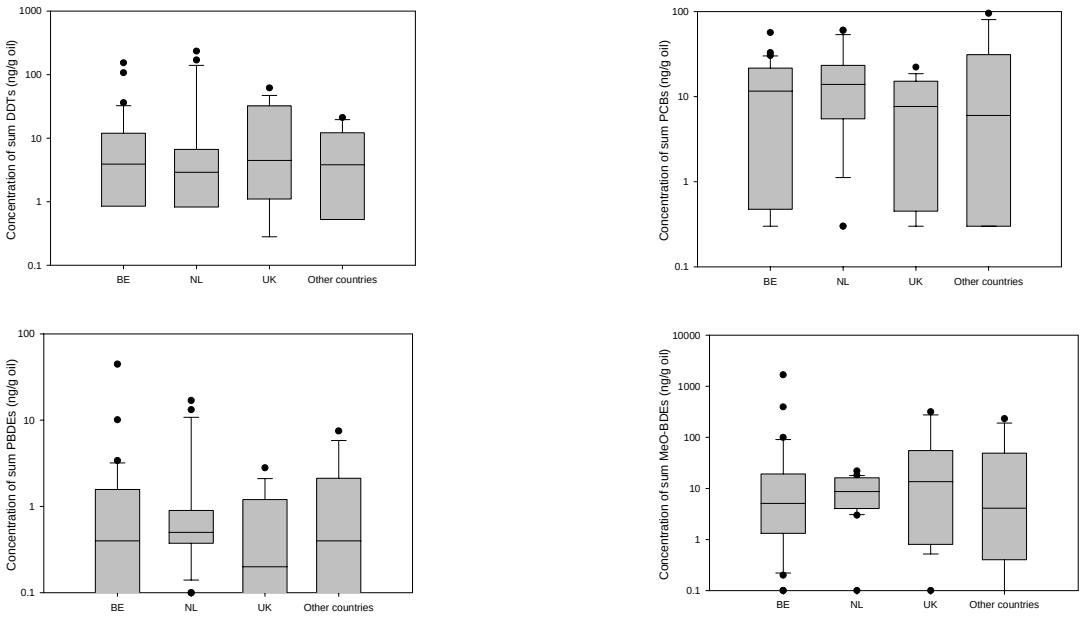

Figure 2. Overview of concentrations (ng/g oil) of anthropogenic and naturally occurring organohalogenated contaminants in fish oil dietary supplements grouped according to their country of production. The box plots show the median, 25 and 75 percentiles, range and outliers.

There was no significant correlation between the levels of BDE 47 and 6-MeO-BDE 47 ($r=0.32$, $p>0.05$) indicating that 6-MeO-BDE 47 is not produced by the metabolism of BDE 47 in the fish species used to produce fish oils. Since the levels of 6-MeO-BDE 47 were highly correlated with the levels of 2´-MeO-BDE 68 ($r=0.93$, $p<0.01$), it is highly plausible that these compounds both accumulate from natural sources.

No significant differences could be found for sum DDTs, PCBs, PBDEs or tetrabrominated MeO-PBDEs between the groups from different countries (Kruskal-Wallis test, $p<0.01$). The use of similar fish sources by the various producers may be one of the main reasons for this observation.

Interestingly, several fish oil supplements with increased content of DHA contained higher concentrations of higher chlorinated PCBs, together with PBDEs and MeO-PBDEs. It is not clear whether these increased levels of high boiling/late-eluting compounds are due to the fish sources used for the preparation of the fish oils with increased DHA content or to the production processes specific for DHA-rich fish oils.

Most fish oil samples showed evidence of purification processes (decreased presence of volatile pollutants) which probably contributed to the reduction in contaminant levels. Comparisons with unrefined fish oils would indicate whether a real reduction in the steady state of these contaminants has occurred during manufacturing[15]. Apart from improvements in refining processes, further reasons for the low contaminant levels include improved sourcing and nutritional profile development to create specific 'optimum' essential fatty acid balances.

While fish and fish oil consumption are associated with numerous health benefits due to the long chain PUFAs, the potential contribution to the human diet of persistent organic contaminants increases if these food sources originate from polluted waters. Nevertheless, fish oils prove to be a powerful source of EPA and DHA compared to vegetables oils, of which approximately eight to ten times more is needed to provide comparative levels of EPA and which do not appear to provide an effective metabolic source of DHA for the average consumer[6].

This analysis strongly supports the need for ongoing monitoring of "classical" persistent organic pollutants in fish oils designated as nutritional supplements. Further, in the public interest, newer anthropogenic, but also naturally occurring contaminants need to be included in monitoring programmes.

Acknowledgements

MINAMI Nutrition N.V. is acknowledged for providing all samples and financially supporting the study. Nathalie Loots is kindly acknowledged for the sample preparation (in the frame of an undergraduate thesis), while Accustandard is thanked for their generous gift of MeO-PBDE standards. Dr. Adrian Covaci was financially supported by the Research Council of the University of Antwerp.

References

1. Jump DB. *J Biol Chem* 2002; 277: 8755-8758.
2. Simopoulos A. *Am J Clin Nutr* 1999; 70: 560S-569S.
3. Sidhu KS. *Regulat Toxicol Pharmacol* 2003; 38: 336-344.
4. Jacobs M, Santillo D, Johnston P, Wyatt C. *Chemosphere* 1998; 37: 1709-1731.
5. Jacobs M, Covaci A, Schepens P. *Environ Sci Technol* 2002; 36: 2797-2805.
6. Jacobs MN, Covaci A, Gheorghe A, Schepens P. *J Agric Food Chem* 2004; 52*:* 1780-1788.
7. Melcher J, Olbrich D, Marsh, G, Nikiforov V, Gaus C, Gaul S, Vetter W. *Environ Sci Technol* 2004; 39: 7784-7789.
8. Teuten EL, Xu L, Reddy CM. *Science* 2005; 307: 917-920.
9. Malmvärn A, Marsh G, Kautsky L, Athanasiadou M, Bergman Å, Asplund L. *Environ Sci Technol* 2005; 39: 2990-2997.
10. Vetter W, Jun W. *Chemosphere* 2003; 52: 423-431.
11. de Boer J, Wells DE, Noren K. *Organohalogen Compounds* 2002; 58: 197-200.
12. de Boer J, Wells DE. *Organohalogen Compounds* 2004; 66: 510-518.
13. Fromberg A. *Organohalogen Compounds* 2001; 51: 352-355.
14. Stapleton HM, Dodder NG, Kucklick JR, Reddy CR, Schantz MM, Becker PR, Gulland F, Porter BJ, Wise SA. *Mar Pollut Bull*, in press.
15. Hilbert G, Lillemark L, Balchen S, Hojskov C. *Chemosphere* 1998; 37: 1241-1252.