INVESTIGATION OF DIOXINS, FURANS AND DIOXIN –LIKE PCBS IN N-3 POLYUNSATURATED FATTY ACID RICH DIETARY FISH AND VEGETABLE OIL SUPPLEMENTS.

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Introduction

Fish oils are widely used as animal feed supplements and have also been used traditionally as dietary supplements, as they are known to have broad nutritional and therapeutic benefits in, for example, cardiovascular, immunological and arthritic disease due to the presence of long chain *n*-3 fatty acids. Fish oils have been shown to be susceptible to contamination with lipophilic organic chemicals that are now ubiquitous contaminants of marine ecosystems. Consequently maximum levels of polychlorinated dibenzo-*p*-dioxins, furans (PCDD/Fs) and dioxin like PCBs (DL-PCBs) have been established in EU legislation related to the presence of PCDD/Fs and DL-PCBs in animal feed [Commission Directive 2006/13/EC of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and DL-PCBs]. Many vegetable oils are sources of the shorter chain precursor forms of *n*-3 fatty acids, and in recent years the dietary supplement specialist market has expanded to include these oils in a variety of different formulations.
This study reports the levels of PCDD/Fs and DL-PCBs, in 21 *n*-3 rich dietary fish and vegetable oil supplements obtained through retail and company outlets in London, United Kingdom in 2002. The data presented here extends the persistent organic pollutants (POPs) data reported in an earlier time trends study report (1) of the same samples. Levels are discussed in relation to comparative studies of dietary supplement fish oil samples obtained also in 2001-2.

Materials and Methods

Sample description: Twenty one dietary supplements rich in *n*-3 fatty acids (7 cod liver oils, 6 whole body fish oils, 4 variable fish and vegetable combination oils and 4 vegetable oils) were obtained from retail and practitioner suppliers of the UK market in December 2001 and January 2002. Sample descriptions have been given previously (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.
Oil density: Densities of oils were determined gravimetrically and were between 0.87 and 0.90 g/ml. Thus, direct comparisons of concentrations given in µg/l with those given in µg/kg or ng/g should allow for an approximate 10% underestimate of concentrations expressed on a volumetric basis.

Materials: All organics solvents used were Dioxin analysis grade (Sigma-Aldrich, Buchs SG, Switzerland). Sulfuric acid 98% extra pure was obtained from VWR International s.r.l. (Milan, Italy). Diatomea earths, Extrelut was obtained from Merck (Darmstadt, Germany). Multi-layer Silica, basic alumina and carbon columns ready to use were obtained Fluid Management Systems (FMS) Inc., (Watertown, MA, USA). 68-CVS and 68-LCS were native and ^{13}C-labelled internal standards for 12 congeners DL-PCBs. EPA-1613CVS, EPA1613LCS and EPA-1613ISS were native, ^{13}C-labelled internal and recovery standards for 17 PCDDs/Fs respectively. The standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada). EC-4058 for 7 indicator PCBs ^{13}C-labelled was obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

Analytical determinations: Oil samples (5-7g), after spiking with internal standards (16 ^{13}C-labelled 2,3,7,8-chlorine-substituited congeners with 400 pg each, except OCDD with 800 pg and 12 DL-PCBs and 7 indicators ^{13}C-labelled with 2000 pg each), were dissolved in 20 ml of concentrated sulfuric acid. The acid phase was adsorbed overnight on pre-cleaned (26g) diatomea earth's column and then eluted with 200ml of n-hexane. After extract concentration to 10ml, the purification was executed with an automated clean-up system (Power-Prep P6, from Fluid Management Systems, Inc., Watertown, MA, USA). Two fractions were obtained, one containing mono-ortho substituted PCBs and one containing coplanar PCBs and PCDD/Fs. The purification method was previously described by Abad et al (2).
Analysis of PCDD/Fs and PCBs was based on isotope dilution using HRGC-HRMS (high resolution gas chromatography – high resolution mass spectrometry). The GC (HP-6890, Hewlett Packard, Waldbronn, Germany), was coupled with a VG Autospec Ultima mass spectrometer (Micromass, Manchester, UK) operating in EI-mode at 34 eV with a resolution of >10000. Quantification was performed on the basis of 1613 and 1668 U.S. EPA methods (3, 4). GC separation of PCBs and PCDD/Fs was performed on BP-DXN 60 m long with 0.25 mm i.d.(inner diameter) and 0.25 µm film (SGE, Victoria, Australia).

