O R I G I N A L A R T I C L E

Fish oil and cod liver as safe and healthy food supplements

Miriam Smutna [1], Kamila Kruzikova [2], Petr Marsalek [2], Vladimir Kopriva [1], Zdenka Svobodova [2]

[1] Department of Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.
[2] Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Correspondence to: Prof. MVDr. Miriam Smutná, CSc., Department of Biochemistry, Chemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 612 42 Brno, Czech Republic. PHONE.: + 420-541 562 602, FAX: +420-541 562 790, EMAIL: smutnam@vfu.cz

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Abstract

OBJECTIVES: The aim of this study is to determine the content of mercury, methylmercury and persistent organic pollutants in the capsules filled with fish oil from marine fish and in the canned cod liver and find out, whether analyse products are safe and suitable for human consumption.
METHODS: Total mercury was determined by cold vapour atomic absorption spectroscopy on an AMA-254 (Altec Ltd., Czech Republic) single-purpose mercury analyzer. Methylmercury in the cod liver in the form of chlorid methylmercury was determined by gas chromatography. Seven indicator congeners of PCB (PCB 28, 52, 101, 118, 138, 153, 180), HCH, HCB, DDT and its degradation products DDE and DDD, were determined by gas chromatography-mass spectrometry.
RESULTS: In capsules filled with fish oil (n=19) total Hg levels were in the range of 0.013 to 2.03 ng/g. All the capsule oil samples analyzed for MeHg were below the detection limit. The highest concentration of total Hg was found in cod liver - can A (0.223 ng/g). The maximum percentage of MeHg in total Hg concentration was found in a liver sample – can B. The values of α-, β-, γ-, δ- HCH and of HCB were found below the detection limits in all the capsule samples analyzed. In eight capsules, the presence of highly lipophilic PCB congeners was demonstrated. DDT and its important DDE metabolite were found in ten samples. In canned cod liver the highest concentration of all PCB congeners was demonstrated in can D. The lowest congener concentrations, however, were found in smoked cod liver – can C. DDT with its main metabolite DDE was detected in can C. No additional DDT and DDD persistent pollutants were detected.
CONCLUSIONS: The consumption of fish oil in capsules, and canned cod liver is safe and healthy and should be encouraged.

Abbreviations

EPA – eicosapentaenoic acid
DHA – docosahexaenoic acid
Hg – mercury
THg – total mercury
MeHg – methylmercury
POPs – persistent organic pollutants
PCBs – polychlorinated biphenyls
HCH – hexachlorocyklohexane
HCB – hexachlorobenzene
DDT – dichlordiphenyltrichlorethane
DDE – dichlordiphenyldichlorethylene
DDD – dichlordiphenyldichlorethane
TCDD 2,3,7,8 – tetrachlorodibenzo-p-dioxin
PHOS – perfluorooctane sulphonate
PHOA – perfluorooctanoic acid
PCDD/F – polychlorinated dibenzodioxine/furane

INTRODUCTION

Omega-3 polyunsaturated fatty acids are highly valued because the enzymatic makeup of the human body with its desaturases and elongases is unable to produce them in sufficient quantities to meet the demand. These include mainly eicosapentaenoic acid ($C_{20:5}$) and docosahexaenoic acid ($C_{22:6}$). The reason for this is that in the period of development when our ancestors consumed diets abounding in omega-3 fatty acids, there was no evolutionary pressure for those acids to be biosynthesized endogenously (Gudmundsen, Gjendemsjo and Cvengroš, 2001). This is why food supplements of fish oils with increased amounts of omega-3 fatty acids (EPA and DHA) stand in the forefront of the contemporary trend of protecting and improving health (Kris-Etherton et al. 2002).

Health benefits of essential ω-3 fatty acids from ocean fish

EICOSAPENTAENOIC ACID ($C_{20:5}$)

- is converted to tromboxane A3, which acts as a mild stimulant of thrombocyte aggregation
- in endothelial cells is converted to prostacyclin (PGI 3), which is a potent thrombocyte aggregation inhibitor
- enhances vasodilation of vascular smooth muscle tissue
- reduces the risk of cardiovascular diseases

DOCOSAHEXAENOIC ACID ($C_{22:6}$)

- lowers plasma LDL cholesterol concentrations
- inhibits thrombocyte conversion of arachidonic acid (ω-6) to tromboxane A2, which enhances thrombocyte aggregation and vascular vasoconstriction of smooth muscle tissue
- is a part of biomembranes and it contributes to their fluidity, flexibility and functionality at low temperatures (fish from cold Arctic seas – salmon, mackerel, cod, tuna fish, herring; Murray, Granner and Rodwell 2006; Mommsen and Moon 2005).

