

Polybrominated diphenylether (PBDE) in poultry meat and eggs

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Introduction

There exist strict regulations concerning fire protection for many things of the daily use, e.g. requirement of treatment by flame retardants. An important class in the group of flame retardants are the polybrominated diphenyl ethers (PBDE) (Hale et al., 2002). In the course of production, processing and use of consumer goods – e.g. computers, TV-sets, furniture and textiles – PBDE are transferred to the environment and afterwards to the food chain where they accumulate due to their persistence and high fat solubility. As a consequence contamination of food-products also happens. Unlike PCB and dioxins, where bans and adequate measures of prevention have led to a decrease in concentration, contamination with PBDE is steadily increasing and will cause problems for several decades.

PBDE are effective neurotoxic agents and interact with the hormones of the thyroid gland (Eriksson et al., 2002). Furthermore mutagenic and cancerogenic effects have to be assumed. This type of contaminants represents therefore a risk for man, which only can be avoided by minimisation of the uptake because of the long retention time in the human organism. The main contamination source for humans is food, especially food-products of animal origin (Salomon, 2005). It is therefore absolutely necessary to be able to determine the concentration of PBDE in these products.

Although there are 209 congeners of PBDE in total only 8 are most important in respect of toxicity and presence in environment. These are BDE 28, 47, 99, 100, 153, 154, 183 and 209. A planned EU-monitoring-project will also focus on these congeners (Scientific panel on contaminants in the food chain, 2006), which are determined by the presented method, too. The method consists of sample extraction, clean up and gaschromatography/high resolution mass spectrometry (GC/HRMS) analysis combined with isotope dilution technique.

Method evaluation as well as results for various PBDE-congeners concentrations in chicken meat and eggs are presented. The analysed samples are from a pool, which is representative for Germany. The data of this publication are the starting point of an ongoing study.

Materials and Methods

The complete method is presented in fig. 1. ASE: The homogenized sample is lyophilised. Afterwards the lyophilisate is extracted by ASE. Extraction cells are filled with lyophilisate, sea sand and drying material, furthermore labelled ¹³C-standards are added. The extraction is performed with hexane at 100°C and 100 bar. The solvent of the extraction procedure is removed by nitrogen flushing in a water bath at 40°C. GPC: The residue of ASE extraction is dissolved in cyclohexane/ethyl acetate and transferred to a glass column filled with Bio-beads. Elution is carried out with cyclohexane/ethyl acetate. Before rotary evaporation 1 ml of toluene is added to the collected fraction. Florisil column: The evaporated eluate of the GPC-procedure is transferred into a SPE column filled with deactivated florisil. Elution is performed with toluene. The eluate is evaporated and transferred into a brown vial. A further volume reduction is attained by nitrogen flushing. The eluate of the florisil column is applied to GC/HRMS analysis.

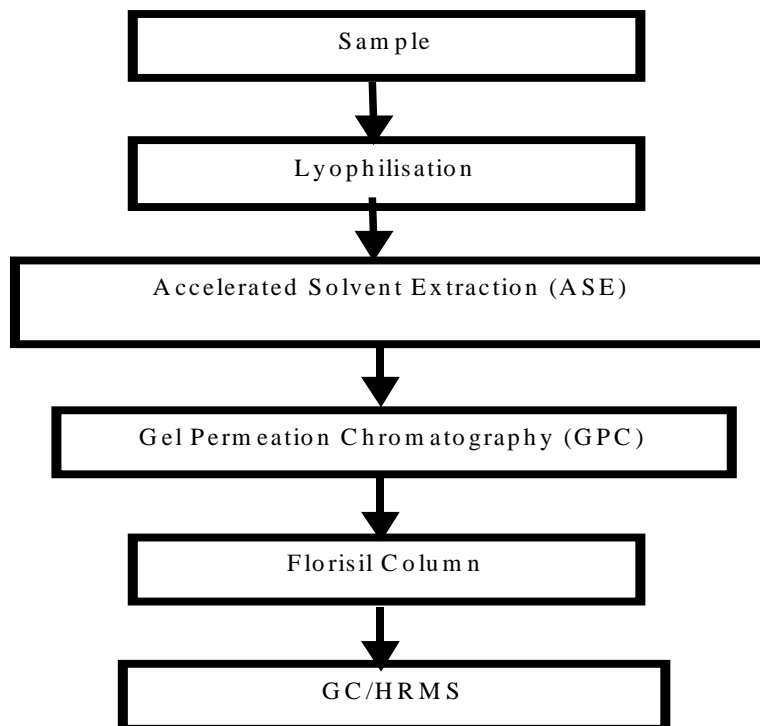


Figure 1: Method for the determination of PBDE in food-products

GC/HRMS: Measurements are carried out with a Thermo Electron DFS-mass spectrometer connected to 2 Trace-GC 2000. GC-HRMS-conditions – Column: J&W DB5-MS (30mx0,25mmx0,1µm), flow: 1ml/min, temperature program: 70°C (2 min) – 230°C (20°C/min) – 330°C (6°C/min) (25 min), split/splitless injector: 270°C, split: 50 ml, injection volume: 1µl, splitless time: 2 min, ion source: 280°C, electron energy: 45 eV, resolution: 10000, transfer line: 280°C. Identification and quantification of PBDE-congeners occur with specific masses. The presented conditions were deduced by method optimisation.

