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**Memo**

**To:** John Hocevar, Greenpeace USA
**From:** Dr David Santillo, Senior Scientist, Greenpeace Research Laboratories
**Date:** 18th January 2007
**Re:** Summary of independent analysis by CEFAS of menhaden fish meal and fish oil samples

**Summary:**

Attached please find the results of the analyses of the four samples of menhaden-derived products (one fish meal and three fish oil samples) which were tested at the laboratories of CEFAS (Centre for Environment, Fisheries and Aquaculture Science) at Burnham on Crouch in the UK.

| Sample code | Sample description |
|---|---|
| MI06020 | fish meal |
| MI06022 | slightly refined fish oil |
| MI06021 | highly refined fish oil |
| MI06023 | fish oil dietary supplement capsules |

The samples were tested for a range of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and the organochlorine pesticides dieldrin, hexachlorobenzene (HCB), DDT and its metabolites and two isomers (alpha & gamma) of hexachlorocyclohexane (HCH).

All three of the menhaden oil products tested, including the dietary supplements, contained a range of PCBs, PBDEs and residues of the pesticides DDT and dieldrin. To our knowledge, the current study provides the first data on PCB contamination in oil derived from menhaden.

At 0.195 ppm, the PCB levels in the dietary supplements (sum of all 25 congeners analysed), when referenced to the EPA screening values for fish tissue (0.02 ppm, assuming fish consumption of 12g/day), indicate an acceptable level of consumption for this fish oil of less than 2 capsules per day, even though concentrations fall below the long-standing FDA limit of 2 ppm (based on all congeners present in commercial mixtures). In this context, it is worth noting that Omega recommends a dose of "1-2 capsules with a meal up to 3 times daily or as directed by a healthcare professional".

On the basis of the sum of the so-called ICES 7 congeners (0.092 ppm), PCB concentrations in the dietary supplement capsules were at the very limit set by the industry as a voluntary standard (0.09 ppm) under the Council for Responsible Nutrition's Voluntary Monograph of March 2006 (CRN 2006). However, it must be remembered that the sum of ICES 7 PCBs, though a convenient standard for inter-comparison, nevertheless represents only a fraction of overall PCB burdens in the oils; for example, they comprise between 47% and 52% of the totals calculated for all 25 congeners included in the current study. Furthermore, many other PCB congeners, which may well have individually made additional contributions to total concentrations, were not included in the analyses. And finally, fish oils represent just one source of exposure to PCBs and other lipophillic contaminants, which would inevitably be compounded by exposure from other sources.

**Analysis:**

The samples arrived at our laboratory on 11th May 2006 and were immediately sub-sampled to retain a portion of each for archived storage. The remainders of each sample were forwarded to CEFAS. The single fish meal sample was Soxhlet extracted for 4 hours using a 1:1 mixture of hexane and acetone. Oil samples were diluted 1:20 using hexane. Following extraction/dissolution, extracts were cleaned and fractionated using alumina and silica columns respectively, resulting in one fraction for PCB analysis and a second for analysis of PBDEs and pesticide residues.

A total of 25 PCB congeners and 7 pesticide residues were quantified using GC-ECD (on a 50m DB-5 capillary column) and individual PCB and pesticide residue standards. A total of 11 PBDEs were quantified using GC-MS-NICI operated in SIM mode. A blank and suitable certified reference material were prepared and run alongside the samples for quality control purposes.

Further details of the methods employed are included at Annex 1.

The results of these analyses are summarised in Tables 1 to 3 below, addressing PCBs, PBDEs and pesticide residues respectively.

Concentrations of all contaminants in the fish meal were either very close to or below detection limits (0.001 ppm for PCBs and pesticide residues, 0.000125 ppm for PBDEs).

In the case of the fish oils, concentrations of contaminants were substantially higher, as may be expected from the nature of the sample matrix given that the contaminants in question are highly lipophillic.

