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Determination of Organochlorine Compounds (OCPs and PCBs) in Fish Oil and Fish Liver Oil by Capillary Gas Chromatography and Electron Capture Detection

A. D. Tsigouri, A. E. Tyrpenou*

Institute of Veterinary Research of Athens - I.V.R.A., National Agricultural Research Foundation-N.Ag.Re.F., 25, Neapoleos Street, Agia Paraskevi, GR-153 10 Athens, Greece

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Previous studies concerning these chemicals have proved that PCB concentrations in cod livers range over 1.2 - 2.6 μg/g wet mass. This means that cod liver poses higher concern to human health since the above figures exceeded the tolerance limit of total PCBs (2.0μg/g for edible parts of fishery products) and also were contained significant residue levels of toxic coplanar PCBs (Falandysz et al.1992; Toepfer 1992). In other countries the authorized level of PCB concentration for fish is 100 μg/kg (WHO 1990). The last few years such food supplements have increased in popularity in our country and various products appeared in the Greek market and sold in, the so called, health food shops, as dietary supplements. Our interest has been focused on these fish oil and fish liver oil supplements, because of the high probability of the presence of persistent organochlorine residues.

Efficient control for the monitoring of these substances requires sensitive and accurate methods of analysis. Several methods with different detectors have been published for the determination of these compounds (Falandysz et al. 1992; Weistrand and Noren 1993; Krahn et al. 1994). In our study we used a previously published method with modifications of the chromatographic part (Tirpenou et al. 1998). The main objective and the aim of this study was to investigate the presence of residues of these toxic chemicals in several types of products sold in the market of Athens, which products seem to be very important to the consumers, as an alternative in balancing their diet for a healthy lifestyle.

MATERIALS AND METHODS

A total of 36 products of fish oil and fish liver oil (bottles and packets with soft gelatin capsules) were analyzed. They were health products produced in European Countries (Norway & UK) and out of them 24 samples were fish liver oil supplements coming from 4 different companies and 12 samples were fish oil supplements represented 2 companies. Fish oil was coming from salmon and halibut fish species and liver oil was coming from cod.

OCPs, 97.6 - 99.9%, Chem Service, *Pesticide Standards & Metabolites,* UK

α-HCH	Nonachlor	α-HCH	p-p' DDE	HCB	Dieldrin
γ-HCH	o-p' TDE	Endrin	p-p' TDE	H.Epoxide	o-p' DDT
Oxychlordane	γ-Chlordane	p-p' DDT	o-p' DDE	α-Chlordane	

Present address: 76, Aigaiou Pelagous Street, Agia Paraskevi, GR-153 41 Athens, Greece
Correspondence to: A. E. Tyrpenou

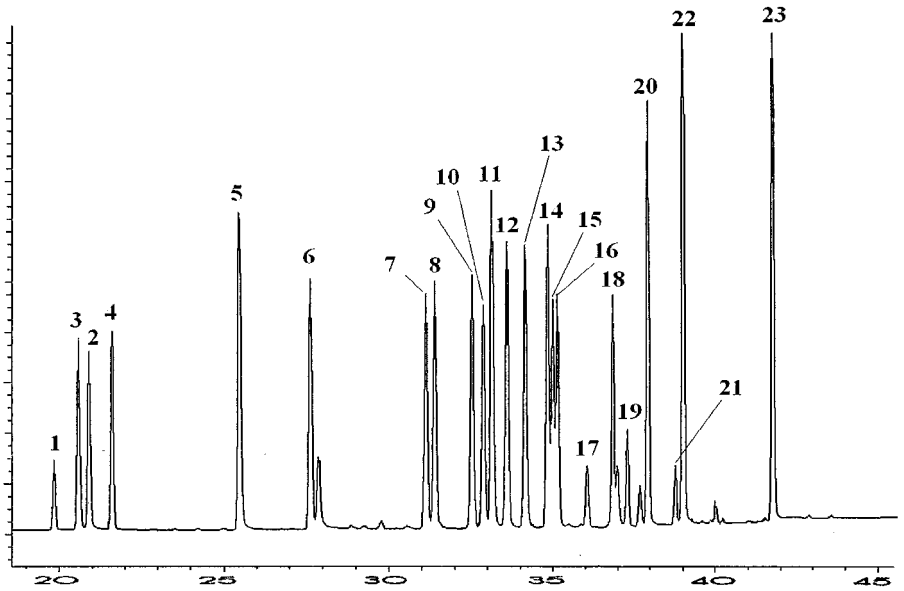

Figure 1. Chromatogram of 17 OCPs and 6 PCB congeners on column HP-1.

