Challenges in analyzing polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in food and feed in the light of the considerable tolerable weekly intake reduction proposed by EFSA in 2018

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Abstract. In November 2018, EFSA published a major risk assessment on the presence of dioxins and dioxin-like polychlorinated biphenyls in feed and food, proposing updated tolerable weekly intake of 2 pg/kg b.w./week, which is a 7-fold decrease over the previous value. This will inevitably result in lowering maximum levels and action levels in food and feed. This paper reflects on the possible consequences of such changes in respect to analytical capabilities in general, and the effort required to ensure suitable performance of the presently available analytical methods. Considerations related to both instrumental and sample preparation aspects are presented, taking into account specific EU legislation in the area of analytical requirements and quality control of analytical methods for dioxins and dioxin-like polychlorinated biphenyls. From the current perspective, it is obvious that any linear decrease of maximum levels and action levels is not feasible and would inevitably require some degree of mitigation of quality control requirements, at least for some food matrices that already have low maximum levels. A sustainable response to tolerable weekly intake decrease will most likely be the combination of gradual decrease of regulatory limits where achievable, inevitable technical progress in analytical instrumentation and adjustment of sample preparation techniques.

1. Introduction

There are several reasons for considering quantitative analysis of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in food and feed as one of the most challenging tasks in food/feed analytical chemistry. The extreme toxicity of some congeners resulting in low-ppt maximum levels (MLs), the necessity to achieve reliable measurements of levels even lower than MLs for the purposes of exposure assessments, stringent legally binding analytical and technical requirements in respect to analytical methods and the financial burden related to establishing and maintaining both instrumental and human resources involved, are just some of the hurdles inherent to PCDDF/dl-PCB analysis.

Basic principles regarding sample preparation and instrumental analysis of PCDDF/dl-PCBs in food/feed are straightforward and have not changed much in the past 30 years. Relatively large size
(e.g. 5-20 g) of raw or previously concentrated sample is extracted in organic solvents, extract is purified chromatographically using several adsorbents in manual or automated systems, and further concentrated to low volumes (e.g. 15-25 µL) prior to injection. An array of internal standards (13C12 substituted congeners of different chlorination levels) are added at various stages of the sample preparation process to facilitate quantification (isotope dilution method), calculation of recovery rates and estimation of extraction and cleanup efficacy. A scientifically and legally recognized confirmatory technique is capillary gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS) (or tandem mass spectrometry [MS/MS] in the cases of compliance assessments as of 2014 in the EU [1]). In the case of HRMS, selectivity is achieved through GC separation of congeners and utilization of internal standards as RT identifiers, while confirmation is accomplished by high resolution (R ≥10000) multiple ion detection of at least two ions of isotopic cluster of the molecular ion, and verification of known isotopic ratios characteristic of chlorinated compounds. Calculated concentrations of individual congeners are corrected with respect to their toxicity by multiplication with an appropriate toxic equivalency factor (TEF [2]), and the sum of individual normalized concentrations is calculated to produce toxic equivalency (TEQ), used for determination of sample compliance.