Over all, a general trend in concentrations was apparent, with lower levels in the more highly refined oil compared to the slightly refined oil and lower levels again in the fish oils sold as dietary supplements. This is particularly clear for the PCBs, whether expressed individually, as sums of concentrations for the 25 congeners analysed or as sums of the ICES 7 PCBs (CB#28, 52, 101, 118, 138, 153 and 180) frequently used as the basis for inter-comparison. For example, sums of concentrations for the 25 congeners together ranged from 0.494 ppm for the slightly refined oil to 0.366 ppm in the more highly refined oil and 0.195 ppm in the dietary supplement capsules. The ICES 7 PCBs contributed approximately half of those totals (at 0.258, 0.188 and 0.092 ppm respectively).

A similar trend was apparent in the case of the pesticides dieldrin (0.044, 0.030 and 0.017 ppm respectively) and the DDT metabolites p,p' DDE (0.110, 0.094 and 0.047 ppm respectively) and p,p' TDE (DDD) (0.058, 0.042 and 0.036 ppm respectively).

| PCB congener | Fishmeal | Slightly Refined Fish Oil | Highly Refined Fish Oil | Fish Oil Supplement Capsules |
|---|---|---|---|---|
| **CB#18** | *<0.001* | *<0.001* | *<0.001* | *<0.001* |
| **CB#28** | *<0.001* | 0.006 | 0.005 | *<0.001* |
| **CB#31** | *<0.001* | 0.001 | 0.002 | *<0.001* |
| **CB#44** | *<0.001* | 0.003 | *<0.001* | *<0.001* |
| **CB#47** | *<0.001* | 0.003 | *<0.001* | *<0.001* |
| **CB#49** | *<0.001* | 0.01 | 0.006 | 0.003 |
| **CB#52** | *<0.001* | 0.017 | 0.009 | *<0.001* |
| **CB#66** | *<0.001* | 0.022 | 0.018 | 0.002 |
| **CB#101** | *<0.001* | 0.039 | 0.025 | 0.001 |
| **CB#105** | *<0.001* | 0.019 | 0.018 | 0.015 |
| **CB#110** | *<0.001* | 0.03 | 0.019 | 0.007 |
| **CB#118** | *<0.001* | 0.029 | 0.022 | 0.009 |
| **CB#128** | *<0.001* | 0.006 | 0.003 | 0.002 |
| **CB#138** | *<0.001* | 0.055 | 0.042 | 0.028 |
| **CB#141** | *<0.001* | 0.003 | *<0.001* | *<0.001* |
| **CB#149** | *<0.001* | 0.041 | 0.029 | 0.013 |
| **CB#151** | *<0.001* | 0.01 | 0.006 | 0.001 |
| **CB#153** | *<0.001* | 0.082 | 0.063 | 0.033 |
| **CB#156** | *<0.001* | 0.008 | 0.012 | 0.007 |
| **CB#158** | *<0.001* | 0.012 | 0.011 | 0.01 |
| **CB#170** | *<0.001* | 0.014 | 0.011 | 0.012 |
| **CB#180** | *<0.001* | 0.03 | 0.022 | 0.021 |
| **CB#183** | *<0.001* | 0.012 | 0.01 | 0.008 |
| **CB#187** | *<0.001* | 0.038 | 0.03 | 0.02 |
| **CB#194** | *<0.001* | 0.004 | 0.003 | 0.003 |
| **Sum of 25 PCBs** | *<0.001* | 0.494 | 0.366 | 0.195 |
| **Sum of ICES 7 PCBs** | *<0.001* | 0.258 | 0.188 | 0.092 |

Table 1: Concentrations of 25 individual PCB congeners (ug/g fresh weight, or parts per million, ppm) in the single menhaden fish meal and three menhaden fish oil samples. The sums of concentrations for all 25 individual PCBs and for the ICES 7 PCBs (CB#28, 52, 101, 118, 138, 153 and 180) have been calculated by assigning zero values to all congeners below detection limits.

