PCDD/PCDF and dioxin-like PCB in spices

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Introduction

Polychlorinated dibenzodioxins and –dibenzofurans (PCDD/F) arise from a multitude of industrial and thermal processes. Polychlorinated biphenyls (PCB) are man-made compounds and were widely used as transformer oils, hydraulic fluids and softening agents. For this reason PCB and PCDD/F nowadays are ubiquitously distributed.

Since 1989[1] productions, import, export and sale of PCB in Germany are forbidden, but today PCB are still found in the environment due to their long half-life times[2].

It seems that food - especially of animal origin - plays an important role in human PCDD/F and PCB exposure. Hecht and Blüthgen[3] analyzed meat and meat products from Germany in a representative study on their PCDD/F contents and observed higher levels in meat products than in meat of the same farmed animal. Therefore, it was under consideration, that spices could possibly contaminate the meat products. Owing to the lack of data about the yield of PCDD/F and PCB in spices a clean-up method was developed[4] to get an adequate number of data about PCB and PCDD/F in a sufficient number of different spices.

Materials and Methods

Material, reagents and samples

$^{13}C_{12}$ -labelled PCDD/F standards solutions (17 WHO-PCDD/F), $^{13}C_{12}$ -labelled PCB standard solutions (PCB 28, PCB 52, PCB 77, PCB 81, PCB 101, PCB 105, PCB 114, PCB 118, PCB 123, PCB 126, PCB 138, PCB 153, PCB 156, PCB 157, PCB 167, PCB 169, PCB 180 and PCB 189) and the recovery standards (deuterized or $^{13}C_{12}$ –labelled) for PCDD/F (D 48, F 69 and F 131) and PCB (PCB 77 and PCB 141) were purchased from Promochem (Wesel, Germany). Internal standard (50 µl of each standard solution) was added before sample extraction.

All solvents (n-hexane, toluene, cyclohexane, ethyl acetate and i-octane) had a purity of “picograde” and were delivered from Promochem (Wesel, Germany).

The spices were received from a commercial company (e.g. pepper, marjoram, ginger, oregano, paprika, chives). The samples were supplemented by herbs from private cultivation and by spices of the marketplace.

Extraction

Extraction cells were filled with dried sample material, drying substance (poly (acrylic acid) - partial sodium salt-graft-poly (ethylene oxide) from Aldrich (Missouri, USA)) and sea sand. Then the extractions were performed by accelerated solvent extraction (ASE 200 from Dionex). For extraction n-hexane was used at a pressure of 100 bar and a temperature of 100 °C. The samples were extracted in two cycles with a static time of ten minutes each.

Clean up

After evaporation, the extract firstly was cleaned on a gel permeation chromatography (GPC) column with an ID of 25 mm that was filled with 60 g Bio-Beads S-X3 (200-400 mesh), a cross-linked divinylbenzene-styrene copolymer, from Bio-Rad Laboratories (München, Germany). The GPC (Abimed Gilson, Langenfeld, Germany) was carried out with a solvent mixture of cyclohexane-ethyl acetate (1:1; v/v) and a flow rate of 5 ml/min. Extract was concentrated and dissolved with 1 ml toluene. The samples were eluted from Florisil columns (8 ml-SPE columns (ID: 12 mm) filled with 3g Florisil deactivated with 4 % water (w/w)) with 60 ml of toluene. After evaporation of the solvent nearly to dryness the samples were dissolved in 1 ml of n-hexane. 8 ml-SPE cartridges with an ID of 12 mm were filled in the following order with 0.3 g of drying substance, 1 g of 33 % NaOH-silica (w/w), 0.25 g of activated silica, 2.5 g of 44 % H_2SO_4-silica (w/w) and 0.5 g of 22 % H_2SO_4-silica (w/w). The sample extracts were eluted with 60 ml of n-hexane. For separation of PCB and dioxins chromatographiy on activated charcoal was used. In the first fraction (20 ml n-hexane/toluene; 99:1; v/v) the di- and mono-ortho-substituted PCB and in the second fraction (20 ml n-hexane/toluene; 75:25; v/v) the non-ortho-substituted PCB were collected. In the third fraction (60 ml toluene) the PCDD/F were collected in back-flash mode. Therefore a LC glass column with an ID of 12.5 mm was purchased from Latek, Germany and filled with 250 mg of activated charcoal (Supelclean ENVI-Carb from Supelco (Missouri, USA)). The last three chromatography steps were operated by a modified ASPEC XLi-system Gilson International (Middleton, USA). The elutions were concentrated to about 100µl (1. fraction) or 15µl (2. and 3.fraction) and after adding a recovery standard, they were analyzed by GC-HRMS.

Instrument and method

The GC-HRMS system used was a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Germany) with an injection in split/splitless mode and combined with a VG Autospec (Manchester, UK) high resolution mass spectrometer. A ZB-5ms column (ID = 0.25 mm, film thickness = 0.25 µm, 5 % polysilarylene, 95 % polydimethylsiloxane) from Phenomenex (Torrance, USA) with a length of 60 m was used. The GC temperature program for the PCDD/F measurement was as follows: 80 °C (1 min) to 210 °C (5 min) at 25 °C/min, to 240 °C (5 min) at 5 °C/min and subsequently at 5 °C/min to 320 °C, then maintained for 10 min at 320 °C. Owing to possible interactions of some native PCB-peaks another GC program was compiled to divide PCB: 70 °C (2 min) to 180 °C (0 min) at 30 °C/min, to 290 °C (0 min) at 5 °C/min and subsequently at 20 °C/min to 320 °C, then maintained for 10 min at 320 °C. PCDD/F and PCB were measured working in the electron impact (EI) positive ionisation mode with an electron energy of 35 eV, the source temperature was set at 250°C. Data were recorded in SIR mode. The resolution of the mass spectrometer was tuned up to 7000 (10 % valley definition). The quantification was made by stable isotope dilution. The limit of detection (LOD) was 0.03 ng WHO-PCDD/F-TEQ/kg spices; the limit of quantification (LOQ) was 0.10 ng WHO-PCDD/F-TEQ/kg spices.