MLs of PCDDFs and dl-PCBs expressed in ng WHO-PCDDF-TEQ/kg (ng WHO-PCDDF-PCB-TEQ/kg) are set by European legislation in food [3] and feed [4]. In 2013, action levels were introduced within the EU [5] as an early-warning tool for the purposes of preventing contamination outbreaks on a large scale, which has happened worldwide on several occasions [6]. Current MLs for PCDDF and dl-PCBs are based on tolerable weekly intake (TWI) of 14 pg TEQ/kg b.w./week, proposed by EFSA in 2001 [7]. Having in mind that existing MLs were (in absolute terms) among the lowest in the entire food/feed contaminants area, analytical methods employed for quantitative determination were developed and validated, predominantly at, and around MLs for any given matrix in most routine official or commercial laboratories analyzing samples for the purposes of official controls. Furthermore, the EU imposed stringent requirements regarding quality control of PCDDF/dl-PCB methods [1]. One of the basic requirements securing reliable quantitation at low levels is the provision that the limit of quantification (LoQ) for each congener must not be less than 1/5 of the respective ML. In the case of PCDDF-dl-PCBs analysis, LoQ is not only the method performance indicator and validation parameter: it actively contributes to the final analytical result, given the legal requirement for upper-bound approach for non-detected congeners in the expression of results [3,4]. Therefore, congener-specific LoQ determination based on legal requirements is of the utmost importance for any PCDDF/dl-PCB analytical method used for the purposes of official analysis. Its overestimation can lead to unrealistically high TEQ values and false non-compliances, while its underestimation can (in borderline cases) lead to the issuing of a false compliant result. However, the upper-bound approach is basically an additional consumer protection measure from the toxicological perspective, given the high toxicity of PCDDF/dl-PCBs. The whole system established in this way proved to be sustainable, and an increasing number of official laboratories took part in dioxin analysis, successfully employing analytical methods and demonstrating competence by participation in numerous proficiency tests.

However, in 2018, EFSA published its “first comprehensive risk assessment of dioxins and dioxin-like PCBs in food and feed, reducing the tolerable weekly intakes seven-fold (2 pg TEQ/kg b.w./week), based on new data and methods and indicating a health concern due to exceedance of the new TWI across the EU population” [8]. This will inevitably lead to lowering of MLs and action levels to a degree yet to be announced, consequently having a significant impact on current performances of analytical methods.

The aim of this paper is to reflect on the consequences of EFSA’s recommendations on the PCDDF/dl-PCB analytical methodology, from the instrumental, sample preparation and systemic aspects, and to propose possible strategies for arriving at a solution for the challenging task put before the dioxin analytical community and regulatory bodies Europe-wide.
It is the opinion of the authors that there are three possible areas where improvements could lead to lowering of MLs: 1. Improvements in current analytical methods with respect to sample preparation; 2. Future improvements in instrumentation design and capabilities, and; 3. Systematic measures e.g. participation in proficiency tests designed to target PCDDF/dl-PCB concentrations lower than current MLs, and joint efforts of official laboratories coordinated by the European Reference Laboratory (EURL) in further method refinements.

2. Improvements in sample preparation methods

The introduction section of this paper outlines the sample preparation steps in PCDDF/dl-PCB analysis. The most obvious way to lower the limit of detection (LoD) or LoQ of any method is to increase sample size and/or the degree of concentration of the final extract. The latter is not easy to accomplish, since final extract volumes are already very low (15-25 µL) and further reduction will seriously impair manipulation of the extract, thus leading to considerable errors. Although extensive quality control is incorporated in the methods (isotope dilution quantification, series of standards for extraction efficiency calculation and calculation of recovery rates) to provide a high degree of robustness, final volume misinterpretation due to technical difficulties in manipulation of the extract (e.g. precise evaporation, rinsing the vessel walls) will inevitably lead to significant analyte losses and, consequently, the inability to meet prescribed recovery rates.

The situation in the case of sample quantity increase is theoretically more promising. It would be the first and obvious choice of any analyst when there is a demand for higher sensitivity. However, there are several limitations in this approach.

Current methods designed for modified PCDDF/dl-PCBs analysis in food/feed already take into account the relatively high sample weights, and the equipment used in laboratories for extraction and cleanup steps is adequately sized for that purpose. In order to meet several-fold decrease in MLs, the increase in sample weight would be considerable, which would lead to replacement of extraction/cleanup systems with physically larger equipment, increase in consumption of solvents and adsorbents and higher costs of analysis. In the cases of automated sample preparation systems, producers of such equipment would have to develop different sizes or types of adsorbents capable of dealing with increased sample loads. All this would undoubtedly pose significant financial burden.