Nevertheless, concerns are being raised about the frequent environmental contamination of ocean fish

- persistent organic pollutants e.g. PCB, HCH, HCB, DDT, PCDD/F
- organo-metallic compounds, heavy metals (e.g. organo-tin compounds, Hg, MeHg, As; Hamasaki et al. 1995)
- natural toxins (e.g. from algae; Sidhu 2003).

Persistent organic pollutants. Persistent organic pollutants are substances that exhibit toxic effects, are bioaccummulative, and are carried in the atmosphere over long distances, even across national boundaries. POPs may cause serious health problems and can have a negative impact on the environment. They are carcinogenic, and can induce changes in the developmental and immune systems. They can also cause endocrine disruption, which may lead to a reduction in reproductive capability (Holoubek and Klanova 2008). The Stockholm Convention in 2001 lists 12 substances (including DDT, PCB and HCB) and obligated the signatory states to stop the production and/or limit their use (DDT). In 2009, Stockholm Convention was extended to include another nine substances including α, β, γ – HCH.

Health risk of mercury. The toxicity of mercury is highly dependent on its chemical form. Both inorganic and organic forms of mercury are highly toxic to humans. Atmospheric Hg enters aquatic systems primarily in an inorganic form and is methylated to the most toxic form - methylmercury in some aquatic systems by sulfate-reducing bacteria *Methanobacterium* living in the sediments (Clarkson and Magos 2006).

MeHg is easily absorbed into the digestive tract, where it forms a complex with the amino acid cysteine. MeHg is not preferentially distributed into fat. MeHg is a neurotoxic agent that affects the development of the central nervous system, resulting in neurological problems, such as having an effect on fine motor and sensory function (Mahaffey 2004). To date, these more severe symptoms have been observed only in people who consumed fish that were contaminated directly by methylmercury from anthropogenic sources, and not from methylmercury that accumulated through the natural methylation process (Clarkson and Magos 2006).

Children are especially vulnerable as they can be exposed directly by eating contaminated fish. Mercury is transferred into the hair and this allows the monitoring of long-term exposure to mercury. Kruzikova et al. (2008) found a positive data correlation between the mercury content in hair and the degree of fish consumption.

MATERIALS AND METHODS

Mercury and methylmercury. The total mercury (THg) content in capsules and cod liver was determined by cold vapour atomic absorption spectroscopy on a single-purpose mercury analyzer (AMA-254 Altec Ltd., Czech Republic) (detection limit 1 ng/g). The accuracy of the results was validated using a standard reference material BCR-CRM 463 (tuna fish). The oil in each capsule was measured six times and the average was calculated. A total of 19 various kinds of capsules were bought in the Czech pharmacy. Six capsules from each box were analyzed (Marsalek and Svobodova 2006).

A total of four samples of canned cod liver was analyzed in this study. Methylmercury in cod liver was determined in the chlorid form of MeHg by gas chromatography (Caricchia et al. 1997). Samples were prepared by acidic digestion and their extraction to toluene. A gas chromatograph (GC 2010A Shimadzu, Kyoto Japan) was used for the analysis. A capillary column DB 608 (30 m × 0.53 mm × 0.83 μm; J&W Scientific) and an electron capture detector (ECD) were used. Evaluations were made using GC Solution software (Shimadzu) and MS Excel software. BCR-CRM 463 (tuna fish) was used as a reference material to validate the methods and to determine the uncertainties. The limit of detection (3σ) was 5.04 ng/g and the limit of quantification was 15 ng/g when a sample amount of 0.2 g is calculated.

