CONGENER PROFILES OF PCDD, PCDF AND NON-ORTHO PCB IN MARGARINES WITH FAT COMPONENTS OF DIFFERENT ORIGIN

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Introduction

About 95 % of the human background exposure to the antropogenic 2,3,7,8 substituted PCDDs, PCDFs and non-ortho PCBs is through the food chain and foods[1]. Polychlorinated biphenyls (PCB) are present at ppb levels in most fatty foods and occasionally found at significantly elevated levels due to technical or industrial circumstances. Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) have never been produced for technical use, but are formed primarily as by-products in various technical applications and processes. They are found at ppt levels in many foods due to enrichment in the food chain.

Persistent lipophilic pollutants are present in the environment at all trophic levels. Since they are toxic, persistent, fat-soluble and tend to metabolise as well as bioaccumulate, their levels and patterns in the food chain and fatty foods should be determined with high accuracy and precision in order to identify the primary and secondary sources, and limit contamination of foods and feed.

Numerous dioxin contamination incidences involving food and feed have been solved, and sources identified, due to pattern recognition of the isomer and congener profiles in the contaminated foods[2,3,4]. Where the industrialised food processing uses fat based raw materials, which are likely to contain traces or even elevated levels of PCDDs, PCDFs and non-ortho PCBs, the continuous monitoring of profiles in consumer fat and feed is of utmost importance.

The analytical requirements for these so called 'chemical finger-prints' are congener and isomer specific chemical analyses at trace, ppt or ppq, level. Conventional identifications of the complex mixtures are done by high-resolution gas chromatography and mass spectrometry (HRGC-HRMS). Pattern recognition by visual comparison or multivariate statistics (MVDA) are commonly used to link the contaminants to a specific source.

Methods and Materials

In a recent food survey, six different types of frequently used margarines were analysed. Two margarines were found to contain significantly higher levels of PCDDs, PCDFs and non-ortho PCBs than the others. One of the margarines (sample A) consisted of plant fat/oil and 6.6 % of marine oil, to provide a high Ω-unsaturated fatty acid content (total 60 % lipids), and the other margarine (sample B) consisted of pure vegetable fat/oil (81 % lipids).

Chemical analytical sample preparation was carried out by a modified Smith et al. procedure[5]. Margarine (10 g lipid) was homogenised with sodium sulphate and fortified with ^{13}C -labelled 2,3,7,8 substituted PCDDs, PCDFs and non-ortho PCBs. The homogenate was transferred to a column consisting of silica based materials. The PCDD, PCDF and non-ortho PCB congeners were eluted onto an activated carbon column with a mixture of 1:1 v/v of dichloromethane and cyclohexane. The PCDD, PCDF and non-ortho PCB congeners were back flushed from the carbon column with toluene. After evaporation, the PCDD/PCDF/non-ortho PCB containing fraction was purified on a chromatography system consisting of two columns in series filled with acidic silica and basic alumina oxide. Then the eluate was concentrated to 30 µl and ^{13}C-labeled 1,2,3,4 – TCDD, ^{13}C-labeled 1,2,3,7,8,9 HxCDD and PCB 189 was added as recovery standards.

The isomer specific analysis was carried out using a Hewlett Packard 5890 Series II gas chromatograph coupled to a VG AutoSpec high-resolution mass spectrometer. The HRGC separation was performed on a 60 m x 0.25 mm ID RTX-5 MS column, with a film thickness of 0.1 μm. Helium was used as carrier gas, with a head pressure of 230 kPa. Injection, 1 μl, was performed in split-less mode at 280°C on a Hewlett Packard 7673 auto-sampler. The mass spectrometer was operated in electron impact ionisation mode with electron energy set to 42 eV and an ion source temperature at 265 °C. Data storage was done in selected ion monitoring, SIM, mode. PCDD/PCDF and non-ortho PCB were analysed in two different runs with different MS descriptors, resolution (R) and GC temperature programs.