Quality Assurance and Quality Control
The quantified isomers were identified through retention time comparison of the corresponding internal standard and the isotopic ratios between two ions was recorded.
Levels of analytical blanks obtained during the clean-up process were 5-10 times lower with respect to the lower reported concentrations both for PCDD/Fs and DL-PCBs. The blank level was not subtracted. The reported detection limits were calculated individually for each sample on the bases of a signal to noise ratio of 3/1.

Results and Discussion

1. Levels of dioxins, furans and DL-PCBs in n-3 rich dietary supplements
All fish oil samples contained detectable residues of PCDD/Fs and DL-PCBs, with cod liver oil having similar levels to those previously reported (5, 6) (with the total mean WHO-PCDD/F-PCB –TEQ (1998) (7) of 8.8pg/g, therefore falling within the current EU maximum feedingstuff WHO-PCDD/F-PCB TEQ level of 24 pg/g, and the greatest levels of PCDD/Fs and DL-PCBs compared to the other supplement groups in this study. Mono ortho PCBs 105, 118, 156, 167 dominated the PCB congener profile. At a total mean WHO TEQ of 7.6 pg/g the whole body fish oils also were within the EU maximum feedingstuff level. However following the manufacturers recommended dose for cod liver and fish oils, in combination with whole dietary exposure intakes could exceed the recommended UK tolerable daily intakes (TDI) of 2pg WHO-TEQ/kg/bodyweight/day and the WHO TDI of 1-4 pg TEQ/kg/bodyweight/day.
Fish and vegetable oil mixtures had similar WHO TEQ levels of PCDD/F levels to the whole fish body oils (mean 0.68 pg/g and 0.87 pg/g respectively), but lower DL-PCB WHO-TEQ than the

whole body fish oils (mean 1.5 pg/g versus 0.21 pg/g respectively), while little PCDD/Fs and DL-PCBs (mean WHO-TEQ 0.11pg/g and 0.01 pg/g respectively) were detected in the vegetable oils, PCB 118 being the greatest with a mean value of 24 pg/g, followed by PCB 105. Results are presented in Table 1 in pg/g wet weight, with mean total sum TEQ pg/g values given using the LOD equal to zero where there were no detections.

2. Comparison with similar studies and risk assessment considerations

These results are in good agreement and of a similar order of magnitude with the levels and congener profiles of PCDD/F and DL-PCBs previously reported for fish oil supplements by the UK Food Standards Agency and the Irish Food Safety Authority, (*5,6*) sampled at a similar time point. However in the present study the fish oil samples showed a trend for slightly lower contamination with POPs compared with the older samples (1) and past reports (5,6). This is probably a consequence of the reduction of organochlorine contaminants (OC) contaminants during refining, as now required by EU regulations, as well as being due to dilution.

Both this study and the UK Food Standards Agency (5) found that the fish oils available to the consumer were often diluted (usually with vegetable oils) compared with the pre-2000 fish oils analyses in the public domain. Thus improved sourcing, processing (e.g. deodorisation) and nutritional profile development of 'optimum' essential fatty acid balances could be improving supplement quality for the consumer.

In terms of risk assessment, the potential contribution to the human diet of PCDD/Fs and DL-PCBs from *n*-3 fatty acids food sources increases if sourced from fish, particularly cod liver. The vegetable oils studied here presented little or no PCDD/F DL-PCB contamination, but approximately ten times the quantity needs to be consumed to provide comparative levels of the long chain form of *n*-3 fatty acids (eicosapentaenoic and docosahexaenoic acid), and this increase in quantity needs to be accounted for when calculating comparative dietary intakes of POPs contaminants from these dietary sources. Furthermore, supplementation with fish oils may impair a person's subsequent metabolic ability to convert the *n*-3 short chain form from vegetable sources to the long chain form required in human metabolism.

The possible contribution to dietary intakes of PCDD/Fs, DL-PCBs and other POPs from cod liver oils available in the UK and international markets could clearly be significant for high consumers, as has been shown previously (1,5,6). Although preliminary, this investigation strongly supports the need for monitoring POPs in fish oils designated both as nutritional supplements for consumers and as animal feed ingredients.

This paper reflects the professional views of the authors and has not been endorsed by the European Commission.

