FROM FOOD CRISIS TO ANALYTICAL STRATEGIES

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Introduction

It all started when more than a million of broiler chickens suddenly and unexpectedly died in the eastern and midwestern parts of the United States in late 1957. The first dioxin crisis record was set. After 10 years of investigation, the dioxin contamination was traced back to by-product fatty acids from tallow collected after hide stripping operations during which dioxin-contaminated pentachlorophenol was widely used as a hide preservative[1]. This motivated the development of the first analytical method to measure selected polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in animal fat samples[2]. Both Yusho and Yu-Cheng catastrophes confirmed the importance of controlling food processing to avoid dangerous episodes of human exposure via food consumption[3,4]. The 1999 Belgian dioxin chicken gate affair ultimately demonstrated the economic damage such a contamination episode could yield to and pushed the European Union (EU) to set an efficient and pro-active monitoring program to ensure proper quality of the European food and feed web and try to maintain most of the population below tolerable weekly intake[5,6].

Therefore, starting back in early 2000, the European Commission (EC) started to regulate dioxin and dioxin-like (DL-)PCB levels in foodstuffs and animal feed, based on maximum (and action) residue levels (MRLs) for PCDD/Fs and DL-PCBs[7]. Originally, because of the complex multi-step approach that is required to 1) extract the analytes from the matrix core, 2) separate undesirable interferences and, 3) finally isolate, separate and quantify analytes of interest, this used to result in long and expensive processing times that were not acceptable in the context of food-feed control where food safety agencies need high sample throughput and fast response strategies to efficiently deal with potential incidental dioxin exposure. A screening-confirmatory approach was thus adopted for the official control[8,9]. In practice, response-binding assays (RBA) like thee chemical activated luciferase gene expression (CALUX) assay are used to screen samples from which positives are confirmed by means of gas chromatography coupled to isotope dilution sector high resolution mass spectrometry, GC-IDHRMS.

Table 1 gives some of the main characteristics of both screening and confirmatory approaches in early 2000, at the time the EU strategy was implemented. Specific analytical criteria had to be developed for the CALUX (BEQ cut-off values,...) to properly be used in the framework of selected contaminant measurement[10]. These criteria became so tailor-made for this cell-based assay that the use of other biological assays like immunoassays is rarely observed. While GC coupled to low resolution (LR)MS is also officially usable for screening, very rare are the cases where it is used in practice as the quality criteria to fulfil are nearly the same than the ones for confirmatory GC-HRMS, except the fact of using a high resolution sector mass analyzer. Therefore, once GC-MS is considered, aiming for confirmatory rather than screening appears to be the right choice as little additional investment is required to acquire a HRMS analyzer capable to reproducibly measure at sub-ppt level and give access to congener-specific data. Replacing HRMS by roughly half-price LRMS would actually not reduce the analytical cost of more than a few %, particularly if large sample numbers are considered (Table 2).

The aim of this paper is to look at the state of play of this strategy and imagine how it could possibly evolve in the future[11].

Table 1 Evolution of the analytical situation regarding the screening-confirmatory approach for the measurement of PCDD/Fs and DL-PCBs in food and feed.

	Cost (EUR)	Delivery (Days)	High Throughput	Congener-specific	Biologicaly relevant
Early 2000					
GC-HRMS	900	5	No	Yes	No
RBA (CALUX)	400	5	Yes	No	No
Early 2010					
GC-HRMS	400	1-3	Yes	Yes	No
RBA (CALUX)	250	2-4	Yes	No	No
Early 2020					
GC-HRMS	300	1-2	Yes	Yes	No
RBA (CALUX)	125	1	Yes	No	Yes

Discussion

More than 10 years after the implementation of the screening-confirmatory approach, the analytical situation has drastically evolved (Table 1). Such an enhancement of the analytical quality has been possible because the EC regulation was built on a performance-based measurement system (PBMS), suitable for analytical improvements, rather than on an inflexible locked non-progressive procedures. The automation and hyphenation of parallel sample preparation techniques allowed both cost and result delivery time to be significantly reduced for the confirmatory GC-HRMS method[12]. For some selected matrices, it is common nowadays to have series of congener-specific data available in day+1 format (less than 30 hours) without even working with two labor shifts, which is significantly faster than performing screening.

This obviously questions the real interest of still performing screening rather than confirmatory, especially in the case of a suspected incident when high rates of MRL non-compliant samples are to be expected. As soon as such rate exceeds 35%, direct performing of confirmatory analyses is more cost effective and further reduces the response time. Additionally, the availability of congener-specific data rather than an estimation of a global level of contamination, is unvaluable to trace the source of contamination and take rapid action. Because of this and because the EU confirmatory capacity (n = 27 official or national reference laboratories, representing a capacity of approximately 1000 samples/week) is much larger than the screening capacity (n = 7 official or national reference laboratories, representing a capacity of approximately 300 samples/week), screening was not considered during a recent food incident[13].