| BDE congener | Fishmeal | Slightly Refined Fish Oil | Highly Refined Fish Oil | Fish Oil Supplement Capsules |
|---|---|---|---|---|
| **BDE#17** | *<0.000125* | 0.0012 | 0.0011 | *<0.000125* |
| **BDE#28** | *<0.000125* | 0.0019 | 0.0011 | *<0.000125* |
| **BDE#47** | 0.00033 | 0.026 | 0.014 | 0.016 |
| **BDE#66** | *<0.000125* | *<0.000125* | *<0.000125* | *<0.000125* |
| **BDE#85** | *<0.000125* | *<0.000125* | *<0.000125* | *<0.000125* |
| **BDE#99** | 0.00014 | 0.0019 | 0.0019 | 0.0015 |
| **BDE#100** | *<0.000125* | 0.0052 | 0.0026 | 0.004 |
| **BDE#138** | *<0.000125* | *<0.000125* | *<0.000125* | *<0.000125* |
| **BDE#153** | *<0.000125* | *<0.000125* | *<0.000125* | 0.0012 |
| **BDE#154** | *<0.000125* | 0.0027 | 0.002 | 0.0033 |
| **BDE#183** | *<0.000125* | *<0.000125* | *<0.000125* | *<0.000125* |
| **Sum of 11 BDEs** | 0.00047 | 0.0389 | 0.0227 | 0.026 |

Table 2: Concentrations of 11 individual BDE (brominated diphenyl ether) congeners (ug/g fresh weight, or parts per million, ppm) in the single menhaden fish meal and three menhaden fish oil samples. The sums of concentrations for all 11 individual BDEs have been calculated by assigning zero values to all congeners below detection limits.

| Pesticide residue | Fishmeal | Slightly Refined Fish Oil | Highly Refined Fish Oil | Fish Oil Supplement Capsules |
|---|---|---|---|---|
| **Dieldrin** | *<0.001* | 0.044 | 0.03 | 0.017 |
| **HCB** | *<0.001* | 0.001 | *<0.001* | *<0.001* |
| **p,p' DDE** | 0.004 | 0.11 | 0.094 | 0.047 |
| **p,p' DDT** | 0.004 | 0.033 | 0.03 | *<0.001* |
| **p,p' TDE** | 0.003 | 0.058 | 0.042 | 0.036 |
| **alpha HCH** | *<0.001* | 0.012 | 0.012 | *<0.001* |
| **gamma HCH** | *<0.001* | *<0.001* | 0.02 | *<0.001* |

Table 3: Concentrations of 7 common pesticide residues (ug/g fresh weight, or parts per million, ppm) in the single menhaden fish meal and three menhaden fish oil samples, including dieldrin, HCB (hexachlorobenzene), DDT and its metabolites and two isomers of HCH (hexachlorocyclohexane).

The summed concentrations of the 11 BDEs were approximately 10 times lower than those for the 25 PCBs, at between 0.0227 and 0.0389 ppm. Once again, the highest values were recorded for the slightly refined fish oil (0.0389 ppm), while concentrations in the highly refined and dietary supplement oils were quite similar to each other, at 0.0227 and 0.0260 ppm respectively.

Comparison of the concentrations of PCBs determined in this study with those reported for fish oils derived from other species and locations is somewhat limited by the fact that most recent studies focus only on those PCBs exhibiting toxicity similar to that of the chlorinated dioxins ad furans (so-called 'dioxin-like' PCBs). In most cases, therefore, studies report concentrations normalised to toxicity equivalents (generally WHO-TEQs) rather than absolute concentrations for a wider range of PCB congeners. Nevertheless, it is clear that the concentrations recorded for the menhaden oils fall in a similar range to those we have previously reported for total PCBs in a range of pharmaceutical and industrial grade fish oils sold as dietary supplements in Europe over ten years ago (ICES 7 <0.046-0.383 ppm, Jacobs *et al.* 1997). Since then, it has been reported by others that PCB concentrations in oils derived from European fish stocks have fallen slightly, with concentrations of ICES 7 PCBs in dietary supplements sold in the UK during the period 2000-2002 in the range 0.0083-0.267 ppm (Fernandes *et al.* 2006). Comparable samples from Ireland in 2001 revealed concentrations towards the lower end of this range (Food Safety Authority of Ireland 2001).