The determination of chlorinated hydrocarbons. The determination of POPs was performed for 16 capsule samples and three cod liver cans (A, C and D). Seven indicator congeners of PCB (PCB 28, PCB 52, PCB

Tab. 1. Overview of capsules and their characteristics

Sample	Name	Description
1	Natures Bounty EPA fish oil	oil from deep-sea cold water fish
2	Bioaktivní Marin Plus	fish oil extract, maximum purity, fortified with folic acid and B12 vitamin
3	Vital salmon oil	contains omega-3 fatty acids + E vitamin (place of origin: Cape Town)
4	Marin Q for children - liquid	exceptional oil for children, an effective EPA/DHA ratio of 3:1
5	Blue Care Fish Oil	mix of salmon, mackerel, herring, sardine and anchovy oils
6	Omega-3 Fish fat	EPA and DHA concentrates from fish fat in gelatin capsules
7	Fish Oil + EPA + DHA	Alaskan deep-sea fish oil
8	Soma® Omega	salmon oil
9	fish oil from three fish species	salmon, mackerel and sardine oil; EPA+DHA = 30%
10	MaxiCor	contains 4 pure omega-3 unsaturated fatty acids
11	Fish oil (Oleum jecoris aselli)	from the liver of fresh cod fish (mainly Gadus morrhua)
12	EPA Marine fish oil	from fish living in the cold Arctic water
13	Omega-3 fish oil	EPA + DHA + vitamins A and D
14	GS - Omega 3	contains omega-3 unsaturated fatty acids
15	Omega-3 for children - liquid	orange flavour, fortified with vitamins A, C, D and E
16	Marin Q for children	capsules, with a higher EPA ratio from quality fish
17	Fish fat 500	omega-3 fatty acid concentrate
18	Food supplement from Canada	gift; no product specification
19	Food supplement from Switzerland	gift; no product specification

Table 2. Overview of canned cod liver

Can	A	B	C	D
Type of can	Cod liver in its own oil	Cod liver in its own oil	Smoked cod liver	Cod liver in its own juices
Country of origin	Poland	Poland (different food producer)	Denmark	Poland

Table 4. Concentrations of DDE in canned cod liver

(mg/kg)	A	C	D
pp' DDE	0.063	0.029	0.083
op' DDE	0.035	0.016	0.033

Table 3. Concentrations of DDT and its metabolites in capsules

(mg/kg)	1	2	4	9	10	12	13	14	15	18
pp' DDE	0.016	0.116	0.033	0.024	0.077	0.026	0.102	0.079	-	0.018
op' DDE	0.016	0.061	0.016	-	0.044	-	0.059	0.066	-	-
pp' DDT	-	0.045	-	-	0.086	-	0.073	-	0.076	-
op' DDT	-	0.033	-	-	0.038	-	0.048	0.037	0.045	-
pp' DDD	-	-	-	-	-	-	-	-	-	-
op' DDD	-	-	-	-	-	-	-	-	-	-

101, PCB 118, PCB 138, PCB 153, PCB 180), HCH, HCB, DDT, and its degradation products DDE and DDD, were determined by gas chromatography-mass spectrometry.

Gas chromatography with ion trap mass spectrometry (GC/IT-MS) was used for determining the amount of chlorinated hydrocarbons. Sample preparation was based on fat extraction and the cleaning on the florisil-packed column (Marsalek et al. 2004). A representative part of codfish liver (50 g) was homogenized and extracted into diethylether (100 ml). The extract was dried by anhydrous sodium sulfate and evaporated in a rotary vacuum evaporator. An aliquot part of a collected portion of fat (0.2 + 0.05 g) was dissolved in n-hexane (15 ml) and 5 ml of solution was cleaned up on florisil-packed column to remove lipids. The cleaned solution was concentrated under the stream of nitrogen into 5 ml and used for analysis. Samples of fish oil were directly weighted (0.2 + 0.05 g), dissolved in 15 ml of n- hexane and cleaned up on the florisil-packed column.

The separation, identification and quantification of analytes were based on the GC/IT-MS method described by Fang and Wang (2007). Gas chromatograph Varian 450-GC with a Varian 220-MS ion trap mass spectrometer and a VF-5ms (30 m x 0.25 mm) column were used for the separation of analytes.

The detection limits (3σ) of individual chlorinated hydrocarbons ranged between 0.010 – 0.075 mg/kg. The expanded uncertainty was 7.5 % on the condition that the coefficient of expansion is $k = 2$.