PCBs, 97.6 - 99.6%, Dr. Ehrenstorfer, *Reference Materials for Residue Analysis,* GmbH Ballschmitter and Zell - IUPAC No. and structure of PCB

PCB 28 2, 4, 4' - Trichlorobiphenyl
PCB 52 2, 2', 5, 5' - Tetrachlorobiphenyl
PCB 101 2, 2', 4, 5, 5' - Pentachlorobiphenyl
PCB 138 2, 2', 3, 4, 4', 5 - Hexachlorobiphenyl
PCB 153 2, 2', 4, 4', 5, 5' - Hexachlorobiphenyl
PCB 180 2, 2', 3, 4, 4', 5, 5'- Heptachlorobiphenyl
Fused silica capillary column HP-1, 25m x 0.31mm i.d. x 0.52µm, HP
Fused silica capillary column HP-5, 30m x 0.25mm i.d. x 0.25µm, HP

Florisil 60-100 mesh activated at 600°C was kept overnight at 130°C and was deactivated before use with 2.5% hyper clean water, Sigma.
All solvents used (acetonitrile, dichloromethane, n-hexane, petroleum ether 40°-60°, isooctane) were of pesticide residue grade, BDH
All glassware must be rinsed thoroughly with acetone before use.

Calibration curves (area versus concentration) were prepared using at least 4 points zero included for each substance. Recovery experiments conducted at the Maximum Residue Limit (MRL) level should give values between 70-110%. Limits of detection and determination were estimated from the results of recovery experiments. Before the analysis of any sample, a bi-distilled water sample blank

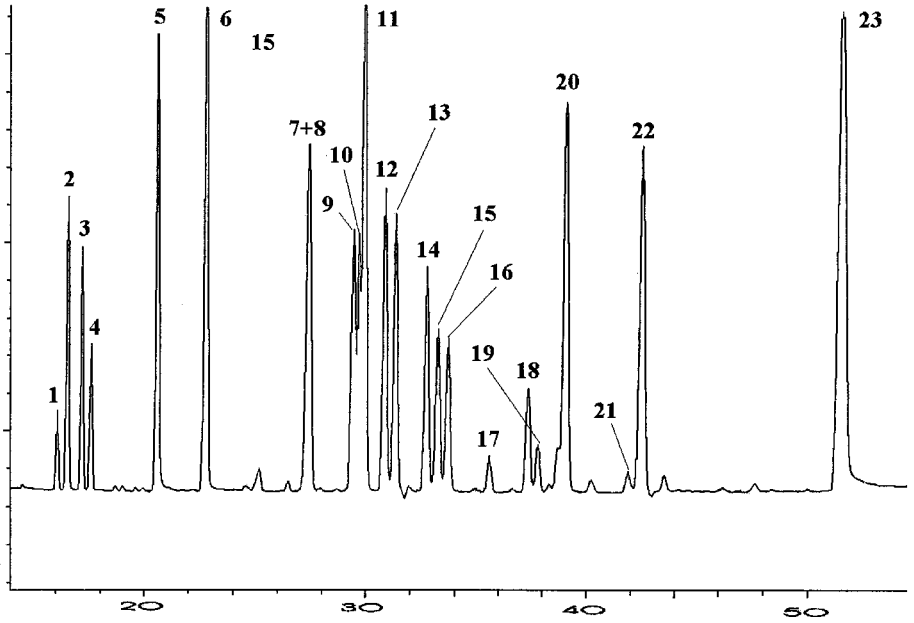

Figure 2. Chromatogram of 17 OCPs and 6 PCB congeners on column HP-5.

was run to determine that all glassware and reagent are interference free. Also, one spiked sample at the MRL or 2*MRL level was analyzed with every batch of samples examined to validate the accuracy of the method. If the recovery did not fall within the method's limits (between 70-110%), then the samples were reanalyzed.