At the same time, however, it is worth noting that the concentrations recorded for all grades of the menhaden oil, including the dietary supplement capsules, were substantially higher than those reported for a wide range of high purity and ultra-refined fish oil supplements analysed under the International Fish Oil Standards' Consumer Report programme (IFOS 2006). To our knowledge, the current study provides the first data on PCB contamination in oil derived from menhaden.

Despite the obvious concerns raised by the accumulation of PCBs and other toxic and persistent halogenated organic contaminants in fish oils, there remain relatively few regulatory limits or guidance levels applicable to absolute concentrations in foodstuffs. Until very recently, PCB-related limits in Europe have been based on tolerable daily intakes of such contaminants from all sources (2pg WHO-TEQ per kg body weight per day for dioxin-like PCBs, in combination with dioxins themselves), rather than on concentrations of PCBs in any particular foodstuff. Concentration-based limits for dioxin-like PCBs finally came into force across Europe for a range of foodstuffs during November 2006, as an amendment to existing limits on dioxins in food set in 2001 and in force since July 2002 (CEC 2006). However, the range of congeners analysed in the current study does not allow direct comparison of the concentrations in the menhaden oils with those TEQ-based limits.

Within the USA, the FDA does apply an overall tolerance limit of 2 ppm for PCBs in fish and shellfish for consumption. On this basis, PCB concentrations recorded for the fish oil dietary supplement analysed in the current study fall significantly (10 to 20 times) below that level (at between 0.092 and 0.195 ppm, depending on the summation method employed). However, a far lower 'screening value' of 20 ppb (0.02 ppm, determined against Aroclor standard mixtures of PCBs) is set by the US EPA for fish consumption by recreational fishers, based on an assumed average daily intake of 12g of material and an 'acceptable' cancer risk level of $10^{-5}$. Against this screening value, the potential dietary intake from even a single menhaden oil capsule taken each day can be seen to be quite significant. Intake from consumption of more than one capsule each day would exceed the EPA guideline, even before consideration of contributions from other sources. Omega recommends a dose of "1-2 capsules with a meal up to 3 times daily or as directed by a healthcare professional".

It is worth bearing in mind that, although indicative of broad levels and trends of contamination, neither the ICES 7 nor the sum of the 25 congeners quantified in the current study encapsulate the totality of possible PCB congeners and nor, therefore, the overall PCB concentrations in these samples. Previous work has shown that sums of individual congener concentrations can fall substantially short of totals determined on the basis of matching against commercial Aroclor standards, for example. Hence it is very likely that both summation methods applied in the current study underestimate total PCB burdens in the menhaden oils.

Furthermore, even with the inclusion of the 11 PBDE congeners and the selected pesticide residues in the current study, it must be remembered that all these analytes together still only represent a subset of the overall range of persistent organic pollutants which are known to have become widespread in the environment and accumulate in fatty foodstuffs. In contrast to regulatory limit approaches which tend to focus on single chemicals or related groups at a time, whether limits are concentration- or intake- based, in practice we are exposed to complex mixtures of individual contaminants, which may contribute in an additive (or sometimes even greater than additive) manner to overall toxicity.

Irrespective of the exceedence or otherwise of regulatory limits or screening values, the results of the current investigation indicate that menhaden oil, in common with oils derived from other species, could be acting as a significant contributory source to overall dietary intakes of PCBs, PBDEs and organochlorine pesticides. The scale of this contribution compared to other sources will depend on the manner in which menhaden oils are already being used in the food chain, whether in livestock feeds or for human consumption directly. Given the indications from this study that concentrations may be particularly high in the slightly refined oil (subject to verification by analysis of a larger sample set), determination of the nature and extent of use of such grades may be of particular interest. Nevertheless, the dietary contribution from all grades, including those sold as supplements, clearly deserves further investigation.

I hope that the results and discussion presented above are of interest and value to you. Please do not hesitate to contact me if you require further information.

