INVESTIGATION OF SELECTED PCBs, PBDEs AND ORGANOCHLORINE PESTICIDES IN OMEGA-3 FATTY ACID RICH DIETARY FISH OIL AND VEGETABLE OIL SUPPLEMENTS

Miriam Jacobs[1], Adrian Covaci[2] Adriana Gheorghe[2], Paul Schepens[2]

[1]School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK
Royal Veterinary College, University of London, Hawkeshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK
[2]Toxicological Center, University of Antwerp (UA), Universiteitsplein, 1, 2610 Wilrijk, Belgium

Introduction

Fish oil is a by-product of the fish meal manufacturing industry and comes from many different parts of the world. Fish oils are sold for dietary purposes due to the presence of long chain omega-3 fatty acids which have certain health benefits and are essential to the human diet. Specific vegetable oils and foods are sources of the short chain form that is the precursor to the long chain form utilised in human metabolism. Essential fatty acids are needed for growth and repair of nervous tissue and for the maintenance of its structure. The consumption of long chain omega-3 essential oils from fatty fish or concentrated sources such as fish oil supplements are thought to have associated benefits in reducing mortality from heart disease and improving symptoms of a number of diseases including multiple sclerosis and rheumatoid arthritis.

However, oily fish and extracted fish oils are a major source of lipophilic contaminants entering the human food chain[1, 2]. Chlorinated compounds accumulate in the lipid compartment of the animal, and oil extracted from fish caught in polluted waters may be contaminated with chlorinated hydrocarbons. UK government officials have met with the fish oil industry to discuss how dioxins and polychlorinated biphenyls (PCBs) can be reduced in dietary supplements and medicinal products containing fish oil[1]. The market for fish oil as a supplement to large sections of the population has been noted in the UK[3, 4]. The Diet and Nutritional Survey of British Adults 1990 found that 17% of the women respondents took dietary supplements, commonly fish oil[4]. Fish oils have also been traditionally given to children to protect against Vitamin D deficiency and rickets. As quantities consumed can vary enormously it is important that close monitoring is undertaken to ensure contaminant levels are greatly reduced.

There is an extensive and growing body of data on the presence of persistent organic pollutants (POPs), including polybrominated diphenyl ethers (PBDEs), in the aquatic environment, biota[5-8] and fish oils designated for aquaculture feed[8]. However, there is little data available in the public domain, for PBDEs in fish oils, and other essential fatty acid containing dietary oils.

This study reports the levels of selected PCBs, organochlorine (OC) pesticides and PBDEs, in 21 omega-3 rich dietary fish and vegetable oil supplements obtained through retail and company outlets in London, UK. The sources and brands of the dietary fish oil supplements were the same as those previously reported[2].

Materials and Methods

Sites and sampling

21 dietary supplements rich in omega–3 fatty acids (7 cod liver oil supplements, 7 whole body fish oil based supplements, 3 vegetable and fish oil combination supplements and 4 vegetable oils) were obtained from retail and practitioner suppliers of the UK market in December 2001 and January 2002

(Table 1). Samples were chosen to cover the products available to the UK consumer, including a neighbourhood pharmacy, a national high street chain pharmacy, whole food retailers and dietary supplement companies selling direct to nutritional therapists. While the samples cover the leading brands available on the London/UK market, this was not a comprehensive survey of all brands available.

Sample preparation

The samples were logged and prepared in duplicate pre-washed glass vials. They were kept at room temperature in the dark. All samples were analysed for 28 PCB congeners n°: 18, 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 153, 156, 163, 167, 170, 174, 177, 180, 183, 187, 194, 196, 199. All samples were also analysed for HCHs (3 isomers), HCB, DDT and metabolites (5 *op*- and *pp*-isomers) and 7 PBDEs congeners (n°: 28, 47, 99, 100, 153, 154, 183).

Sample analysis

0.5-0.7 g of the sample oils were solubilised in 3 ml hexane, internal standards (10 ng of PCB 46 and 143, 5 ng of e-HCH and 1 ng of BB 80, BB 103 and BB 155) were added and the mixture equilibrated in an ultrasonic bath for 5 min. The extract was applied to a hexane pre-washed SPE cartridge filled with 5 g acidified silica and eluted with 15 ml of hexane and 10 ml of dichloromethane. The final eluate was concentrated with a rotary evaporator and under nitrogen to approximately 100 µl (ready for GC analysis).