A Hewlett Packard Gas-Chromatograph, model HP 5890 Series II equipped with an electron capture detector (^{63}Ni-ECD) and an HP 7673 automated sampler fast injection and with HP Vectra PC ChemStation data handling, was used. The rest of the apparatus used, was usual laboratory equipment.

All capsules from each test bottle were cut using a sharp blade and the oily content was squeezed out of them into a glass vial. The capsules' contents from each test bottle were then thoroughly mixed by stirring and an aliquot of 0.5g ± 0.01g was taken in duplicate for the analysis according to the method of Tirpenou et al (1998). The final extracts of each test sample (duplicates) were first analyzed on a fused silica capillary column coated with 100% dimethyl-polysiloxane and film thickness of 0.52 μm (HP-1). Helium (purity 99.999%), was used as a carrier gas and nitrogen (purity 99.999%), as the make up gas. A volume of 1μL of the final extract was injected into the gas chromatograph automatically at splitless mode. The retention times of the compounds detected were compared with those of the standards and confirmation was accomplished using another fused silica capillary

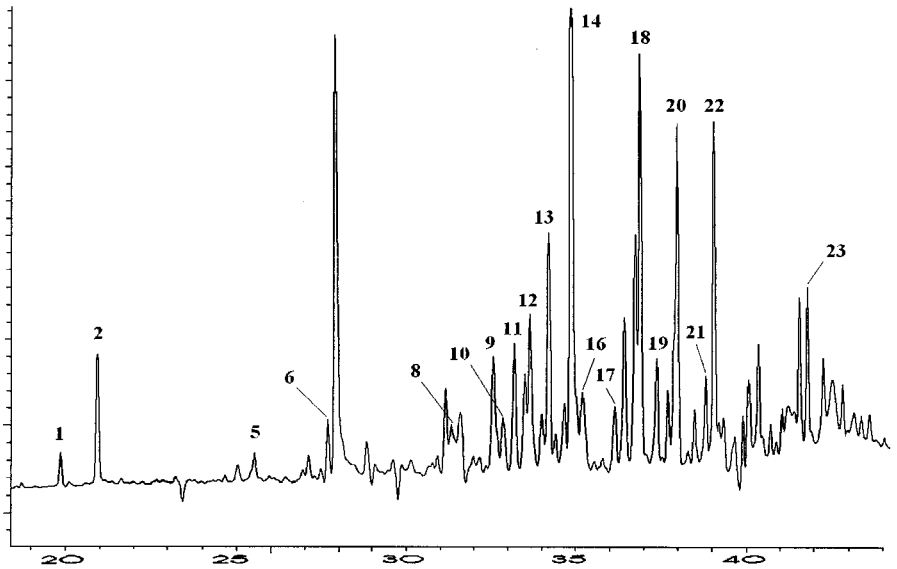

Figure 3. Chromatogram of a cod liver extract on column HP-1.