**References:**

CRN (2006) Council for Responsible Nutrition Omerga-3 Voluntary Monograph, USA: 6 pp. http://www.crnusa.org/pdfs/O3FINALMONOGRAPHdoc.pdf

CEC (2006) Maximum levels set for dioxins and PCBs in feed and food. Commission Press Release IP/06/119, Commission of the European Communities, Brussels, 03 February 2006 http://europa.eu.int/rapid/pressReleasesAction.do?reference=IP/06/119&format=HTML&aged=0&language=EN

Fernandes, A.R., Rose, M. *et al.* (2006) Dioxins and polychlorinated biphenyls (PCBs) in fish oil dietary supplements: Occurrence and human exposure in the UK. *Food Additives & Contaminants* 23(9): 939-947

Food Safety Authority of Ireland (2001) Investigation on PCDDs/PCDFs and several PCBs in fish liver oil capsules. Analysis and report provided by ERGO Forschungsgesellschaft mbH, Germany: 55 pp.

Jacobs, M.N., Johnston, P.A., French, M.C., Wyatt, C.L. & Santillo, D. (1997). Organochlorine pesticides and PCB residues in pharmaceutical and industrial grade fish oils. International Journal on Environment and Pollution. 8, (1/2) 74-93

IFOS (2006) International Fish Oil Standards Consumer Reports, 12th April 2006: http://www.nutrasource.ca/ifos_new/index.cfm?section=ifosconsumer,

## Annex 1: details of sample preparation and analysis methodologies

*Sample extraction*

The fish meal sample was extracted by Soxhlet using a 1:1 (v/v) acetone:*n*-hexane mix (de Boer *et al*. 2001). For this, 10 g of sample were mixed with sodium sulphate, transferred to a Soxhlet thimble and topped with 1 cm of sodium sulphate. Extraction took place over a 4 h period with an average of 9 - 10 cycles $h^{-1}$.

The oil capsules were cut open with a scalpel and 0.5 g of oil were diluted up to 10 ml using *n*-hexane.

*Sample extract cleanup for PBDEs, organochlorine pesticides and PCBs*
An aliquots of the Soxhlet extract and 1 ml of the diluted oil were cleaned up and fractionated using alumina (5% deactivated) and silica (3% deactivated) columns respectively. The silica column fractionation results in two fractions, the first fraction containing PCBs and the second fraction containing PBDEs and organochlorines pesticides.

*Analysis of PBDEs using GC-MS-NICI*
Residues of selected PBDEs were determined by GC-MS-NICI after the method of De Boer *et al.* (2001). In brief, sample extracts in iso-octane were analysed by gas chromatography mass spectrometry in the negative ion chemical ionisation mode. A seven point calibration curve was constructed using BDEs17, 28, 47, 66,100, 99, 85, 154, 153 & 138, representing the dominant congeners in "penta" mix formulations, plus BDE183, representative of "octa" mix formulations. Samples were injected in the pulsed splitless mode onto a 50m x 0.25mm x 0.25µm DB-5 column and bromine ions at 79/81 amu were monitored in selected ion monitoring (SIM) mode. Quantitation was performed by internal standard procedures using CB#200 as a reference.

*Analysis of PCBs and Organochlorine Pesticides by GC-ECD*
An Agilent 6890 GC with microcell ECD was used to determine PCBs. The separation was performed on a 50.0 m × 200 µm, 0.33-µm-film-thickness DB-5 capillary column supplied by Agilent Technologies (Waldbronn, Germany). The carrier and ECD make-up gas were hydrogen (32.2 psi constant pressure, initial velocity 50 cm/s) and argon/methane (95:5), respectively. The initial oven temperature was 90°C, held for 2.00min, then increased to 165°C at 15°C/min, to 285°C at 2°C/min, and finally held for 23 min. The injector temperature and detector temperature were 270°C and 300°C, respectively. A 1-µl extract was injected in splitless mode with a purge time of 2 min. The identification of PCBs and organochlorine pesticides was based on the retention time of individual standards in the calibration mixtures.

de Boer, J., Allchin, C.R., Law, R.J., Zegers, B and Boon, J.P. (2001) Method for the analysis of polybrominated diphenylethers in sediments and biota. Trends in Analytical Chemistry, vol. 20, no.10, 2001