Statistical evaluation. Statistical evaluation of the results was carried out using Statistica software 8.0 for Windows (StatSoft). The data were first tested for normality (Kolmogorov-Smirnov test) and homoskedasticity of variance (Bartlett's test). If those conditions were satisfied, a one-way analysis of variance (ANOVA) was employed to determine whether or not there were any significant differences in the measured variables between groups. When a difference was detected ($p<0.05$), Tukey's multiple comparison test was applied to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, a non-parametric Kruskal–Wallis test was used (Zar 1996).

RESULTS

An overview of ocean fish oil-based food supplements and their characteristics is given in Table 1. An overview of the cans of cod liver marketed in the Czech Republic is given in Table 2.

Mercury and methylmercury. Total mercury content in capsules filled with fish oil (n=19) is given in ***Fig. 1***. The total mercury content (ng/g) in samples 1 and 2 was statistically significantly higher ($p< 0.05$) than the mercury content found in samples 11, 8, 15, 4 and 9. No statistically significant differences in total mercury content were found among the other samples. The total mercury levels in oil from the capsules were in the range of 0.013 to 2.03 ng/g.

An analysis of the capsule samples 1, 2, 10 and 5 shows a large spread of total mercury content values from individual capsules. For example, the maximum range of total mercury content values from sample 5 was from 0.1 to 2.0 ng/g oil (mix of salmon, mackerel, herring, sardine and anchovy oils).

It follows from Fig. 1 that, from the total mercury content point of view, capsule samples 11, 8, 15, 4 and 9 can objectively be labeled as „high quality food supplements". Capsule samples 17, 3, 14, 16, 13, 12, 6 and 19 fall into the „medium quality" group, and capsule samples 1, 2, 10, 5 and 18 are the „poor quality" group of food supplements.

All the capsule oil samples analyzed for methylmercury were below the detection limit.

Canned cod liver. Total mercury concentrations (ng/g) in cod liver (n=4) are summarized in ***Fig. 2***. The highest concentration of total mercury was found in cod liver cans from Poland (0.223 ng/g; sample A). The total mercury concentration in that sample was statistically significantly different ($p< 0.01$) from the total mercury concentrations found in cod liver cans B, C and D.

Methylmercury concentrations (ng/g) in the cod liver are given in ***Fig. 3***. The methylmercury concentration from the cod liver in its own oil (A) was low compared with that from canned cod liver samples (B, C and D), but the differences between the samples were not statistically significant.

The percentage of MeHg in THg concentration is given in ***Fig. 4***. The minimum percentage of methylmercury in total mercury level was found in cod liver from can A. This finding also corresponds with the low arithmetic mean of 19%. The percentage of methylmercury in cod liver from can A was statistically significantly different ($p< 0.01$) from that in the cod liver from cans B and C. Compared with sample A, a higher percentage of methylmercury was also found in sample D (arithmetic mean = 43%). The maximum percentage of MeHg was found in the liver sample from can B (arithmetic mean = 56%).

Persistent organic pollutants in capsules. An analysis of ocean fish oil in capsules demonstrated the presence of persistent organic pollutants. Values of α-, β-, γ-, δ-HCH and of HCB were found below the detection limits in all the capsule samples analyzed.

To demonstrate the presence of PCBs, a total 16 capsule samples were examined, of which eight were found to be below the detection limit (samples 2, 4, 6, 7, 11, 12, 14 and 18). In the remaining eight capsule samples, the presence of highly lipophilic PCB congeners was demonstrated (samples 1, 5, 8, 9, 10, 13, 15 and 17). It follows from ***Fig. 5*** that stable high-chlorinated congeners of PCB 118, PCB 138, PCB 153 and PCB 180 were predominant in samples 9, 10, 13 and 15, while low-chlorinated congeners of PCB 28, PCB 52 and PCB 101 were predominant in samples 5 and 8.