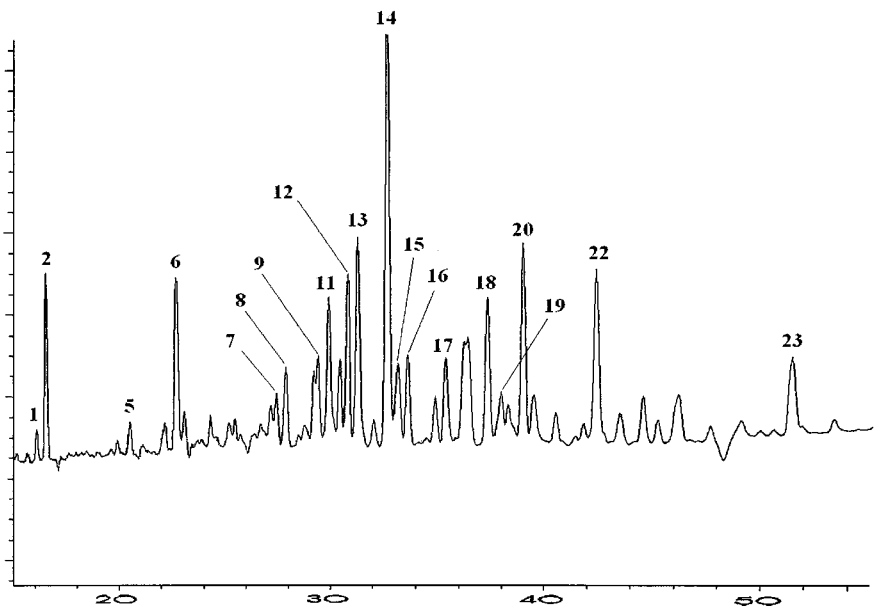

Figure 4. Chromatogram of a cod liver extract on column HP-5.

column coated with 5% diphenyl and 95% dimethylpolysiloxane with film thickness of 0.25 μm (HP-5).
Operating conditions: The injection port temperature was set at 250°C and the detector temperature at 300°C. The temperature programming of the oven was set as follows:

Column HP-1: initial temperature 80°C for 1 min, 1st rate 10°C/min ↗up to 150°C, 2nd rate 3°C/min ↗up to 230°C, 3rd 8°C/min ↗up to 280°C, final isotherm at 280°C for 3.08 min. The helium flow rate was 1.52 mL/min.

Column HP-5: initial temperature 80°C for 1 min, 1st rate 25°C/min ↗up to 190°C, 2nd rate 2°C/min ↗up to 210°C, 3rd 1°C/min ↗up to 250°C, final isotherm at 250°C for 0.60 min. The helium flow rate was 0.5 mL/min.

In figures 1 and 2, characteristic gas chromatograms and the corresponding retention times of the 17 chlorinated pesticides and 6 polychlorinated biphenyl isomers and congeners chromatographed on HP-1 and HP-5 columns respectively, can be seen.

Also, in figures 3 and 4, characteristic chromatograms of the same cod liver oil sample can be seen chromatographed on the two columns HP-1 and HP-5. Peak identification can be done according to the numbering series below.

1	α-HCH	7	Hept.Epoxide	13	Nonachlor	19	o-p' DDT
2	β-HCH	8	Oxychlordane	14	p-p' DDE	20	PCB 153
3	HCB	9	γ-Chlordane	15	Dieldrin	21	p-p' DDT
4	γ-HCH	10	o-p' DDE	16	o-p' TDE	22	PCB 138
5	PCB 28	11	PCB 101	17	Endrin	23	PCB 180
6	PCB 52	12	α-Chlordane	18	p-p' TDE		

The concentrations of the peaks of interest were compared with those of the standards and calculated using the calibration curves prepared and the software provided. Individual PCB and DDT concentrations were then summed (ΣPCB and ΣDDT).

RESULTS AND DISCUSSION

Firstly, it should be pointed out that these products provide a source of fatty acids Eicosapentaenoic acid-EPA (18%) and Docosahexaenoic acid-DHA (12%) from fish oils. These fatty acids can be used by anyone who wants to increase intake of Omega 3 fatty acids which are difficult to obtain, except from fatty fish such as salmon, mackerel, herrings and sardines.

Secondly, the oil quantity per capsule ranges from 250 mg to 1000 mg and the normal consumption rate of these capsules, (according to the producers' instructions), is 1 to 4 capsules per day and for some products over 6 capsules per day e.g. salmon oil capsules.

Table 1. Daily intake quantities of ΣDDT and ΣPCB determined.

