FAST SAMPLE PREPARATION FOR ROUTINE DETERMINATION OF PCDD/F, PCB AND PBDE IN FOOD AND FEED

Thorsten Bernsmann[1]*, Michael Albrecht[2], Peter Fürst[1]

[1]Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL), Joseph König Straße 40, Münster, Germany; [2]Bavarian Health and Food Safety Authority, Veterinärstraße 2, Oberschleißheim, Germany

Introduction

The recent years in POPs analysis have illustrated the need for fast and high throughput methods to identify and confirm non-compliant samples in the feed and food chain. While the development of measurement technology progressed with the introduction of GC-MS/MS as confirmation method, the clean-up in many laboratories is still done manually. The clean-up from fat extraction until the final solution can take up to several days. For this purpose, a highly efficient clean-up procedure is required to purify raw extracts prior to the final analytical separation and quantification step. With the DEXTech system™ (LCTech), it is possible to get purified extracts within 90 minutes, which can be measured by GC-HRMS or GC-MS/MS. All results from different laboratories demonstrate the suitability of the automated LCTech sample preparation system for a fast and reliable routine analysis of PCDD/F, PCB and PBDE congeners in foodstuffs and feedstuffs that meet the requirements of European Union legislation.

Materials and methods

Two different laboratories, the Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe (CVUA-MEL) and the Bavarian Health and Food Safety Authority (LGL) analyzed their samples with their different manual sample preparation and the automated DEXTech system.

CVUA-MEL: The principle of the manual sample preparation method is based on the clean-up of the acid stable PCDD/F, PCB and PBDE on silica gel coated with sulfuric acid. A separation of the PCDD/F from PCB/PBDE is subsequently performed on a Florisil column. For further purification, both eluates of the Florisil column are cleaned up on two different carbon columns which contain a different active carbon. The PCB/PBDE fraction can be split into a group of non-ortho PCB, and a fraction containing the mono- and di-ortho PCB, and PBDE. This is important because the non-ortho PCB fraction includes PCB 126 and 169 which were assigned the highest toxic equivalency factors of the PCB (WHO 2005). If PCB 126 is measured along with the other PCB, it may cause interferences, which can lead to a substantial overestimation of the PCB 126 concentration depending on the separation column. It is therefore essential to separate the non-ortho PCB from the other PCB. The PCDD/F fraction also needs to be cleaned up on a carbon column to separate matrix substances which may potentially interfere especially with the tetra-, penta- or hexa-CDD/F traces in the mass spectrometric analysis[1].

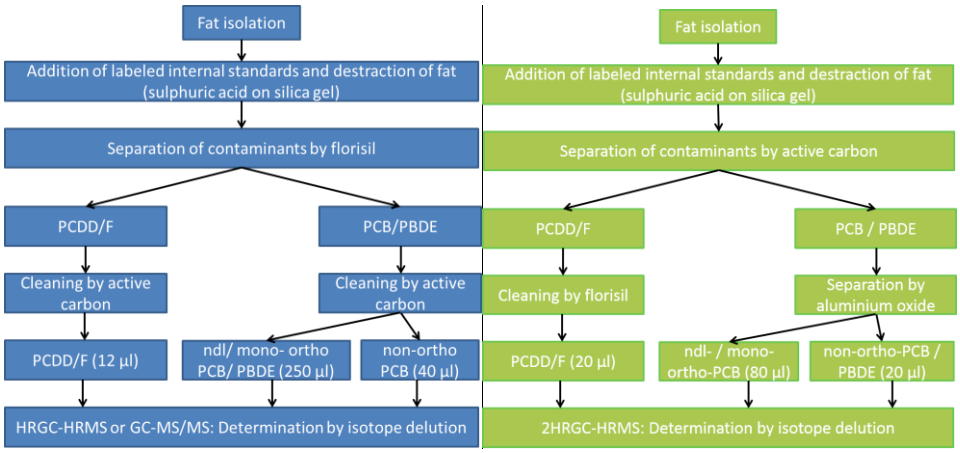

Figure 1: Manual sample preparation at CVUA-MEL (left) and at LGL (right)