PCB and OCP determination

One µl of extract was injected in pulsed splitless mode into a GC-µECD equipped with a 50 m x 0.22 mm x 0.25 mm, HT-8 capillary column. For confirmation, one µl was injected in pulsed splitless mode into a GC/MS equipped with a 25 m x 0.22 mm x 0.25 mm, HT-8 capillary column. The MS was operated in electron impact ionisation and SIM mode. Two most abundant ions were monitored for each level of chlorination for PCBs or for each pesticide. Method limits of determination ranged for individual compounds ranged between 0.1- 0.2 ng/g oil. Recoveries of internal standards ranged between 75 and 85 %.

PBDE determination

One µl of extract was injected in cold splitless mode into a GC/NCI-MS equipped with a 10 m x 0.10 x 0.10 µm, HT-8 capillary column. The MS was operated in the negative chemical ionisation in SIM mode (ions: 79 and 81). Method limit of determination ranged between 0.05 and 0.1 ng/g oil for individual PBDE congeners.

Quality Control

All samples were analysed with GC-µECD and GC-MS systems. The lowest result for each compound obtained in both systems was considered for further calculations. The procedure was validated through regular analysis of procedural blanks, certified material CRM 350 (PCBs and organochlorine pesticides in mackerel oil) and through successful participation to Quasimeme interlaboratory studies (PCB determination in sediment and fish).

Densities of oils were determined gravimetrically and were found to lie between 0.87 and 0.90 g/ml. Thus, direct comparisons given in mg/l with those given in mg/kg or ng/g (ppb) should an approximate 10 % underestimate of concentrations expressed on a volumetric basis.

Results and Discussion

All fish oil samples contained detectable residues of organochlorine contaminants, with cod liver oil (Samples 1-7) having similar levels as previously reported[1, 2, 9] and the greatest levels of POPs contaminants compared to the other supplement groups in this study. Fish and vegetable oil mixtures (Samples 14-17) had lower levels than the whole fish body oils, while no PCBs were detected in the vegetable oils (Samples 18-21). On the whole, the fish oils showed less contamination compared with past reports[1, 9-11] suggesting that improved sourcing, developments in processing such as deodorisation and nutritional profile development in creating specific ‘optimum’ essential fatty acid balances (Samples 8, 14-17, 21) are improving the quality of such supplements for the consumer.

With most of the samples, the predominant pesticide isomers were *p,p'*-DDE and g-HCH. Results are presented in Table 1, with total sum (S) values given using the LOD equal to zero where there were no detections. In comparison with the samples purchased eight years ago[2], there is a clear reduction of contaminant loadings. Most fish oil samples showed evidence of steam distillation, and this probably contributed to the reduction in contaminant levels. HCHs and HCB levels were very low, only the a- and g-HCH isomers were detected. DDTs were present in all but one sample (Sample 21, a vegetable oil mix from Canada), while PCBs were under the detection limit for some samples. The oils with higher concentrations of PCBs had low concentrations of volatile compounds, an indication of thermal processing. The PCB profile was dominated by hexa and penta congeners followed by hepta, with some variation in percentages of different PCB congeners depending upon the type of fish oil and sample formulation. In the fish oils, higher levels of DDTs were correlated with higher PCB concentrations (r^2= 0.78). PCB concentrations in the fish oil samples were between ND and 90.9 ng/g lipid. These concentrations are generally of the same order of magnitude, but are not as high as previously reported based on ICES 7 PCBs[2]. This is probably a consequence of the reduction of OC contaminants during refining[12] as requested by UK government departments[1]. Comparisons with unrefined fish oils would indicate whether a real reduction in the steady state of PCBs has occurred[10, 11]. The ICES 7 PCBs consistently constituted over 50 % of the sum of PCB congeners in all cod liver oil and fish oil samples where PCBs were detected.

The PBDE levels in cod liver oils ranged from 14.6 to 34.2 ng/g and were greater than those reported in samples collected three years previously[8]. The fish and fish/vegetable formulations showed PBDEs levels which were an order of magnitude lower than the cod liver oils and all but one fish/vegetable oil sample (Sample 17) had detectable levels of PBDEs. No PBDEs were detected in the pure vegetable oils. The most abundant PBDE, (BDE 47) was detected at concentrations comparable to PCB 118. PBDEs showed the same trend as PCBs (r^2= 0.94). Our results are in good agreement with the levels of PCB and OC pesticides previously reported for fish oil supplements[9, 13], but with higher levels of PBDEs than those recently reported[8].