Fish Liver Oil		Fish Oil	
ΣDDT [p-p'DDT, o-p DDT, p-p'DDE & p-p'TDE(DDD)]	0.008μg - 1.1μg	**ΣDDT** [p-p'DDT, o-p DDT, p-p'DDE & p-p'TDE(DDD)]	0.076μg - 0.6μg
ΣPCB [PCB 28, PCB 52, PCB 101, PCB 153, PCB 138, PCB 180]	traces - 0.5μg	**ΣPCB** [PCB 28, PCB 52, PCB 101, PCB 153, PCB 138, PCB 180]	traces - 0.122μg
Daily Intake in capsules (mean value of two determinations)			

Our results which can be seen in table 1, showed that consumption of fish liver oil in capsules fortifies consumers with 0.008 to 1.1μg total DDT and with up to 0.5μg total PCB (6 congeners) per day. Consumption of fish oil supplements fortifies consumers with 0.076 to 0.6μg total DDT and with up to 0.122μg total PCB (6 congeners) per day. The chlorinated pesticides α-HCH, HCB, α-chlordane, γ-chlordane, nonachlor and endrin were also detected in traces in some samples but the amounts determined were much lower. Also, in figures 5 and 6 we can see histograms indicating the concentrations of ΣDDT and ΣPCB of the 36 samples analyzed.
Considering all the above information, the consumers' preference to increase the number of capsules per day and the intrinsic properties of high lipophilicity and low biodegradability of these toxic chemicals, we concluded that consumers fortify themselves daily with quantities which might long-term cause toxic concern (Tanabe 1988). Also, the presence of the chlorinated pesticides and polychlorinated biphenyls in such health products has been confirmed by another research program, where we can see that samples from 22 brands of fish oils such as cod liver oil obtained from Norway, Japan, Spain, UK, Iceland, Germany and they found that from 22 samples, 21 contained high levels of hazardous contaminants namely the organochlorine pesticides DDT and Lindane and PCBs (Santillio 1995).

WHO has recommended Provisional Tolerable Daily Intake (PTDI) of DDT [any combination of DDT, DDD & DDE] of 0.02mg/kg/bw (JMPR 1998). For an adult person (body weight 60kg) this means 1.2mg/day and for children of 10kg is 0.2mg/day. So, the quantities calculated of 0.008 to 1.1μg are below these values.

According to the provisional level set by some European Countries of 0.4μg/kg/bw, the daily intake for an adult person (body weight 60kg) is 24μg/day and for children of 10kg is 4μg/day. The Maximum Daily Intake of ΣPCB by using the investigated food supplements, as we calculated, is 0.5μg. For adults this is 2.0% of the provisional level and for children (10kg) the maximum daily intake of ΣPCB is 12% of the provisional level. Although these percentages are higher for ΣPCB compared to the situation for ΣDDT, the calculated Maximum Daily Intake of PCBs by food supplements do not exceed the provisional level of 0,4μg/kg/bw/day. Taking into account that Daily Intake of PCBs by the food

