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Dioxins & Robotics

Innovative rapid HTPS dioxin/PCB biodetection systems

author



Dr. Peter A. Behnisch, Commercial Director of BioDetection Systems (BDS), www.bds.nl

In the last few years we learned a lot more about the complexity of dioxin and dioxin-like PCB traces in the food/feed life cycle. It seems as if we have been moving quickly from one Dioxin/PCB pollution situation in the feed/food chain to the next. Since more than a decade we have yearly dioxin/PCB crises in feed/food in Europe. The last one in 2011 in Germany did prove again that not enough testing is routinely done as well as the testing capacities from the existing laboratories are too low – leading to long turn-around-times for dairy producers.

It is certainly now the time to learn from the countries who have experienced dioxin crisis situations and to learn from their individual solutions. In the Netherlands already twenty years ago, experts noticed higher dioxin levels in milk and therefore a project was funded in 1989 to establish at the research team of Prof. Brouwer at the Wageningen University a cheap, easy, robust and sensitive screening system for dioxin-like compounds called Chemically Activated Luciferase Expression (DR CALUX). This screening method for Dioxins/dl-PCBs has now been established for routine analysis in most of countries who have experienced with such dioxin crisis such as e.g. Australia (2003), Belgium (1999), Chile (2008), Ireland (2009), Italy

Extraction

Clean-Up

Seeding/Dosing

Measurement



	Confirmative HRGC/HRMS	Cell-based Screening e.g. DR CALUX	Chemical screening, e.g. LC/MS/MS
Congener-specific	yes	no	yes
ISO 17025 accredited	yes	yes	required
False negative < 1%		yes	
False positive food < 5%		yes	
Sensitivity 1/5 th EC level	yes	yes	required
Continues PTs	yes	yes	required
Equipment costs (EURO)	ca. 450-500	ca.50-60	ca. 320-350
Costs per sample (EURO/sample)	ca. 320-400	ca. 80-120	ca. 220-300
Weekly sample capacity/pp and laboratory equipment	ca. 10-15	ca. 40-60	ca. 20-30
Typical market prices	320 - 1200	130 - 260	no data available
Acceptance in Quality Systems e.g. QSS, GMP+ and FEDIOL	yes	yes	no

Table 1: Key evaluation points for cell-based and chemical based screening methods compared to confirmative chemical HRGC/HRMS method for dioxins and dl-PCB-TEQ

(2009), Netherlands (1995), Taiwan (2002) or as well as for National Surveillance Programs (China 2008, Cyprus 2002, France 2008, Israel 2011, Japan 1998, Russia 2011, Slovakia 2005, Spain 2009).

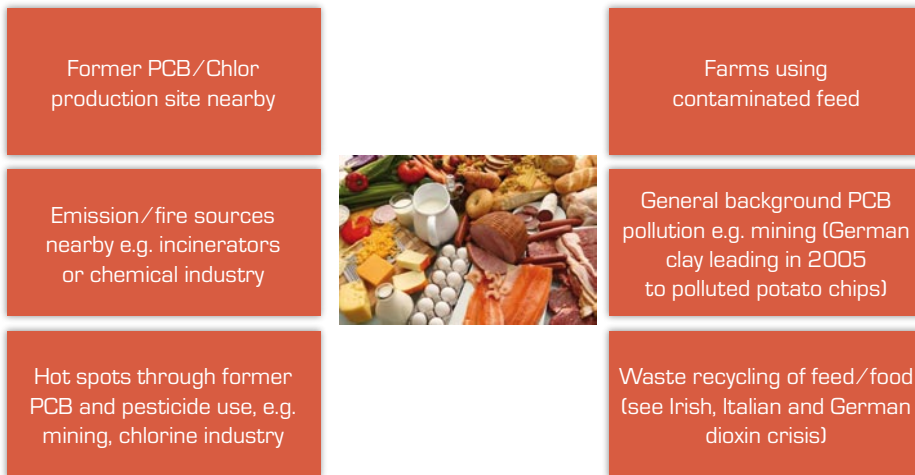
Can we crack the 100€ barrier for dioxin/PCB-TEQ testing?

Over the past decade, several official governmental laboratories as well as feed/food companies have moved from the traditional chemical method HRGC/HRMS to regulatory compliance, easier-to-learn, high-through-put capacity, faster, more appropriate for their local situation and reliable cell based biological methods such as DR CALUX. The investment for laboratory equipment costs are about 1/10th and the service costs around 1/3 of the chemi-

cal HRGC/HRMS analysis. This modern bioanalysis is as sensitive as the chemical analysis (using HRGC/HRMS; 1/5th of the regulated level from the current EC guidelines). Now more than 70 laboratories in more than 30 countries as well as more and more laboratories from the CRL/NRL network have experiences with this biotechnology (in their last proficiency test, CALUX and confirmative HRGC/HRMS had same success rate, 80%). These bioanalysis tools fulfil the European Union regulation already since 2002 (69 and 70/EC), and are recommended ('should be used') in the latest guidelines from 2006 (EC/1883 for food) and 2009 (EC/152 for feed). It is also possible to learn this method in less than ten days and to establish your laboratory in less than six weeks – the solution if you are in the crisis situation and need locally a quick analysis tool.