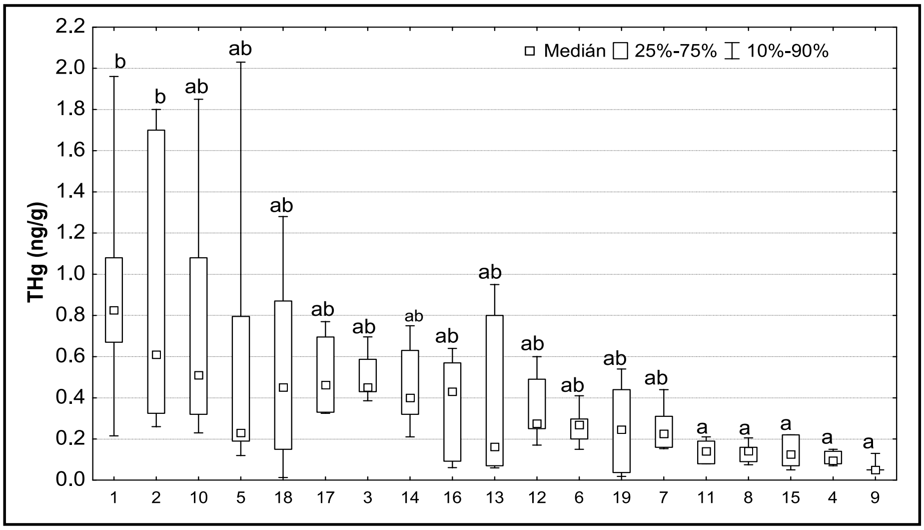

Figure 1. Total mercury content in capsules. a, b different alphabetic letters differ significantly (p<0.05)

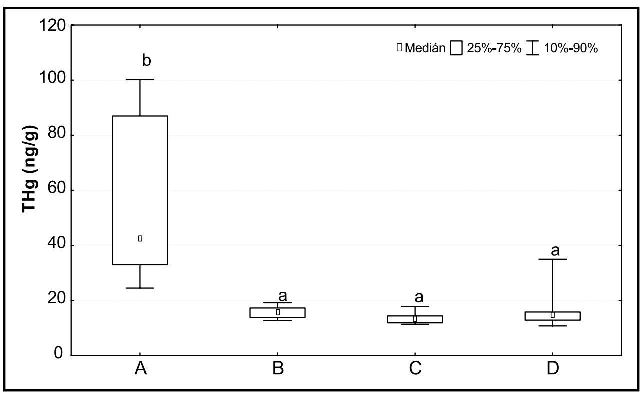

Figure 2. Total mercury content in cod liver. a, b different alphabetic letters differ significantly (p<0.01)

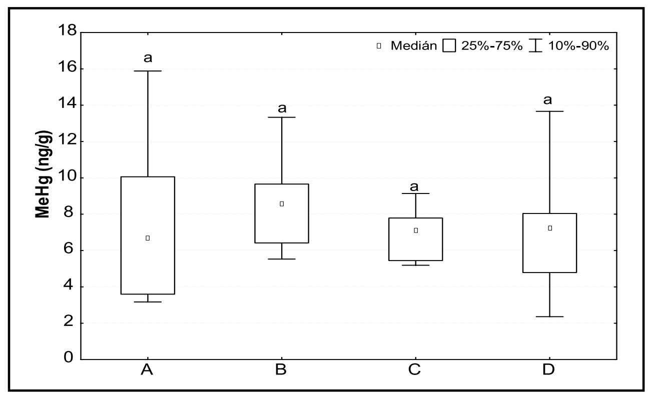

Figure 3. Methylmercury content in cod liver

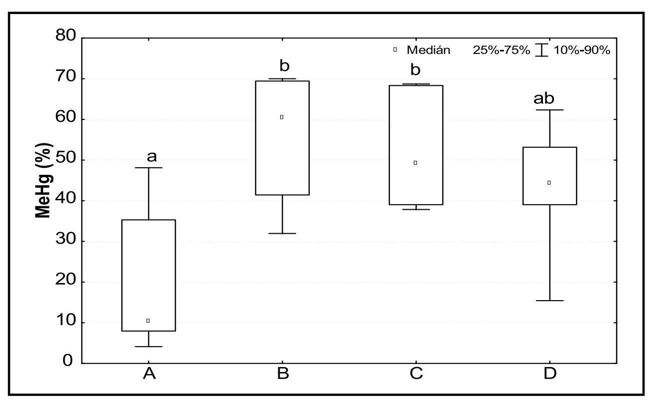

Figure 4. Percentage of methylmercury in total mercury. a, b different alphabetic letters differ significantly (p<0.01)

Concentrations of DDT and its metabolites in capsules are given in Table 3. In six samples, the values found were below the detection limit. In the remaining samples (n=10, samples 1, 2, 4, 9, 10, 12, 13, 14, 15 and 18), an important DDE metabolite (p, p'- DDE > o, p'- DDE) was discovered to be present. The presence of DDD (p, p'- DDD; o, p'- DDD) was not demonstrated.