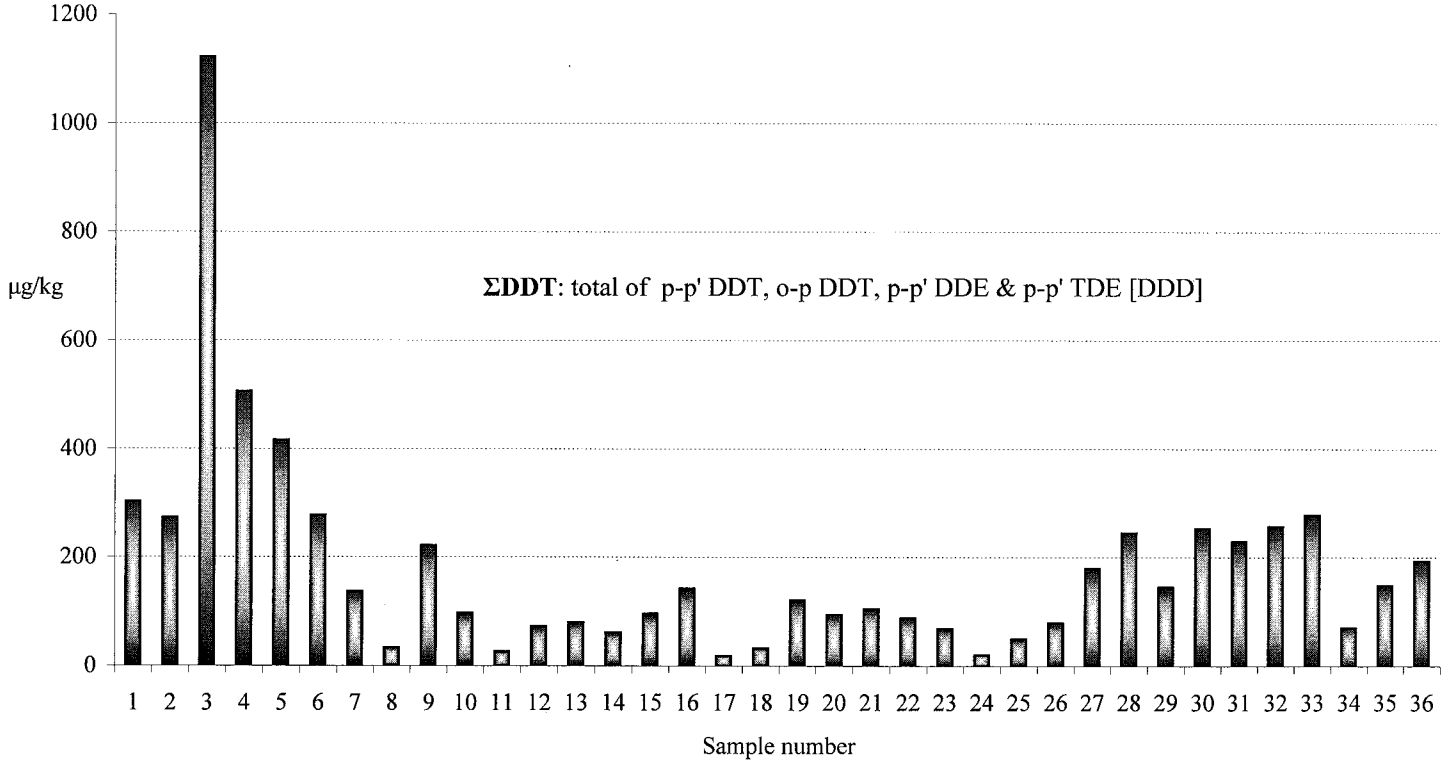

Figure 5. Concentrations of ΣDDT in 36 samples.