Results and Discussion

So far more than one hundred samples have been analyzed, including 26 pepper, 12 paprika, 11 oregano, 10 marjoram samples as well as a multiplicity of other spices.

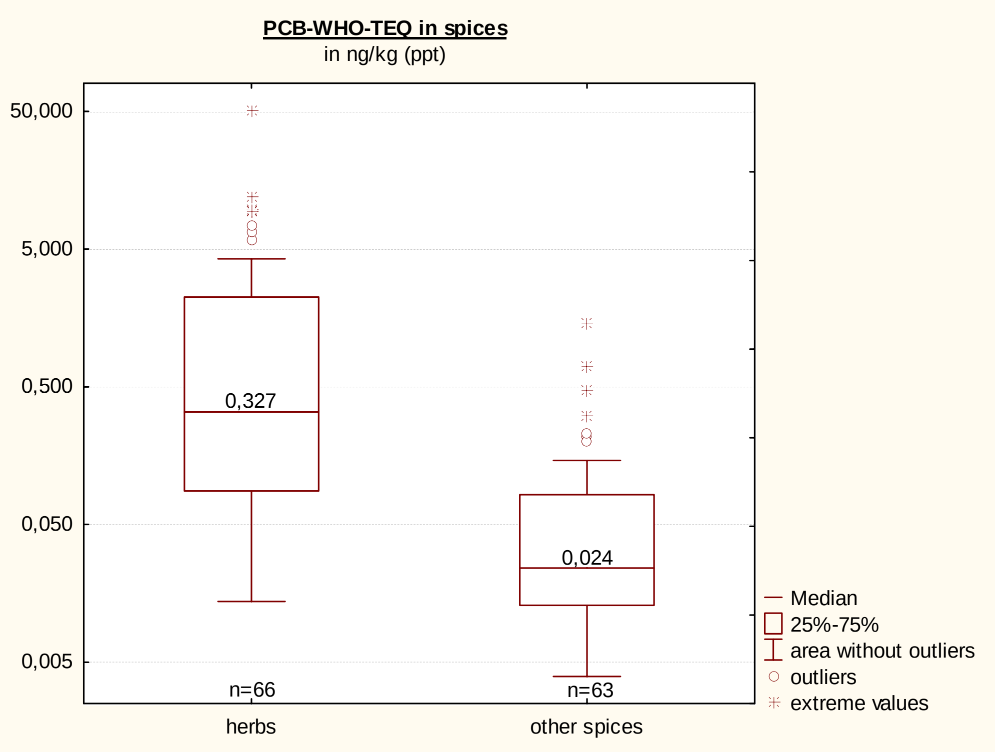

Figure 1: WHO-PCB-TEQ of the analyzed spices in ppt (logarithmic representation) presented as herbs and other kinds of spices, presented as box-whisker-plots. The respective medians are indicated in the figures.

It was found that the PCB yields in herbs (median = 0.327 ppt) are more than ten times higher than the concentrations in other kinds of spices (median = 0.024 ppt) (fig. 1). The uptake of dioxins by the root of plants is negligible[5]. Possibly this observation could be transferred to the substance class of the PCB. The measured data of PCB concentrations in herbs indicate that PCB contamination occurs on the surface of the leaves of herbs. This assumption hold true also for dioxins.

Comparing the two groups of spices in their dioxin content we found similar low dioxin contents in both groups (fig. 2).

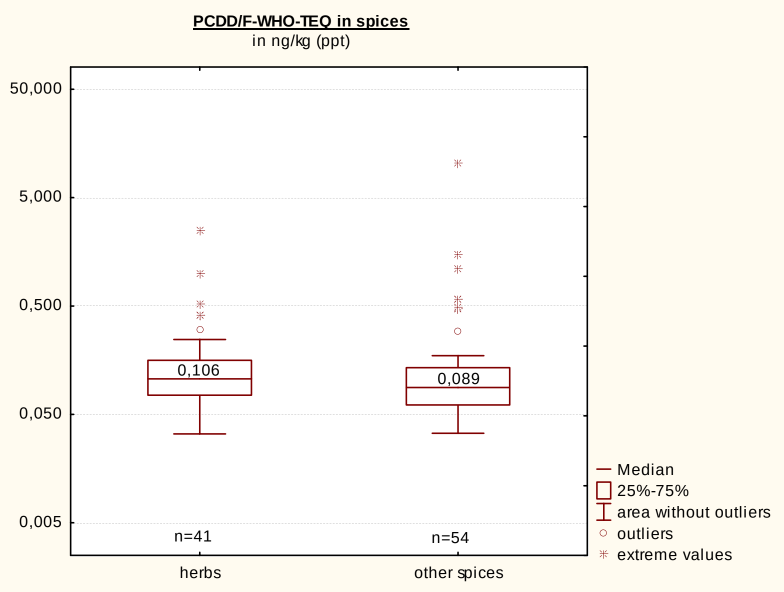

Figure 2: WHO-PCDD/F-TEQ of the analyzed spices in ppt (logarithmic representation) presented as herbs and other kinds of spices, presented as box-whisker-plots. The respective medians are indicated in the figures.

Conclusion

The investigated spices are contaminated with PCDD/F and PCB but the measured concentrations are low. The concentration in meat products by the usual addition of 2% is negligible. The reasons for higher PCB contents in herbs compared with the other kinds of spices are unsolved and needs further investigations.

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