Persistent organic pollutants in canned cod liver. The qualitative and quantitative overview of the 7 indicator PCB congeners found in canned cod liver is given in ***Fig. 6***. The highest aggregate concentration of all PCB congeners was measured in the canned cod liver in its own juices (D). The lowest congener concentrations, however, were found in smoked cod liver in its own oil from Denmark.

The only DDT metabolite and degradation product detected in the cod liver samples was the DDE metabolite (o, p'- DDE and p, p'- DDE; Tab. 4), no other DDT or DDD persistent pollutants were detected.

DISCUSSION

Over the past century, the total intake of omega-3 fatty acids has dwindled by 80% as a result of changing life styles. The enzyme systems of the human body represented by desaturases and elongases are unable to rapidly and sufficiently meet the demands of omega-3 fatty acids. Moreover, these enzymes are known to be inhib-

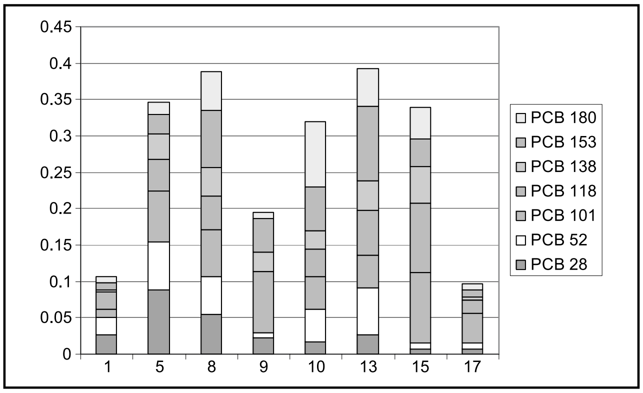

Figure 5. Qualitative and quantitative content of PCB in capsules (mg/kg)

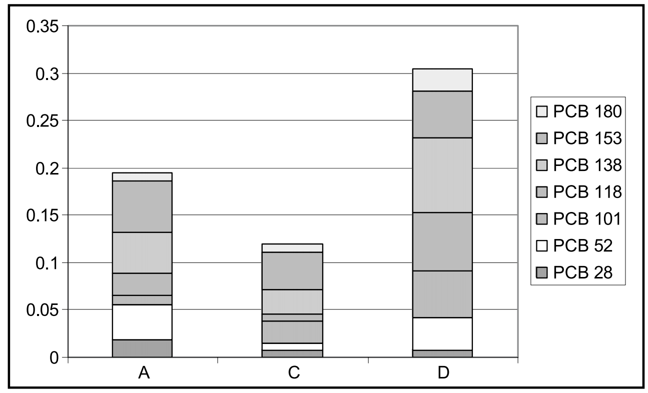

Figure 6. Qualitative and quantitative content of PCB in cod liver (mg/kg)

ited by the intake of large quantities of the linoleic acid (ω- 6, C18:2), trans-fatty acids and saturated fatty acids. Under such circumstances, a deficiency of fatty acids with long chains (EPA and DHA) will develop even if the above polyunsaturated fatty acids are ingested through the diet (Bjerve 1989). Also, our present-day diets are characteristic of the predominance of fatty acids from vegetable oils and meat, which dramatically changes the ω-3 to ω-6 ratio (ratio < 1/25) in contrast to our ancestors who were first and foremost hunters and whose diet was significantly richer in ω-3 fatty acids (ratio < 1/2; Gudmundsen, Gjendemsjo and Cvengroš 2001).

It follows from the scientific findings above that an adequate intake of food supplements based on oils from marine fish is essential for the optimum development and function of live organisms, e.g. biomembrane, brain, retina, myocardium, etc. (Sidhu 2003).