A better strategy would be to test, in a systematic and organized manner, the existing sample preparation capacities for accepting increased sample loads, especially manual glass chromatographic equipment (increased adsorbents quantity, alterations in elution, solvent selection etc.). If proved feasible, some degree of sensitivity increase would certainly be achieved, although not in the order of magnitude required.

On the other hand, increased sample size without adequately increased cleanup efficiency would introduce more interference in the extraction and could result in a reverse effect, raising the PCDDF/dl-PCBs levels in blanks and, consequently, increasing LoQ values. According to internationally recognized analytical methods [9], the actual limitations in dioxin analysis are related to the (in)ability to produce sufficiently low contaminated pseudo-blanks, rather than any sensitivity deficiency of HRMS. Instrumental LODs of modern sector instruments are in the concentration ranges of low pg/L.

One more approach is perhaps worth considering, regarding existing method performance. PCDDF/dl-PCBs analysis is, as already mentioned, heavily regulated in many respects. The legal requirement for the LoQ to be 1/5 (or less) of the respective ML is one of the key guidelines during method evaluation and validation. In some instances (i.e. for some matrices for which higher MLs have been set), due to inertia, laboratories might have only verified this requirement rather than tested it, leaving reported LoQ at or around the required limit. This would mean actual LoQs are realistically lower than are reported by those laboratories. If this is proved correct, revalidation of existing methods would yield some increase in method performances, although it would be unrealistic to expect significant lowering of the existing LoQs. Reasons for this are twofold: firstly, this “underestimation” of the method performance cannot be large, and hence, the contribution of re-evaluation would not be
3. Improvements in technical aspects of instrumentation

This is by far the best and obvious approach when there is a demand for increase in analytical capabilities. However, this process cannot be governed administratively, while the results of such changes cannot be held to any predicted or imposed timeline. Mass spectrometry has rapidly expanded, becoming an analytical technique of choice in the past decade, especially in the area of official analysis of food/feed. High resolution instruments having other than sector type analyzers, are already on the global market at competitive prices. Although they currently lack the sensitivity needed for PCDDF/dl-PCBs detection, it stands to reason that in the future they might compete with, or even replace the “gold standard” in dioxin analysis. Magnetic sector instrumentation is currently the dominant technique, but nevertheless, it suffers from several deficiencies in the practical, rather than the analytical domain. The price of these sector instruments is still among the highest in the field of routine mass spectrometry. Furthermore, their size, limited number of manufacturers, limited service support (compared to e.g. ubiquitous quadrupole instruments), steeper learning curve for the analyst and more complex/demanding skills needed to operate and maintain such instruments, are still impeding factors especially in high-throughput, routine, official or commercial laboratories.

Together with approval by the European Commission in 2014 of MS/MS instruments for official controls at the levels (MLs) of interest, it seemed that magnetic sector instrumentation, as providing the technique of choice for PCDDF/dl-PCBs analysis, would become a somewhat smaller niche within the reference laboratories and research centers. The language used in current Commission Regulation (EU) 2017/644 and its predecessor (589/2014) is cautious and balanced, limiting the use of MS/MS to compliance assessment only, while leaving exposure assessment (background contamination) to HRMS exclusively. Having in mind the magnitude of the TWI decrease proposed by EFSA in 2018, and the almost certain significant lowering of the MLs by the European Commission, the analytical community might be faced with the situation in which today’s background levels would be close to or even surpass tomorrow’s regulatory levels (one should not forget that action levels are regulatory as well). Other researchers [11] involved in exposure assessments in humans have demonstrated that new sector instruments are technically capable of reaching as low as atogram levels on columns, for analysis of human plasma and milk samples. However, this level is not achieved without difficulties and with questionable reproducibility, even in a laboratory far beyond any routine food/feed control facility.