LGL: Samples are homogenized and freeze-dried, depending on the water content. Mixed with anhydrous sodium sulfate, fat was extracted with hexane/acetone (2/1). For isotope dilution, all labeled internal standards were added. Fat and instable compounds were removed on a silica gel column coated with sulfuric acid (44%) by pentane. This fraction which containing the target analytes was subsequently separated by an active carbon column. Dl- and ndl-PCBs as well as PBDEs were eluted in the first fraction with hexane, cyclohexane and dichloromethane, followed by PCDD/Fs in the second fraction (eluted with toluene). While the PCDD/F fraction was further cleaned up on a Florisil column (1% water, rinsed with hexane, eluted with toluene), the PCB/PBDE-eluate was applied to an aluminum oxide/anhydrous sodium sulfate column to separate the mono-ortho- and ndl-PCBs (eluted with hexane/dichloromethane, 98/2) from the non-ortho-PCBs and PBDEs (eluted with hexane/dichloromethane, 1/1). Extracts were dissolved in 20 µl and 80 µl (mono-ortho- and ndl-PCBs) with a recovery standard solution.

The automated sample clean up with DEXTech:

Since the last dioxin congress 2013[1] there were further developments of the system. The fat or in case of samples of by plant origin, the organic solvent extract, e.g. gained with Soxhlett or Twisselmann are dissolved in 2 ml toluene and 3 ml n-hexane and loaded directly into the sample loop of the system. For rinsing the sample vessel, syringe and injection port, additional flushing with smaller amounts of hexane is useful up to the maximum of 15 ml for the used sample loop. The ready-to-use LCTech columns (acid silica, Florisil and two activated carbon columns) are placed into the column holder. The system starts with a conditioning step of the columns, injects the samples automatically and collects three fractions per sample. The automated separation process follows the same well-proven principle as the manual CVUA-MEL clean-up. The whole sample clean-up takes 90 minutes. Half of the time is needed for conditioning the columns. The fractionation process performed with the DEXTech automated clean-up system enabled a rapid and separate analysis of PCDD/F, non-ortho, mono-ortho and di-ortho PCB[1] as well as PBDE.

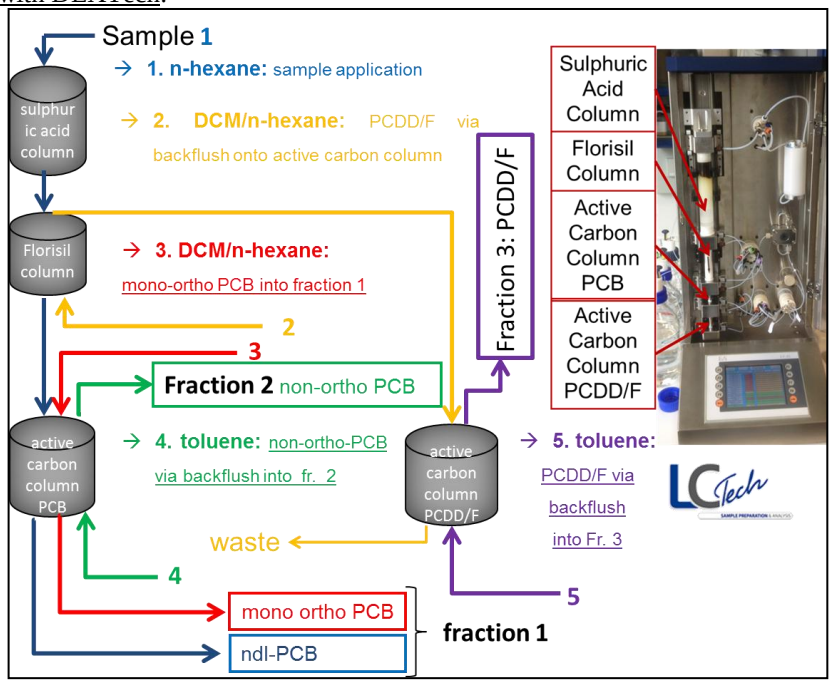

Figure 2: Scheme of the DEXTech system

Results and discussion

Recoveries:

A comparison study was performed on quality-control samples, food samples of animal origin and feeding stuff samples to evaluate the robustness of the new automated sample clean-up system compared to the two manual standard methods of the different laboratories. The LGL analyzed 52 different samples (15 egg, 13 beef, 12 standard solutions, 5 cattle liver, 3 blank, 2 Oil and 2 milk QC samples). The CVUA MEL analyzed 146 different samples (44 sheep liver, 25 fish, 21 feed (from corn, grass to compound feed), 20 blank, 19 egg, 10 milk QC and 7 beef samples). Figure 3 showes the recoveries of the ^{13}C-labeled internal standards summarized over all sample types analysed at LGL and CVUA MEL. Each column includes the error bars, which represent the standard deviation over all samples. The recoveries for all analyzed 198 samples were below 120 % and for nearly all samples over 60 %. In some cases, the recoveries were below 60 %, which is due to matrix interferences and can also be seen with the manual sample preparation. The shown recoveries are not only based

on the sample clean up. They also include the sample extraction and measurement. In comparison to the manual sample preparation, it can be said that the recoveries and the results are very robust with the automated DEXTech sample clean up system.

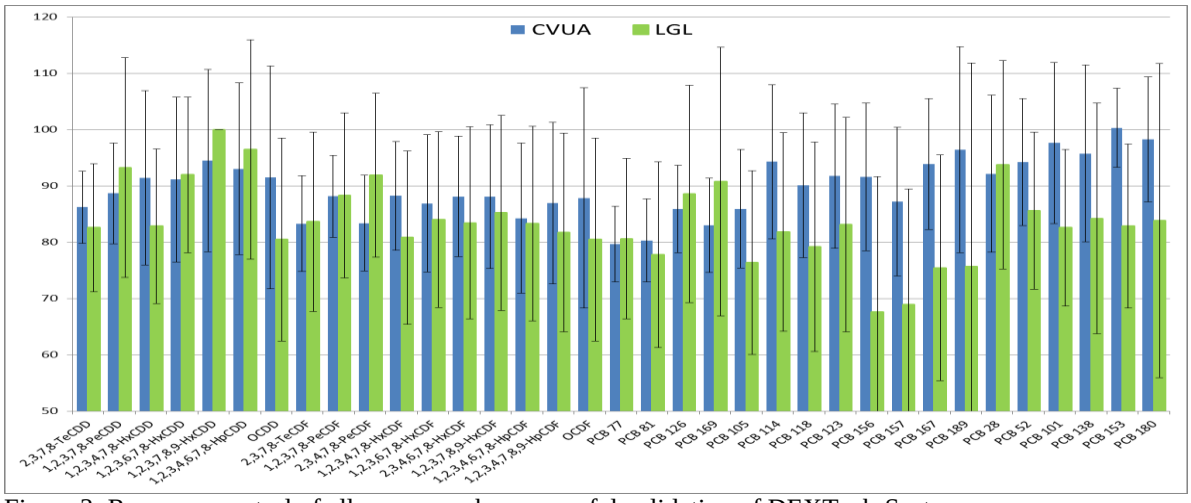

Figure 3: Recovery control of all congeners by successful validation of DEXTech-Systems

Proficiency tests:
The DEXTech systems were also used in both laboratories during the recent proficiency test of the EURL for dioxins and PCB, Freiburg, for sample preparation of Sepiolite extracts. The results are shown in Figure 4 and 5.