In addition to the omega-3 fatty acids beneficial for health, fish are a source of quality proteins, minerals (e.g. I, Se and Ca) and vitamins (e.g. A and D). Concerning health risks, there is the presence of lipophilic persistent organic pollutants (DDT, PCBs, HCH, HCB, TCDD, dieldrin, heptachlor) and toxic forms of mercury, the dominant being methylmercury. Orally ingested, PCBs and MeHg have long half-lives in human bodies. The elimination of the half-lives of PCBs from whole blood ranges from 4 to 12 months, and that of MeHg is 70-90 days (Rowe et al. 1996). PCB intake can be minimized by removing skin and trimming fat before grilling fish. Methylmercury, on the other hand, gets deposited in skeletal muscles (it makes up 75% to 90% of total mercury content; Mahaffey 2004) and no skinning or fat trimming can eliminate it.

The total mercury levels in capsules filled with oil from ocean fish (cod, halibut, tuna, salmon or shark) were analyzed by Vazquez (2005). These food supplements came from commercial outlets, pharmacies and health food shops from England, Scotland, Wales and Northern Ireland. Mercury levels above the detection limit of 0.0014 mg/kg were found in only 9 of 100 oil capsules analyzed, and the minimum and maximum mercury concentrations were 0.0017 mg/kg and 0.0030 mg/kg, respectively. In comparison, the results of our study were lower by an order of magnitude, and ranged from 0.0011 mg/kg (min., capsule 4) to 0.0093 mg/kg (max., capsule 1). The overview of food supplements analyzed by Vazquez (2005) listing their names and producers shows no commonality with our food supplements, nor even in the places of their acquisition (commercial outlets *vs.* pharmacies).

It follows from our results in the present study that methylmercury makes up about 50% of the total mercury in fish liver, which compares with the findings of Marsalek et al. (2006), who also reported the presence of MeHg *inter alia* in muscle tissue, where it made up about 80%.

Environmental contaminants from among the PCB persistent organic pollutants were present in the form of both high-chlorinated stable congeners (PCB 118, 138, 153, 180) and low-chlorinated congeners (PCB 28, 52, 101). E.g. the liquid syrup (sample 4) intended for children from 3 years of age was free of all of the PCB congeners listed above, while a similar syrup (sample 5) contained residua at a 0.339 mg/kg total concentration.

Rawn et al. (2009) stated that the lowest PCB concentration they found in food supplements based on a mix of anchovy, mackerel and sardine oils (0.711 ng/g). The highest concentration of PCB residua was found in shark oil (10.400 ng/g).

None of our set of samples of food supplements (capsules, syrups) based on ocean fish oils exceeded the hygienic limit for PCB residua concentrations in edible parts of fish, which is 2 mg/kg (Commission Regulation (ES) No 1881/2006 of 19 December 2006 which sets the maximum levels for certain contaminants in foodstuffs).

Cod livers are characterized by having an abundance of lipids (50 – 80%) and are a significant source of omega-3 polyunsaturated fatty acids. Consumption of at least 5 g of canned cod liver a day is a sufficient source of the recommended daily intake of EPA and DHA. To receive the same amount of omega-3 fatty acids from fish meat, we would have to eat 700 g of cod or 40 -70 g of herring (Kołakowska et al. 2002).

Although it is common knowledge that persistent organic pollutants accumulate in fish fat as well as in fish oil, no POP daily intake limits have been considered for food supplements, nor are there any hygienic limits set for them. For this reason other possible contaminants or pollutants, e.g. perfluorinated compounds such as perfluorooctane sulphonate (PHOS) and perfluorooctanoic acid (PHOA) should also be taken into account. They are ubiquitous, persistent and bioaccumulative in the environment (Kovarova and Svobodova 2008). In 2009, PHOS were included in the Stockholm Convention. It is very difficult and problematic however, because their concentrations may change due to their dependence on the length of fatty acid chains and to the degree of saturation depending on the season, location, diet and water temperature at the site. Seasonal changes in lipid composition cause simultaneous changes in the proportion of lipid tissues in the fish body and, consequently, also in the POP dynamics (Elskus et al. 2005).

In conclusion, the potential risks associated with the consumption of fish oil capsules should include, e.g.:

- fish smack, gastrointestinal upset
- elevated cholesterol in combination with hyperlipidaemia
- increased bleeding time, nose bleeds, easy bruising and hemorrhagic stroke
- oxidative damage
- vitamins A and D toxicity with some dietary preparation
- some fish oil (not highly refined) may contain contaminants (HCH, HCB,DDT, PCBs, dieldrin, heptachlor, dioxin, Hg, MeHg)
- the expensive cost compared with fish intake (Stone 1996).

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