Regulatory analytics is the field where predetermined stipulations have to be met, and it is obvious that a considerable amount of time and effort will be required in order to achieve several-fold lower levels on a routine basis. On the other hand, in the case of MS/MS analyzers, the question of the competency of current instruments with respect to the required sensitivity for enforcement purposes has to be raised. That question is not addressed solely to the analytical community; it is a legal question par excellence. What would be expected from the analytical community is to demonstrate (or not) the ability of such instruments to comply with the new requirements, without any mitigation in regulatory requirements. The competent authority i.e. the European Commission, would have to respond if the outcome of the testing proves to be non-satisfactory.

Undoubtedly, technical progress will, at some point, deem MS/MS instrumentation fit for use in PCDDF/dl-PCBs analysis at the new levels, as was the case with current requirements.

Another consideration is worth mentioning: for obvious reasons, the European Commission could not endorse or impose any particular producer and/or model of instruments. That is not an issue in the sector, since any commercial manufacturer offers a singular model, which is by default capable of reaching the required MLs (in general, magnetic sector instruments are traditionally used for regulatory purposes only in limited areas of analysis of halogenated persistent organic pollutants (POPs), sport doping control and certain petrochemical applications). However, in the much broader
realm of MS/MS, a considerably wider gamut of instruments are available in any manufacturer’s portfolio, and they are not all capable of performing PCDDF/dl-PCB analysis. In the light of potential considerable ML reductions, an even lower number of such instruments will qualify for this task. Naturally, manufacturers themselves advertise their products for certain applications. However, the plethora of available models might cause a degree of confusion or even misuse. Since having the regulators endorse the equipment is not an option, recommendations by the scientific community or reference laboratories would be advisable.

4. Systematic measures within the framework of the EU Reference laboratory

Probably the most important set of measures to be taken before the considerable decrease in MLs of PCDDF/dl-PCBs is brought into force is to demonstrate and ensure the feasibility of such actions in the long term. The role of the EURL for Halogenated POPs would certainly be crucial in that domain. A network of National Reference and official laboratories within the EU has been established, and coordination between laboratories by the EURL in identifying and conducting the necessary steps in adjusting to the much lower MLs is of great importance.

EURL is also the organizer of the proficiency testing schemes that provide the most accurate insight into individual laboratory competency. However, due to the nature of PCDDF/dl-PCBs analysis, i.e. very low MLs, most of the proficiency testing schemes are conducted with samples contaminated at or around the current MLs. Therefore, it would be necessary to re-assess the ability of laboratories to perform at lower levels through several cycles of proficiency testing where samples are contaminated at lower levels, in accordance with the TWI published by EFSA.

Another role of the EURL in this matter would be in the coordination of efforts to modify or adjust analytical methods in order to lower existing LoQs and to govern the testing of such modifications throughout the network. Having in mind that current MLs also vary considerably between matrices, it would be safe to assume that some “high” MLs (e.g. fish oils) could be reduced with no additional effort, since the existing lower-end working range would allow it without modifications of the actual method or re-validation. However, many of the matrices would have to be thoroughly revisited, which would require a considerable amount of time and funds. Hence, it will be essential that those efforts are efficiently coordinated by the EURL.

5. Conclusion

It is evident that the review on risk to human and animal health from dioxins and dioxin-like PCBs in feed and food, published by EFSA in November 2018, will result in considerable decrease of current MLs for these contaminants. This poses a challenge for the analytical community in conforming to the new, stricter requirements without sacrificing any of the criteria and requirements for quality control of the analytical methods. This adjustment process will not be rapid, and cannot be implemented without considerable obstacles. For that reason, it is important the adjustment is conducted systematically and supervised by the EURL at all stages. Some of the current MLs can be lowered immediately, but others will require time, scientific and technical effort and some funds to be accomplished. Having in mind that stringent analytical and regulatory requirements in dioxin analysis are the cornerstones of reliable analytical results at ultra-trace (low ppt) levels, a sustainable response to TWI decreases would most likely be the combination of gradual decrease of regulatory limits where achievable, inevitable technical progress in analytical instrumentation and adjustment of sample preparation techniques, all under the supervision and coordination of the EURL.

Acknowledgments

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