Although fish are a significant source of POPs in the diet, fish and fish oil consumption are associated with numerous health benefits due to the long chain omega-3 essential fatty acids. There are alternative sources of omega-3 fatty acids with evident lower contamination levels. a-linolenic acid (abundant in rapeseed and flax (linseed) oil) is the short chain form found in the vegetable oil based nutritional supplements used in this study. A number of human clinical studies have demonstrated good conversion of a-linolenic acid to the metabolically active eicosapentaenoic acid (EPA), but to date this has not been reported for decosahexaenoic acid (DHA). At the bottom of the food chain, DHA is originally synthesised by phytoplankton and krill (*Euphausia pacifica*)[14]. Species further along the food chain preferentially accumulate DHA, but appear to be unable to synthesise it effectively. DHA is of nutritional importance in fetal development, so nutritional sources of DHA are often promoted for pregnant and breast feeding women.

In terms of risk assessment, the potential contribution to the human diet of POPs from omega-3 fatty acids food sources increases if sourced from fish, particularly cod liver. The vegetable oils studied here presented little or no POP contamination, but do not provide an effective metabolic source of DHA for the average consumer. The possible contribution to dietary intakes of POPs from cod liver oils currently available in UK and international markets could clearly be significant for high consumers. Although preliminary, this investigation strongly supports the need for monitoring POPs, including the newer POPs, in fish oils designated both as nutritional supplements for consumers and as animal feed ingredients.

References

1. MAFF (1997) Food Surveillance Information Sheet 106, June 1997. FSA, London UK.
2. Jacobs MN, Johnston PA, Wyatt CL, Santillo D, French M (1997). Int J Env Pollut 8, 74-93.
3. Hamilton RJ, Rice RD Eds. Fish Oil: Technology, Nutrition and Marketing. PJ Barnes & Associates 1995
4. Gregory J, Foster K, Tyler H, Wiseman M (1990) The Dietary and Nutritional Survey of British Adults. Ministry of Agriculture Fisheries and Food, Dept of Health. HMSO. London.

5. de Boer J, Allchin C (2001) Organohalogen Compounds 52, 13-17.
6. Jackson LJ, Carpenter SR, Manchester-Neesvig JB, Stow CA (2001) Environ Sci Technol 35, 856-862.
7. Manchester-Neesvig JB, Valters K, Sonzogni WC (2001) Environ Sci Technol 35, 1072-1077.
8. Jacobs MN, Covaci A, Schepens P (2002) Environ Sci Technol In press.
9. Jacobs MN, Johnston PA, Santillo D, Wyatt CL (1998) Chemosphere 37, 1709-1731.
10. Falandysz J, Kannan K, Tanabe S, Tatsukawa R (1994) Ambio 23, 2188-293.
11. Falandysz J (1994) Arch Environ Contam Toxicol 27, 266-271.
12. Hilbert G, Lillemark L, Balchen S, Schriver Højskov C (1998) Chemosphere 37, 1241-1252.
13. Fromberg A (2001) Organohalogen Compounds 51, 352-355.
14. Saito H, Kotani Y, Keriko JM, Xue C, Taki K, Ishihara K, Ueda T, Miyata S (2002) Marine Chemistry 78, 9-28.

Table 1: PCBs, PBDEs and organochlorine pesticides in fish and vegetable oils rich in omega –3 fatty acids: Lipid Adjusted (ng/g, ppb)