Results and Discussion

1) Evaluation of the method

Main factors influencing performance and sensitivity of the GC/HRMS system, i.e. GC-column, temperature program, column flow, settings of the split/splitless injector and temperature of the ion source were checked and optimised. Optimum parameters are described in Materials and Methods. Linearity, repeatability, limits of detection and quantitation, recovery and blank values were checked for the method as well as the suitability of the applied mass traces for different matrices. The relevant mass traces for HRMS were tested for meat products and eggs after extraction and clean up. The criteria of the corresponding EU-guidelines (2002/657/EG and SANCO/10232/2006) were always fulfilled, so that applied quantifier and qualifier masses are suitable for identification and quantification of PBDE-congeners in the indicated matrices. The linearity for the single PBDE-congeners was tested for the GC/HRMS system as well as for the total method over a range of 4 orders of magnitude by standard mixtures and spiked food samples. The coefficients of determination R^2 for the regression lines were always $> 0,99$. The repeatability was tested for the total method in original and spiked food matrices by 5 independent repetitions each. The values for the relative standard deviation (RSD) of the single congeners ranged from 2 to 15 %. Only if the congener concentration was very low (< 10 ppt) RSD increased up to 40 %.

Limits of detection (LOD) and quantitation (LOQ) were determined both for the GC/HRMS system alone and for the total method of analysis, GC/HRMS system included. LOQ

corresponds to three times of the value of LOD. LOD for the GC/HRMS system was 0,07 pg (absolute amount) for all congeners with the exception of BDE 209 (1,7 pg). LOD for the total method was independent of congener 1 ppt or lower related to fresh weight (BDE 209: 100 ppt). Corresponding LOQ was 3 ppt or lower related to fresh weight (BDE 209: 300 ppt). Recovery was determined by standard mixture, by addition of labelled standards to food matrices and by spiking of food samples with known amounts of unlabelled standards. For all congeners recoveries were always between 60 and 109 % (exception BDE 209: 15-25 %). The blank values for the method were < 2 ppt (BDE 47: 10 ppt). For BDE 209 blanks varied from 200 to 400 ppt.

BDE 209 is the only PBDE-congener which is currently allowed to be used in the EU and therefore it is frequently present in environment and also in laboratories. The deca-BDE is very unstable, poorly volatile and soluble and these are the reasons for occurring difficulties in analysis. BDE 209 is up to now the only congener for which the method has to be optimized, that means especially the blank value has to be reduced.

2) Concentrations and distribution of PBDE-congeners in chicken meat and eggs

The presented method was applied to chicken meat (fig. 2) and hen egg samples (fig. 3). BDE-congeners 28 to 183 were determined.