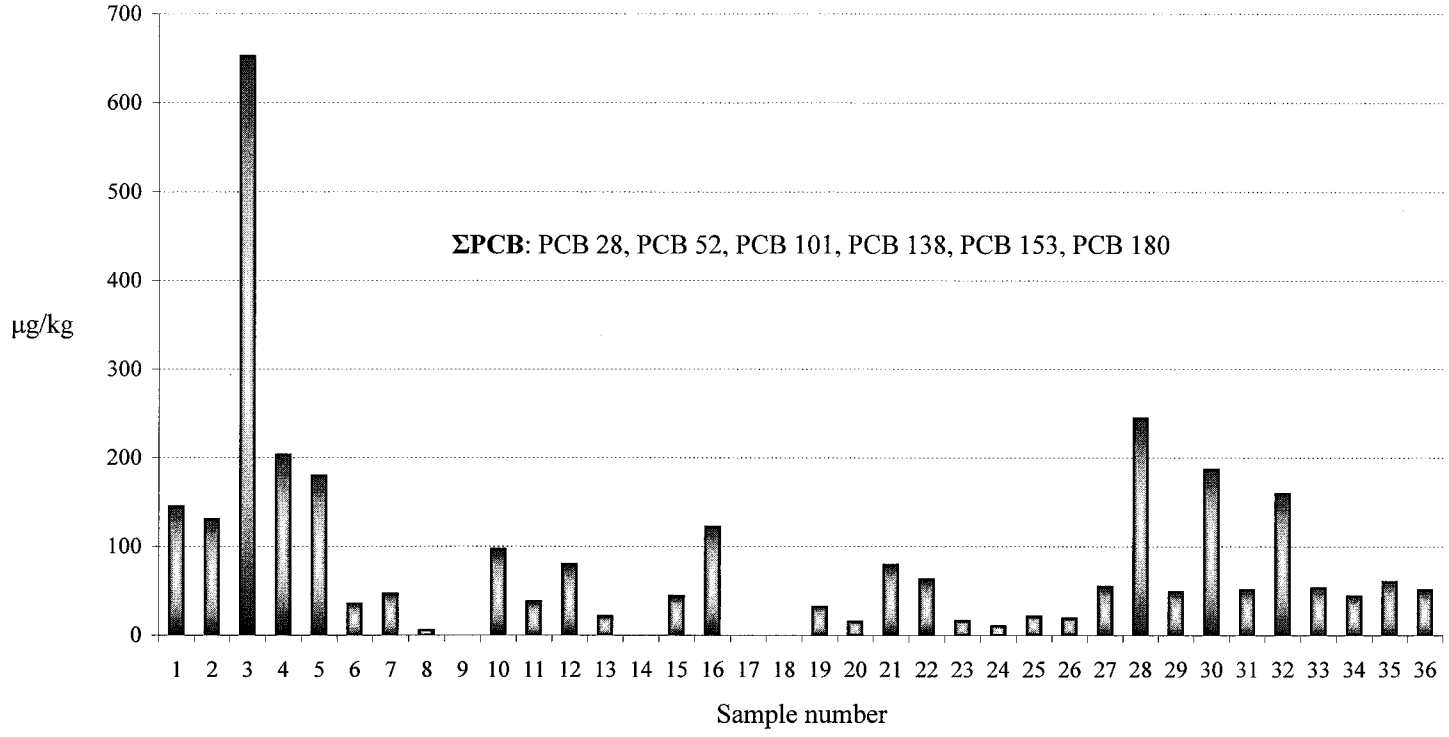

Figure 6. Concentrations of ΣPCB in 36 samples.

supplements is of the same order of magnitude as average daily intake of PCBs by consuming meat and/or fish, but also that some of the congeners have dioxin-like toxic properties (Safe 1990), the use of these studied health food supplements leads to a significant contribution to the overall PCB intake through food consumption.

In the present study it was shown, that the occurrence of organochlorine pesticides-OCPs and polychlorinated biphenyls-PCBs in such health food supplements as well as of other toxic chemicals, although they were found in small quantities, could be considered long-term hazardous for the consumers especially for children and for people with special needs.

REFERENCES

Falandysz J, Yamashita N, Tanabe S, Tatsukawa R (1992) Isomer-specific analysis of PCBs including toxic coplanar isomers in canned cod livers commercially processed in Poland. Zeitschrift fur Lebensmittel-Untersuchung und-Forschung 194:120-123

JMPR (1998) Inventory of IPCS and other WHO pesticide evaluations and summary of toxicological evaluations performed by the Joint Meeting on Pesticide Residues (JMPR) through 1998, UNEP-ILO-WHO, WHO Distribution and Sales, Geneva, Switzerland, pp.19

Krahn MM, Ylitaio GM, Buzitis J, Sloan CA, Boyd DT, Lam Chan S, Varanasi U (1994) Screening for planar chlorobiphenyl congeners in tissues of marine biota by high-performance liquid chromatography with photodiode array detection. Chemosphere 29:117-139

Safe S (1990) Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51-88

Santillo D (1995) Cod liver oil contaminated by toxic chlorine chemicals says Greenpeace. Greenpeace report shows fish oils containing organochlorines http://www.greenpeace.org/~comms/cod/cod.html

Tanabe S (1988) PCB Problems in the Future: Foresight from Current Knowledge. Environ Pollut 50:5-28

Tirpenou AE, Tsigouri AD, Gouta EH (1998) Residues of Organohalogen Compounds in Various Dolphin Tissues. Bull Environ Contam Toxicol 60:216-224

Toepfer H (1992) Detecting PCBs in Fish Tissue. Hewlett-Packard PEAK, News for users of analytical instrumentation 2:1-3

Weistrand C, Noren K (1993) Liquid-gel partitioning using Lipidex in the determination of polychlorinated biphenyls in cod liver oil. J Chromatog 630:179-186

WHO (1990) Evaluation de certains additifs alimentaires et contaminants. Trente-cinquième repport du Comité mixte FAO/OMS d'experts des additifs alimentaires.Série de Rapports techniques 789.Organisation Mondiale de la Santé, Genève