PCDD/PCDF: The column temperature program was 100 °C isothermal for one minute, followed by a gradient of 22 °C per minute up to 187 °C, then 3 °C per minute up to 300 °C held for 3 minutes. Resolution (R) was set to 10,000.

Non-ortho PCB: Initial temperature was set to 90 °C isothermal for one minute, raised by 40 °C per minute to 190 °C, then 0.8 °C per minute to 218 °C and at last 30 °C per minute to 300 °C held for 3 minutes. Resolution (R) was set to 8,000.

The instrument detection limits, DL, were between 0.05 and 0.25 pg/μl for the tetra to octa PCDD/PCDF and below 0.1 pg/μl for the non-ortho PCB congeners in the calibration solution. The method detection limits, MDL, for the different PCDD/PCDF congeners were calculated to be between 0.02 and 0.15 pg/g lipid, the corresponding values for the non-ortho PCB congeners were 0.03-0.09 pg/g lipid.

Results and Discussion

The profiles, 'chemical finger-prints', of the 2,3,7,8-substituted PCDD and PCDF congeners in margarine samples A and B are shown in Figures 1 and 2. The two 'finger-prints' are quite different. Sample A, consisting of vegetable fat added an amount of marine oil, has a profile dominated by OCDD, tetraCDF, pentaCDF and hexaCDF. While hexaCDD, heptaCDD and OCDD are the major congeners in sample B, which is pure plant fat/oil.

Margarine A has a congener profile similar to that in fish, where the tetra, penta and hexa PCDFs dominate. Precursors for the formation of PCDDs, which dominate in margarine B, have demonstrated to include chlorophenols and chlorobenzenes[6,7]. The PCDD and PCDF pattern of margarine B is reflecting a profile from processes including chlorinated chemicals, a so called 'pentachloro phenol profile', suggesting that the plants used to process margarine B have traces from this type of sources[6].

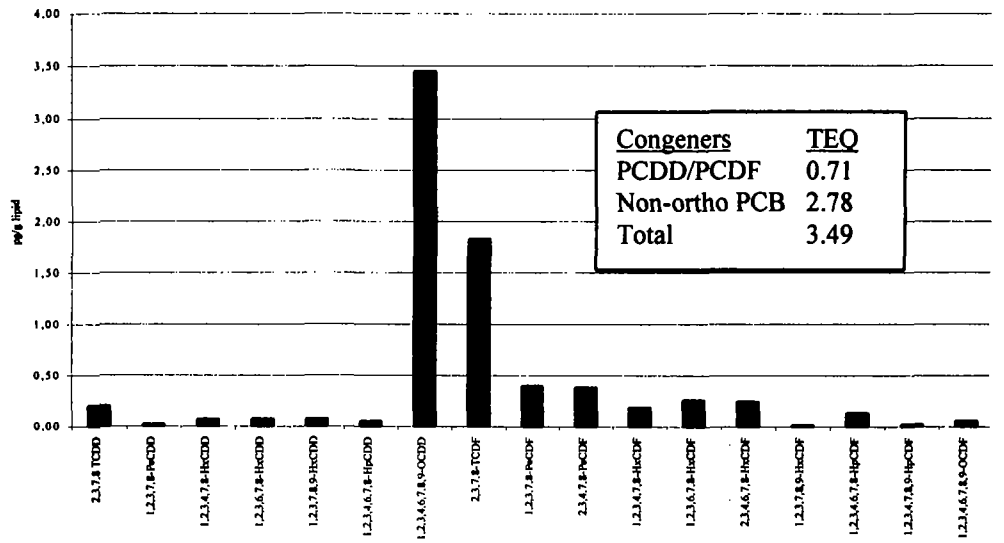

Figure 1: Levels in pg/g lipid and profile of PCDDs and PCDFs in margarine consisting of vegetable fat and some amount of marine oil (sample A).