Sample No.		Further Information	Country of Origin	Sample Wt (g)*	PCBs Σ tri	Σ tetra	Σ penta	Σ hexa	Σ hepta	Σ octa	Σ PCBs[a] (ND=0)	Σ 7 marker PCBs[b]	Σ 7 PBDEs	HCB	γ-HCH	Σ HCHs	*pp*-DDE	*pp*-DDT	*pp*-DDD	Σ DDTs	*pp*DDT/ ΣDDT
1	Cod Liver Oils	Enriched with FO	UK	0.54	ND	2.2	39.0	90.3	27.6	1.6	**160.7**	**92.1**	**14.9**	ND	ND	ND	56.4	22.7	44.1	**137.7**	0.17
2		Pure CLO	UK	0.60	ND	0.7	36.9	102.7	31.4	2.0	**173.7**	**96.6**	**20.0**	ND	ND	ND	56.7	26.0	55.1	**155.9**	0.17
3		CLO, soyabean oil	NA	0.62	2.8	4.9	69.5	79.0	18.6	0.5	**175.4**	**86.7**	**14.6**	9.1	5.1	9.0	44.9	12.0	32.9	**99.6**	0.12
4		Pure CLO	UK	0.66	2.9	6.7	120.9	183.7	43.6	360.1	**360.1**	**201.9**	**34.2**	2.9	0.9	1.8	106.3	28.3	73.7	**224.1**	0.13
5		Pure CLO	UK	0.62	8.3	4.2	73.1	96.9	27.1	210.4	**210.4**	**110.9**	**20.3**	7.3	1.2	5.4	62.0	7.9	36.8	**116.7**	0.07
6		Enriched with FO	NA	0.61	6.9	4.3	78.4	93.3	26.4	210.3	**210.3**	**107.7**	**15.6**	4.9	1.3	3.2	50.5	7.4	38.7	**104.9**	0.07
7		CL oil+taste masks	NA	0.61	0.2	3.8	54.1	120.5	44.0	225.1	**225.1**	**133.3**	**22.3**	ND	ND	ND	75.3	34.2	65.2	**193.3**	0.18
8	Fish Oils	Salmon oil	N/USA	0.62	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**0.8**	ND	ND	ND	1.6	1.9	1.3	**4.8**	0.40
9		Marine lipid conc.	NA	0.60	ND	2.1	28.6	34.4	9.9	ND	**75**	**38.7**	**0.8**	3.4	ND	0.5	14.5	5.6	10.2	**30.3**	0.19
10		Fish body oil	NA	0.60	ND	ND	32.9	24.8	2.5	60.3	**60.3**	**35.1**	**1.4**	0.2	ND	ND	10.5	4.5	11.4	**31.4**	0.14
11		Fish body oil	NA	0.61	ND	ND	13.4	28.6	8.1	50.1	**50.1**	**27.8**	**2.6**	ND	ND	ND	10.3	6.3	7.6	**35.9**	0.18
12		FO concentrate.	NA	0.62	0.2	ND	17.1	32.5	8.1	57.9	**57.9**	**32.0**	**1.9**	ND	ND	ND	6.3	7.1	1.9	**15.4**	0.46
13		Pure FO	UK	0.60	0.2	3.8	38.5	40.4	8.0	90.9	**90.9**	**49.0**	**2.7**	0.8	0.3	0.8	20.8	8.1	12.7	**47.1**	0.17
14	Fish+ Veg Oils	EPO, Tuna fish oil	NA	0.60	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**0.3**	ND	0.2	0.2	12.3	2.2	2.7	**21.4**	0.10
15		EPO, Marine FO	NL	0.60	1.0	2.5	15.4	28.8	3.5	ND	**51.2**	**28.9**	**1.9**	4.2	0.7	1.7	14.4	6.2	11.7	**39.8**	0.16
16		FO, garlic, vit/min lecithin enriched	NA	0.55	ND	ND	13.6	20.0	0.7	34.4	**34.4**	**18.7**	**1.1**	ND	ND	0.4	48.6	77.8	10.2	**142.9**	0.55
17		EPO, marine FO	NA	0.64	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	ND	1.5	2.5	05	ND	0.6	**1.1**	-
18	Veg Oils	Unrefined LO	NA	0.61	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	ND	5.9	5.9	0.7	5.4	0.8	**8.2**	0.66
19		Organic FSO	NA	0.61	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	ND	1.3	1.3	0.5	2.3	0.5	**3.9**	0.59
20		Organic CP LO	Germany	0.60	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	ND	0.6	0.6	0.3	ND	0.7	**1.0**	-
21		FSO, SSO,SeSO, MCT, EPO, RO, OO	Canada	0.61	ND	ND	ND	ND	ND	ND	**ND**	**ND**	**ND**	ND	0.3	0.3	ND	ND	ND	**ND**	-

CLO=Cod liver oil; EPO=Evening primrose oil; FO=fish oil; CP=cold pressed; LO=linseed oil; FSO=flax seed oil; SSO=sunflower seed oil; SeSO=sesame seed oil; MCT=medium chain triglcerides; RO=rice bran and rice germ oils; OO= Oat bran and oat germ oils; Veg= vegetable; vit/min=vitamins and minerals; N= Norway; NA =Not Available; ∗ Sample Wt taken for analyses; ND= Non Detects (treated as 0 for Σcalculations); [a]28 PCB congeners - IUPAC no's: 18, 28, 31, 52, 74, 95, 99, 101, 105, 110, 118, 128/174, 132, 138, 149, 153, 156, 163, 167, 170, 177, 180, 183, 187, 194, 196, 199; [b] ICES 7 marker PCBs: IUPAC no's; 28, 52, 101, 118, 138, 153, 180; 7 PBDE's no's: 28, 47, 99, 100, 153, 154, 183.