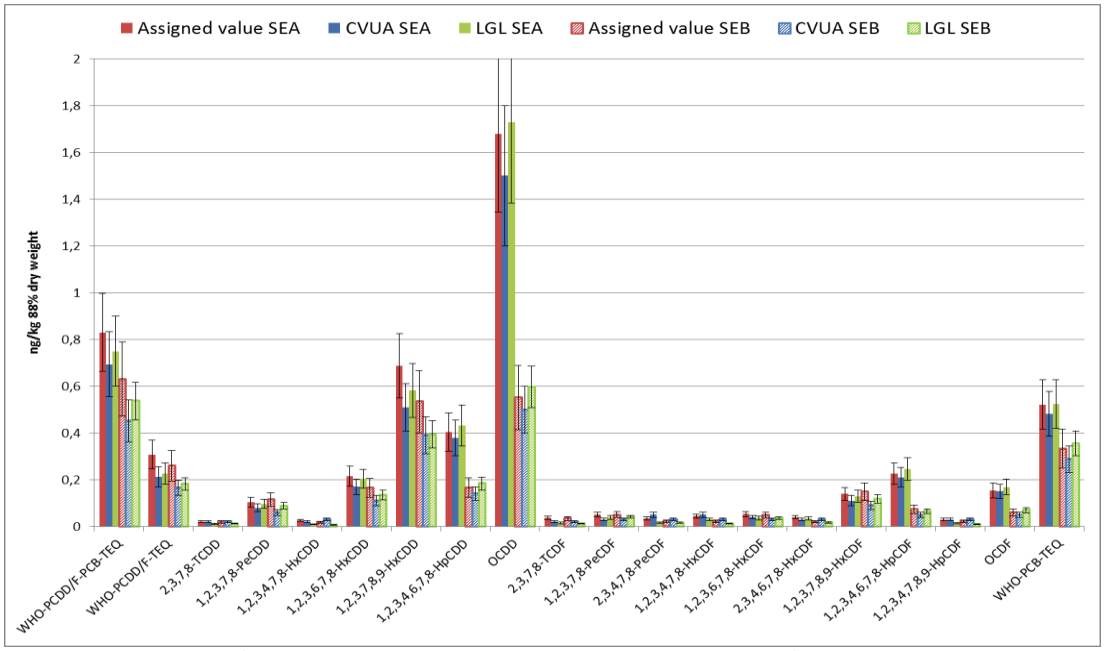

Figure 4: Comparison of PCDD/F-congeners and their Sum-TEQ including PCB-TEQ of both laboratories with assigned values from proficiency test of EURL

For all sum-TEQ levels and the sum of the 6 ndl-indicator-PCB (ICES-6) as well as PCDD/F- and PCB-congeners, the regulations pertaining to compliance with the performance criteria have been met. This means that the participation was successfully carried out on the last EURL-ring test after validation of DEXTech systems in two different laboratories and the successful application of different matrices. This confirmes the statement of the last paper[1] where the proficiency test (PT) material "Feed Fat 2013" from the EURL for Dioxin

and PCB (Freiburg) was analyzed. The results were, like in this PT, very close to the assigned values. This indicates the robustness of the automatic DEXTech system over different laboratories and different matrixes.