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	Congener	Cod liver oil (n=7)		Fish oils (n=6)		Fish + vegetable oils (n=4)		Vegetable oils (n=4)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Dioxins/Furans	**2,3,7,8-TCDD**	0.24	**0.02**-0.45	0.06	**0.03-**0.11	0.05	0.02-0.11	**0.02**	0.01-0.02
	1,2,3,7,8-PeCDD	0.40	**0.03-**0.93	0.12	0.01-0.2	0.13	0.08-0.21	**0.02**	0.01-0.04
	1,2,3,4,7,8-HxDD	0.14	**0.02-**0.29	0.09	0.1-0.2	0.26	0.06-0.81	**0.03**	0.02-0.03
	1,2,3,6,7,8-HxDD	0.29	0.07-0.65	0.19	0.03-0.47	0.84	0.15-2.7	**0.03**	0.02-0.06
	1,2,3,7,8,9-HxDD	0.12	**0.03-**0.22	0.22	0.01-0.81	1.21	0.18-3.92	0.04	0.02-0.07
	1,2,3,4,6,7,8-HpDD	0.82	0.33-1.43	1.97	0.38-7.97	7.97	1.10-35.1	0.31	0.19-0.52
	OCDD	3.63	0.42-7.62	3.52	1.56-5.26	5.27	3.74-49.7	1.26	0.81-2.15
	2,3,7,8-TCDF	3.73	0.10-8.11	1.08	0.17-1.79	0.66	0.09-1.38	0.05	0.02-0.07
	1,2,3,7,8-PeCDF	0.64	0.10-1.28	0.21	0.07-0.4	0.19	0.05-0.19	0.04	0.02-0.06
	2,3,4,7,8-PeCDF	1.4	0.13-3.14	0.5	0.13-0.87	0.35	0.05-0.67	0.06	0.02-0.12
	1,2,3,4,7,8-HxDF	0.24	0.10-0.43	0.15	0.06-0.23	0.22	0.15-0.32	0.06	0.03-0.08
	1,2,3,6,7,8-HxDF	0.26	0.15-0.37	0.13	0.05-0.2	0.23	0.2-0.26	0.06	0.05-0.07
	2,3,4,6,7,8-HxDF	0.37	0.2-0.69	0.21	0.14-0.26	0.33	0.28-0.38	0.12	0.11-0.16
	1,2,3,7,8,9-HxDF	0.13	0.03-0.23	0.06	0.02-0.1	0.14	0.08-0.27	0.02	0.02-0.03
	1,2,3,4,6,7,8-HpDF	0.62	0.32-1.49	0.55	0.3-0.85	1.07	0.82-1.7	0.3	0.22-0.46
	1,2,3,4,7,8,9-HpDF	0.14	0.06-0.36	0.09	0.04-0.12	0.16	0.1-0.19	0.05	0.02-0.11
	OCDF	0.88	0.34-2.55	0.75	0.34-1.65	1.42	0.74-2.72	0.42	0.30-0.69
	Human-WHO-TEQ*	1.92	0.39-4.06	0.68	0.16-1.07	0.87	0.36-1.35	0.11	0.08
DL PCBs	**TeCB-77**	157	6-392	161.3	5.1-264	16.0	2.7-36.5	**1.2**	0.8-1.6
	TeCB-81	4.1	1.0-9.2	2.0	0.3-3.1	0.73	0.24-1.58	**0.09**	0.07-0.12
	PeCB-126	53.4	4.6-107	60	15-104	8.21	0.53-17	0.13235	0.06-0.17
	HxCB-169	9.4	1.8-18.0	5.6	2.0-8.8	1.66	0.05-3.65	0.049	0.03-0.06
	Non-ortho PCB WHO-TEQ	5.45	0.48-10.9	6.1	1.52-11.0	0.839	0.053-1.7	0.0	0.01.02
	PeCB-105	5485	2687-11400	3063	101-4534	284	24-701	10	6-16
	PeCB-114	324	162-716	136	6-240	18	1-47	0.6	0.4-3.5
	PeCB-118	14481	7450-30720	5772	250-8904	814	56-2153	24	18-34
	PeCB-123	279	103-593	127	6-213	18	2-38	0.6	0.4-0.8
	HxCB-156	1731	846-3522	858	211-1343	137	10-306	3	1-3
	HxCB-157	467	215-988	213	45-343	30	3-62	0.6	0.2-0.8
	HxCB-167	978	438-1833	431	137-600	93	4-193	1.4	1-2
	HpCB-189	165	67-312	67	47-92	21	1-50	0.30	0.1-0.4
	Mono-ortho PCB WHO-TEQ	3.3	1.8-6.9	1.5	0.17-2.1	0.207	0.015-1	0.01	0.003-0.01
	Total PCDD/F + DL-PCB WHO-TEQ	8.8	2.4-14.5	7.6	1.7-12	1.05	0.08-2.01	0.019	0.01-0.02

Notes: * Upper bound; Combined concentrations of dioxins and dioxin like PCBs may not equal the sum of the separate concentrations due to rounding; N=number of samples analysed; Bold = Limit of detection (LOD)