Bioanalysis

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Bioanalytical methods have been shown to offer a good alternative for the determination of dioxins and PCBs (reviewed by Behnisch and Hoogenboom). This is primarily due to the relatively low costs and the possibility to screen large numbers of samples in a relatively short period of time. In addition these assays, and in particular the cell-based assays, may contribute to the identification of novel contaminants with a similar action as the target compounds, since they are effect-based rather than looking for known structures. The combination of bioanalytical and chemical-analytical methods offer a good team for tackling any crisis and for identifying novel risks. However, novel approaches and techniques are required for the identification of such compounds. More in general, more data are required in support of the use of these assays for official control of samples.

Novel assays and clean-up procedures

One more or less novel bioassay was presented by Xu et al., based on the quantification by PCR of a specific piece of DNA following binding to a dioxin-Ah-receptor complex. This test was shown to be very sensitive, and might be easily introduced into laboratories that are experienced with PCR technologies. Further data on its use on actual samples are required to demonstrate the robustness of this assay.

Chahbane et al. addressed the issue of metabolic activation, which is common in genotoxicity assays, but much less in Ah-receptor and estrogenicity assays. Wild-type H4IIE-cells were used to activate methoxychlor prior to incubation with the YES estrogenicity assay. This resulted in an increased response. The metabolic activity of this cell-line does play a crucial role in the specificity of the DR CALUX assay for dioxins, since certain other agonists are metabolised by these cells. Furthermore, the luciferase expressed by the first-generation assays, is unstable and as a result the initially increased production of this enzyme will no longer be visible at longer incubation times. This was once more demonstrated by Masunaga et al., who presented data on the time-related (6-72 hr) effect of different PAHs in the DR CALUX assay, using the H4IIE-cells. Relative potency factors, initially being in a range of 0.003-0.2, showed a 100 to 1000 fold decrease after longer exposure times, which can be explained by the degradation of these compounds by the cells. This stresses the need to use longer exposure times for ruling out the contribution of unstable Ah-receptor agonists in these assays. At the same time it demonstrates one of the major advantages of these cell-based bioassays over assays without metabolic degradation, unless the specificity of clean-up procedures is good enough to exclude such compounds from the extracts.

Novel clean-up procedures may help to further decrease the time required for analysis, and may also improve the reproducibility of the clean-up and as such the performance characteristics of the assays. Two Japanese groups presented automated sample preparation systems in combination with immunoassays (Kishino et al., Fujita et al.). Both groups showed that these procedures are very suitable for clean-up of flue gas and fly ash samples. Another Japanese group presented an on-site clean-up procedure, in combination with an antibody-based strip test, for the detection of PCBs in soil samples (Okuyama et al.). Nording et al. presented further data on the use of a specially designed ASE-cell for clean-up of food and feed prior to the use of a cell based bioassay expressing green fluorescent protein instead of luciferase. Introduction of a carbon column in the ASE cartridge allows the on-line separation of dioxins and PCBs, which may be of interest under certain conditions (e.g. separate legislation dioxins and dioxin-like PCBs).

Novel applications

Takigami et al. demonstrated that the DR CALUX assay is very suitable for controlling the degradation of PCBs by a sodium dispersion process. Zoric et al. used the EROD assay with primary rat hepatocytes to examine the TEQ levels in sediment samples from two different rivers contaminated with PCBs. Different types of extracts were prepared, showing clear differences in the response obtained in the assay. In addition, very different correlations were obtained between the GC-ECD analysis and the bioassay for the two different rivers, indicating the presence of high levels of unidentified agonists in one of the rivers.

Tsutsumi et al. used an immunoassay for PCB 118 in order to determine the levels in fish products. A very good correlation was obtained between the levels of this congener as determined by ELISA and HRGC/HRMS. The correlation with total TEQ levels was less good and requires a relatively low action limit that may result in too high levels of false-positive results. This should be compared with data from other rapid screening assays.

Identification of novel agonists

A second important development is the application of bioassays for detection of novel agonists. One study, presented by Suzuki et al. investigated the novel agonists in environmental samples, by using a fractionation method on HPLC. Another Japanese group presented data on nonylphenol congeners in water samples from the sea, rivers and lakes in the Tokyo area (Kim et al.). Both HPLC and gas chromatography combined with a preparative fraction collector (GC-PFC) were used to prepare fractions that were tested with the YES estrogen-assay and analysed by GC/MS. Responses were compared to levels and activities of a number of nonylphenol congeners and showed that only part of the activity could be explained by these compounds. Houtman et al. used a similar approach to investigate the nature of the compounds responsible for the estrogenic and dioxin-like activities in sediments from a harbour in the Netherlands. Following HPLC fractionation, GCxGC combined with TOF-MS was used to point out 17ß-estradiol and estrone as the major estrogenic compounds, and polycyclic aromatic hydrocarbons as the compounds that are most likely responsible for the effects in the DR CALUX assay.

Validation studies

Thus far the most sensitive and therefore most promising screening assay for controlling the very low limits in food and feed, is currently the DR CALUX bioassay, based on the increased production of luciferase by rat or mouse hepatoma cells after exposure to dioxin-like compounds. However, only a small number of institutes and laboratories have thus far presented in house validation data and are actually applying the test in practice, among them the companies that provide the tests. Two Belgium groups compared the clean-up procedure and bioassay of two different suppliers (Goeyens et al.) and showed that both seem to perform well, but that there are also differences in sensitivity for both dioxins, as well as for possible matrix related disturbances of the test. During this conference several new sets of validation data were presented for food (van Overmeire et al., Besselink et al.), feed (Besselink et al.) and human plasma (Brouwer et al., van Wouwe et al.), further supporting the use of the test. Using novel clean-up procedures, LOQs were reported that are required to meet the very low EU-limits in food and feed, and can also detect the very low levels in small amounts of plasma. Additional, these results show that these kind of screening methods are able to fulfill the quality criterias set by the European Union for screening methods (EC 69/2002 and 70/2002). In addition, data from an international ring trial were presented by Gizzi et al., aiming at investigating the properties of the test. In this study a set of samples of both feed and fish oil were tested blind by a consortium of both experienced and newly trained participants. It was shown that the detection part of the test, i.e. the actual bioassay, could

easily be introduced into the laboratory. However, a very critical step turned out to be the following the instructions for the planned clean-up of the samples, which requires enough experience to reduce e. g. recovery losses and variation. Similar results may be expected for other screening methods that will also require purified extracts for testing, without using internal standards for recovery control.

Another study by Ota et al. focussed on fly ash and flue gas and showed that several different screening assays, like reporter gene assays, Ah-immunoassay, or the AhR PCR assay seems to be suitable for detection of dioxins in this type of samples. At the same time it was concluded that the immunoassays, directly developed against dioxins still have technical difficulties, showing both strong under- and overestimation of the expected levels.