Performing only confirmatory analyses for the official regular food-feed control could also be thought through as the difference of cost between biological screening and instrumental confirmatory method (especially when the cost of analyzes reported by tons of food-feed ranges between 0.01 and 0.1 Euro) is so small for similar delivery times and throughput. In such conditions, that would become to be against logic not to produce congener-specific data that are a crucial component of the action level strategy that is supposed to be a key pillar to stimulate a pro-active approach to reduce the presence of PCDD/Fs and DL-PCBs in food and feed.

Table 2 Distribution of the analytical cost (%) for both screening and confirmatory approaches for the measurement of PCDD/Fs and DL-PCBs in food and feed.

	Confirmatory by GC-HRMS	Screening by RBA
Extraction	10-15	10-15
Clean-up	15-20	20-25
GC	≤ 5	-
HRMS	10-15	-
Standards	≤ 5	≤ 5
Cell culture	-	10-15
Licence fee	-	15-20
Personnel	≈ 50	30-40

Perspectives

If the viability of biological screening can be questioned in the context of the implementation of congener specific TEQ regulatory values, one should perhaps think back to what such assays are capable to offer us in terms of global exposure information. Instead of spending time, money, and efforts on tailor development of dioxin-dedicated sample preparation procedures to ensure specificity and reduce false positive rates, RBAs could be used with a minimalist sample preparation procedure. That would allow most toxicants present in the sample to interact with the AhR to give a general toxicity information rather than an estimation limited to regulation compliance. This would be much more biologically relevant as the assay mimics the biological process that is thought to be a significant part of the mechanism of dioxin-like toxicity. In that context, if the RBA procedure would be simplified and further automatized, one could expect a 50% cut in prices (see Table 2 for price distribution). One could thus imagine that, in another 10 year period, the MRL control for PCDD/Fs and DL-PCBs would be performed exclusively by instrumental method, simultaneously to a large AhR-related screening for global toxicity assessment. This could be achieved at a price close to today's target analyses (Table 1). Such a dual approach would make the best use of each technology by applying them where they perform the best.

This comprehensive method is actually needed if we want to enlarge current food safety practices to other known and unknown persistent organic pollutants (POPs). Sample showing high response for the biological screening should be further analyzed in the hope of identifying new compounds. Efforts on the development of new instrumental analytical approaches that would allow to extend the list of target compounds to more 'exotic' (un)suspected persistent molecules present in our food should be made. Such a more proactive and exhaustive approach is probably needed to more appropriately ensure high level of food quality.

Major analytical challenges would then be at the level of instruments themselves. Both chromatographic resolution and instrumental limits of detection (iLODs) have to be improved. Using comprehensive two-dimensional GC (GCxGC)[14,15] or cryogenic zone compression (CZC)[16] GC coupled to HR time-of-flight MS (HRTOFMS) operating in full scan (FS) mode[17] could nicely complement classical GC-sector HRMS performing in selected ion monitoring (SIM) used for target analyses. This would open the possibility to screen for other compounds than the one under current regulation (organochlorine pesticides, halogenated flame retardants, GC-amenable perfluorinated compounds, …), without any more work nor additionally sacrifice of sensitivity.

Recent advances in coupling between GCxGC and HRTOFMS nicely put this approach one step further as it allows to produce elemental composition data for unknown compounds present in complex mixtures. Furthermore, when considering halogenated compounds, such a system can be granted by a tremendous improvement in sensitivity (back to low fg iLODs) by operating in negative chemical ionization (NCI) rather than electron impact (EI).

Conclusions

Although so called 'dioxin analyses' have been performed since mid-1970's, the need for controlling our exposure via food-feed monitoring is still up to date. Analytical procedures have quite evolved to make such measurements more rapid and straightforward. Improving sensitivity and enlarging the task to other more emerging compounds are probably the two most important challenges now. Several alternative approaches do exist and offer some interesting features. The analytical chemistry of POPs has still some work ahead…

Because of all efforts provided by the EU on both analytical and food-feed continuous control aspects, one often says that Europe has one of the highest levels of food safety in the world… The last few years only, several potential food incidents have been identified (e.g. citrus pulp pellets, recycled fats, mineral clays, choline chloride component, hydrochloric acid related to gelatin production, guar gum thickener, biodiesel-related fatty acids, …) and related potential human exposure has been avoided. So far, however, only a very limited set of analytes is included in those monitoring programs. What about all the other potential harmful molecules present in our food but that we do not look for? What about potential synergic or antagonistic effects of mixture of untargeted toxicants? The question is still open.

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