

**FINAL RESULTS OF THE EUROPEAN RESEARCH PROJECT DIFFERENCE:
NEW ALTERNATIVE METHODS FOR DIOXIN ANALYSIS**

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The European research project DIFFERENCE (“Dioxins in Food and Feed – Reference methods and New Certified Reference Materials”) was focussed on the development of alternative methods for the analysis of polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) using comprehensive multi-dimensional gas chromatography (GC×GC), gas chromatography combined with low resolution ion-trap mass spectrometry (GC-LRMS/MS), the CALUX bioassay and an Ah-PCR technique. GC combined with high resolution mass spectrometry (HRMS) was used as a reference method in all comparisons. Given the need for a regular monitoring of PCDD/Fs and PCBs in Europe, which was further enhanced by the implementation of the maximum residue levels (MRLs) for PCDD/Fs in food and animal feed per 1 July 2002, and the relatively high costs of GC-HRMS and the limited capacity for HRMS analyses in European laboratories, cheaper, faster, but reliable methods were badly needed. The method development part included a developmental phase, a validation phase and a standardisation phase. In addition to the method development, attention was also paid to alternative extraction and clean-up methods, in particular on accelerated solvent extraction. A feasibility study on the preparation and certification of reference materials was also included.

Three out of the four methods have successfully been developed and validated. CALUX may be considered as a valuable screening method that can be used in times of crisis for a large number of samples to indicate if those samples are below, around of above the EU MRLs. Quantitative total-TEQ values may also be produced by this method, but due to the variability of those data, in particular for the mono-ortho substituted PCBs, the use of CALUX in that way is not recommended. The application of recovery correction appeared to be essential in any case. GC-LRMS/MS may be used as a reliable routine method that will produce congener-specific data. GC×GC-ECD or GC×GC-ToF-MS emerged as viable routine methods for dl-PCBs and PCDD/F measurement. No doubt for environmental samples, and for food and feed materials in which dioxins and dl-PCBs occur at higher concentrations (around the MRLs in fatty fish, fish oil), GC×GC is able to serve as an excellent routine method and alternative for HRMS. As soon as dioxin and dl-PCB concentrations drop to low levels, i.e. lower than the MRLs and action levels, a lot of time is needed to produce congener-specific data, to integrate the chromatograms, to adjust the baseline, to compare relative retention times, etc. However, even then GC×GC may still serve as a screening method. GC×GC-ToF-MS is an improvement compared to GC×GC-ECD, but obviously the instrument is considerably more expensive. The fourth method that was studied, an Ah-PCR analysis, is still under development. It is not excluded that that technique may finally also result in a suitable alternative screening method.