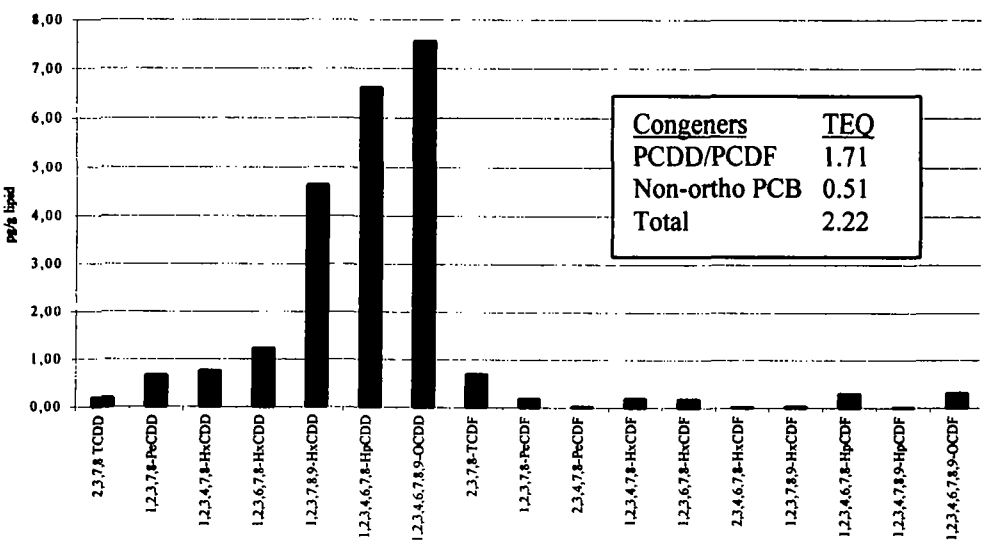

Figure 2: Levels in pg/g lipid of PCDDs and PCDFs in margarine consisting of pure vegetable fat (sample B)

For risk assessment of complex mixtures of PCDD, PCDF and non-ortho PCB, toxic equivalent factors have been developed[8]. For the non-ortho PCB congeners, it is noticed that sample A contains 2.78 pg TEQ/g lipid, while sample B is approximately 20 % of this, with 0.51 pg TEQ/g lipid. This is supporting the suggestion that the PCDD, PCDF and non-ortho PCB congeners in sample B, is from a non-marine source.

In this survey the sum of the toxic equivalents, including PCDDs, PCDFs and non-ortho PCBs, of margarine sample A was 3.49 pg TEQ and of sample B 2.22 pg TEQ per gram lipid. WHOs latest recommendation of maximum tolerably daily intake (TDI) of PCDD/PCDF and non-ortho PCB is 1-4 pg TEQ/kg bw/day[9]. A person with a weight of 60 kg is recommended to have an intake of less than 60-240 pg TEQ/day. In SNT report 9, 1997, the estimated Norwegian consumption of margarine is approximately 30 g per day[10]. Using this estimate, the intake of PCDD, PCDF and non-ortho PCB per day for samples A and B is 63 and 54 pg TEQ per day, respectively, which is in the same range as the lowest recommended TDI. For a child weighing 15 kg a consumption of 30 g of margarine per day corresponds to 4.2 and 3.6 pg TEQ/kg/day for sample A and B, respectively. This is at the same level as the maximum TDI of 4 pg TEQ/kg/day.

The primary sources of intake through food are usually believed to be marine organisms, meat and dairy products. This study has shown that other fatty foods can be of great importance. Earlier cases have demonstrated that consumer fat can be contaminated with PCDD, PCDF and PCB congeners. A continuos monitoring program for consumer fat is therefore necessary. The PCDD/PCDF profiles of the two margarines also show that congener specific chemical analyses are of utmost importance to eventually identify primary and secondary sources of contamination.

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