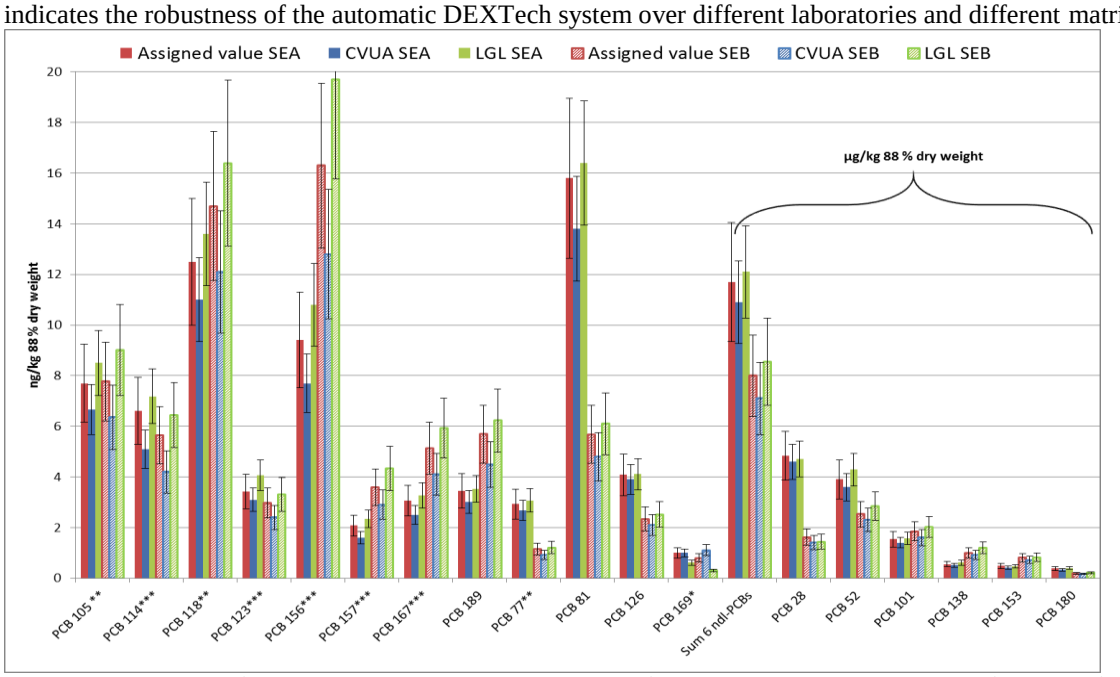

Figure 5: Comparison of PCB-congeners, their sum-TEQ and ICES-6 of both laboratories with assigned values from the proficiency test of the EURL; * concentration of these compounds 10 times higher; ** concentration of these compounds 100 times higher

At last, the system was tested for polybrominated diphenyl ethers (PBDE) and polybrominated biphenyl (PBB 153) in 21 fish samples. During the sample clean up, these compounds get into the first fraction together with the mono-ortho-PCB and the ndl-PCB. As can be see in figure 6, the recoveries of these compounds are within 60 to 110 %. These results indicate that other polybrominated compounds which were not destroyed or changed by concentrated sulphuric acid can be measured with this system very easily together with dioxins and PCB. Further investigations are ongoing and will be developed in more detail in the future.

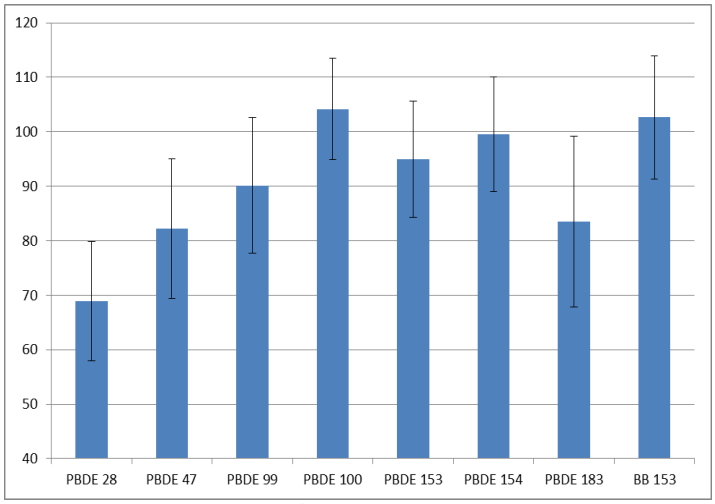

Figure 6: PBDE/BB recoveries in fraction 1

Conclusion

The recoveries for all PCDD/F and PCB congeners in up to 200 different samples from two different laboratories with the automated sample preparation system are in good agreement with the legal requirements laid down in Commission Regulation (EU) No 252/2012 and range between the prescribed limits of 60 % to 120 %. Also PBDE/BB congeners can be measured with satisfying recoveries. A comparison of analytical results obtained for the EURL PT material "**Sepiolite**" prepared with the automatic sample preparation DEXTech system demonstrates the suitability as a fast and reliable routine analysis of PCDD/F and PCB congeners in foodstuffs and animal feed at the level of interest that meet the requirements of European Union legislation. The easy to use system with all valves visible makes a fast sample clean-up also in an emergency case possible.

References:

1. Bernsmann, T, Möhlenkamp, U, Fürst, P, Aulwurm, U, Baumann, M (2013); Organohalogen Compounds Vol. 75, 728-732