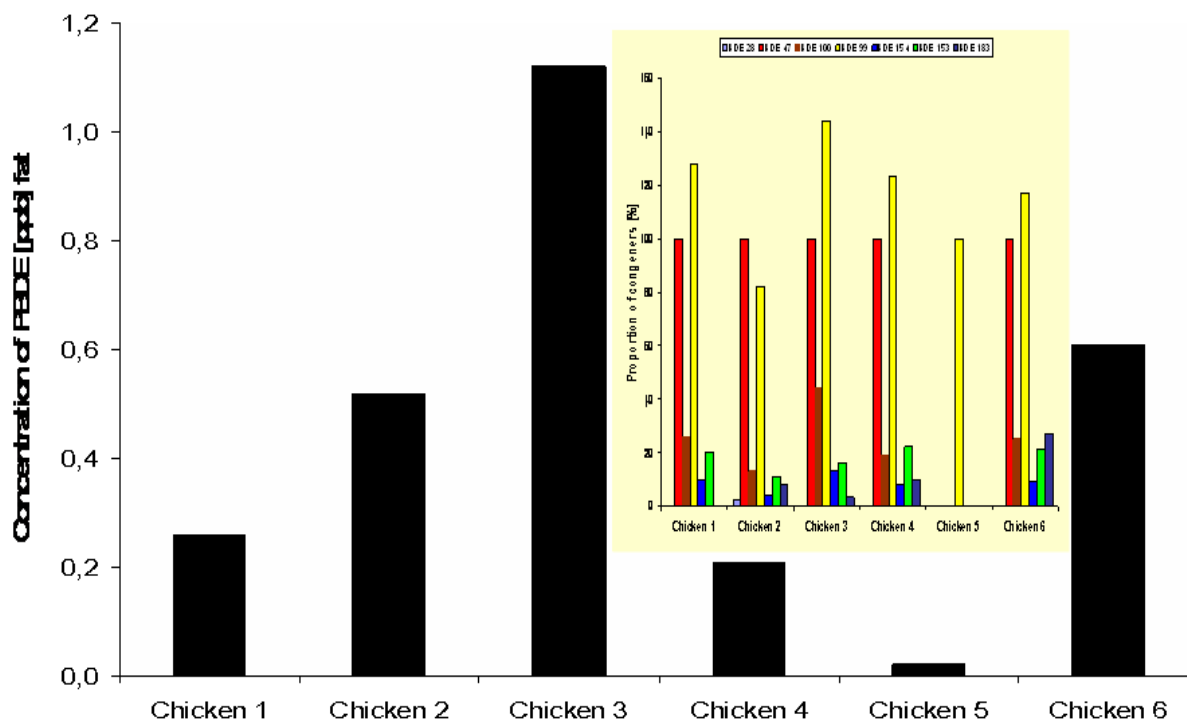


Figure 2: Concentrations of PBDE in chicken meat, related to the fat content and distribution of single congeners in reference to BDE 47 (100 %) (small diagrams)

PBDE-concentrations for the analysed chicken meat samples varied from 0.02 to 1.1 ng/g fat, for the egg samples from 0.1 to 0.9 ng/g fat. That means there is a variation of the PBDE-concentration within the chicken meat products of approximately a factor of 50, within the egg samples of a factor of 9. The consequence is, that the human intake of PBDE can widely vary in dependence of the various consumable products.

BDE 47 and 99 dominate in chicken meat as well as in hen eggs, but there are some meat as well as egg samples for which BDE 47 and others for which BDE 99 appears to be the main congener. It seems that an increase of BDE 99-percentage relative to BDE 47 is

correlated with an increase of corresponding BDE 153- and 154-percentages for both matrices.

In summary these facts indicate that the PBDE-concentration and congener distribution in eggs are influenced by the hen itself.

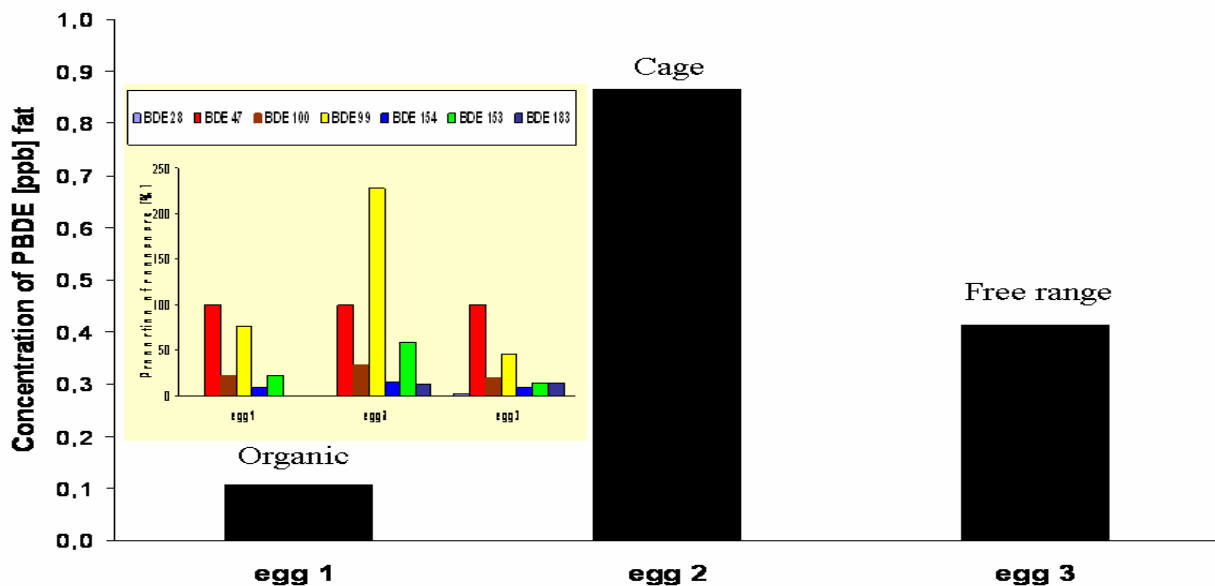


Figure 3: Concentrations of PBDE in hen eggs, related to the fat content and distribution of single congeners in reference to BDE 47 (100 %) (small diagrams)

In the case of hen egg samples different animal husbandry – cage, organic and free range – was taken into consideration. Fig. 3 shows, that eggs originating from different animal keeping vary in PBDE-concentration, resulting in higher levels for “caged eggs”. The congener-patterns of the eggs originating from organic and free range production are very similar, but both differ from that of the “caged eggs”. Hens of organic and free range animal husbandry are in contact with soil, “cage-hens” on the other side are missing this contact. That could mean, that the patterns of organic and free range eggs are influenced by the PBDE-congener pattern of soil. This hypothesis has to be proven by a larger number of samples. In general the database has to be enlarged by analysing additional poultry meat and egg samples to confirm or disprove present results and to get some data for turkey meat, which is less dominant than chicken meat, but still important for Germany.

Acknowledgement

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