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Methods for the analysis of dioxins and dl-pcbs

a) Method for the analysis of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like Chlorobiphenyls using GC-HRMS.

The detection of polychlorinated dibenzo-p-dioxins (PCDD's), dibenzofurans (PCDF's) and dioxin-like chlorobiphenyls (non-ortho and mono-ortho substituted CB's) in biological and environmental samples has been subject of intensive research. Due to the toxicological behaviour of the total 210-dioxin congeners only the 2,3,7,8-chlorine substituted compounds are of interest. In biological species these toxic compounds are accumulated in fat so the applied methods are mainly focused on the analysis of these toxic compounds. The concentration in biological samples is very low, in general in the low pg/g range expressed as toxic equivalents to 2,3,7,8-TCDD (TEQ) and in environmental samples somewhat higher. Therefore highly sensitive and specific methods are required combining, after extraction, several clean-up procedures. Prior to extraction ¹³C labelled dioxins (internal standards) are added to the samples for identification and quantification purposes. The first step when analysing biological samples is a quantitative extraction of the fat. At RIKILT-DLO separation between dioxins and fat is carried out using acidified silicagel. After an additional clean up with activated AL₂O₃, separation between planar compounds (dioxins) and non-planar compounds e.g. chlorobiphenyls is carried out with carbon. The final extract is concentrated and analysed with gas-chromatography-high-resolution-mass spectrometry (HRGC-MS), which is present, the best-suited technique combining sufficient sensitivity and specificity. The mass spectrometric method to determine the tetra through octa dioxins is based on United States Environmental Protection Agency protocols. These protocols describe the basis tuning and calibration of the hardware as well as criteria for identification and quantification with isotope dilutions and procedures for quality assurance and quality control. Included in our analysis is a standard QA programme e.g. determination of recovery of internal standards, accuracy of spiked samples and blanks. To express the toxic potency of the mixture of dioxins, the toxic equivalency factor (TEF) approach was used. A TEF value was assigned to the dioxins, which represents their relative toxic potency towards 2,3,7,8-TCDD, the most toxic dioxin congener which TEF value is 1.0. By multiplying the TEF value of each congener with the concentration of that congener in pg/kg product, the toxic value of that congener was calculated (pg iTEQ/ kg product). Summarising the TEQ's of all congeners gives the total TEQ value in each sample.

b) Alternative methods for dioxin analysis

Regarding the high costs and low sample throughput of the GC/MS analysis, several alternative methods have been developed. Immunoassays have been developed for this purpose but at present their limited sensitivity does not allow their use for food samples. More promising is the use of bioassays, like the DRE-CALUX assay, which are based on the detection of dioxins by the

effects that underlie their toxicity. As a result, the tests obey the TEQ-principle. Since other compounds capable of binding to the Ah-receptor would also show a response in the test, a clean-up procedure (e.g. acid silica) is required to increase the specificity. Positive samples require confirmation by the GC/MS reference method. The method has been extensively validated for milk fat and citrus pulp and to a limited extent for other animal fats, egg fat, fish oil and animal feed. International validation is required. The test has been used in the Netherlands during both the citrus pulp and Belgian dioxin incidents.

The CALUX-bioassay is based on the biological response of cells exposed to dioxins, being an increased production of several proteins after binding of the dioxin to the cytosolic Ah-receptor and the subsequent transport of this complex to the nucleus and binding to a so-called dioxin responsive element (DRE). The hepatoma cells used in the test have been modified by the introduction of a DNA construct containing DNA encoding for the firefly enzyme luciferase under control of a mouse DRE. As a result, the cells will produce luciferase in response to dioxins, which can be measured by light production in an enzymatic assay.

The response shows a sigmoid-like dose-response curve and in principle this allows a quantitative determination of dioxins in a sample extract. In practice results should be corrected for results obtained with blank samples (correction for impurities introduced by used chemicals) and for the extraction recovery for which a positive control sample with levels around the action limit (e.g. Maximum Residue Limit) is included in each series of samples. Since in practice all samples above the action limit will have to be confirmed by the reference method, RIKILT uses the test primarily as a screening assay by comparing results obtained with the unknown samples with that of a reference sample with a dioxin content at a residue or action limit. Any sample showing a lower response is declared negative, any sample with a higher response is declared suspected.

c) Method for the analysis of polychlorobiphenyls using LC-LVI-GC-LRMS.

The method is developed for the automatic determination of the seven indicator Chloro Biphenyls (28, 52, 101, 118, 138, 153, 180) in a wide variety of fat containing commodities. The system consists of an automatic clean-up system based on HPLC, which is coupled to a GC-MS system via a Large Volume Injector. Fat/oil is dissolved in hexane and an aliquot is injected on a silica column which is eluted with hexane to obtain separation between fat/oil and polychlorobiphenyls (PCB's). The fraction, which contains the PCB's, is transferred to the GC-LRMS using the LVI. In the LVI the solvent is evaporated and blown off while the compounds of interest are trapped. Heating up the oven of the GC starts thereafter chromatography. The method is very recently tested for the indicator CB's in a collaborative study organised by ISPRA

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