Innovations of bioanalysis tools

To optimise the effectiveness of the DR CALUX method now high-through-put robots and 96 well stackers (for the automated measurement) are in routine used to keep pace with the demands of



Risk sources for having Dioxins and PCBs in dairy products

the international feed/food chain. The new tools lead to the possibility to extract (see Graph 1), clean-up (see Graph 2), seed/dose (by robot, see Graph 3) and measure (by luminometer and stacker, see Graph 4) more than 30 samples/hour (per each step) for a separate dioxin- and dl-PCB-TEQ analysis.

Regulatory compliance

Several research and evaluation projects (e.g. EC Project DIFFERENCE; International training and proficiency testing by Gizzy et al. 2005; Japanese Millennium Project 1998-2002, USA project called SITE; German § 64 working group) intensively evaluated existing screening methods (kit based: ELISA, PCR; cell based: CALUX, EROD; chemical based: ECD, LC/MS/MS) for dioxins and dl-PCB-TEQ. Also the NRL/CRL network is organising since a couple of years international proficiency testing's (PTs). So far, only the CALUX method fulfilled all from the current valid EC guidelines established quality criteria's such as sensitivity, standard deviation of triplicate measurement (15%), precision (below 30%), false negative rate (below 1 – 5%), continues successful PTs and linearity of the TCDD curve (see also Sanco/10557 and 10376/2011). So far in the EC, only CALUX laboratories have mastered the ISO 17025 accreditation with the current strict guidelines of EC/1883/2006 (food) and EC/152/2009 (feed) for screening tests. According to these EC guidelines, samples are compliant in case the result of the dioxin and/or dioxin/dl-PCB-TEQ of the screening test is below 25% of the current valid EC regulated levels. In table 1 key evaluation points for cell-based and chemical based screening methods compared to confirm-

ative chemical HRGC/HRMS method for dioxins and dl-PCB-TEQ are listed.

Cell based screening tests for dioxin dl-PCB-TEQ offer – around 90% lower costs for starting laboratory equipment, 70% lower analysis costs and 50% higher weekly capacity – and the further possibility to speed up the process with future automatisations robots

High capacity = Fast testing

Customs made training and cell technology transfer that are capable to set-up quickly these dioxin/PCB screening tests have been available since more than 15 years. These systems require much less instrument maintenance and calibration efforts as the chemical HRGC/HRMS method, which dedicated such methods for routine analysis for all kinds of biological and chemical laboratories instead of the at the moment existing highly sophisticated HRGC/HRMS laboratories. Such bio-analysis tools are easily to speed up for handling more samples in crisis situations by setting up the laboratory in a 24 h/7 d rhythm with two labour shifts (such as BDS performed during the last dioxin crisis in feed/food in Germany).

German dioxin crisis 2011

Reported levels of dioxin and dioxin-like PCBs have been measured separately by DR CALUX for mostly pig meat, poultry meat, eggs and all kinds of feed materials with results in more than 99% compliant samples. During this time the measured confirmative analysis results with HRGC/HRMS proved to be compliant. In crisis situations for the matrices analysed, the DR CALUX has proven to

have a lower than 1% false negative and false positive rate.

Dioxins in dairy products

In the last few years in a few countries dioxins/PCBs have been tested in dairy and egg products in National Monitoring Projects or due to crisis situations. In case of National Monitoring Projects in most cases more than 99% of milk products are below the EU guided levels, while in case of feed additives and egg products more products are reported to exceed the EC limits (see also the EC alert system RASSF).

Handling of dioxin/PCB crisis

In general, several routine monitoring projects have shown that several food/feed matrices have frequently been higher than expected results for dioxin/dl-PCBs. Peak levels can arise due to local Dioxin/PCB crisis situations. The demand for feed/food inspections will continually rise, not at least due to the new EU legislation with the ultimate goal to reduce faster these Dioxins and PCBs limits significantly. More efforts will be promoted on country level as well as by the EU Commission to receive more data about the dioxins and dioxin-like PCBs of products with all member states and an increasing number of countries exporting to the European union – leading to more analysis and therefore also a higher frequency of non-compliant samples.

Safer food and feed

Advancements of luminometer-based measurement tools combined with cell based biological assays have allowed cell culture providers to develop new testing methods, in addition to the standard regulated testing. Now the technology provider offers also highly sensitive, reliable and easy-to-learn/adapt test systems for obesity (PPAR receptor), hormones (ER, AR receptor), painkillers (dexamethasone), pharmaceuticals, plastic additives (e.g. phtalates, bisphenol A), car exhaust gas (PAHs), waste recycling, eco-toxicological endpoints (such as genotoxicity and P53 receptor), UV-sun oils, and for healthy soils, wildlife reproduction, mother/baby health biomarkers, anabolic steroids and many more for wildlife and human health relevant issues such as the panel of CALUX screening tests.