TOWARDS BIOLOGICAL OR PHYSICO-CHEMICAL SCREENING FOR DIOXINS ?

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Introduction

Since few years, significant advances have been observed in the field of dioxins analysis. Many reports have been published concerning evolution in, not only sample preparation but also in the way in which resulting extracts are analyzed. The combined use of new extraction [1] and clean-up [2,3] techniques as well as last developments in the area of mass spectrometry [4] made it possible. However, most of the analytical procedures still include labor intensive sample preparation steps as well as high-cost equipment such as high resolution mass spectrometers (HRMS) only available in some well specialized laboratories and the overall process is both time and resource consuming. For these reasons, it is currently not yet possible to enlarge the analyses to sufficient number of samples in acceptable cost allowing early detection of contamination problems [5]. In order to edge their way towards high sample throughput capabilities, analysis protocols have to be simplified. Efforts are then now more focused on development of alternative methods. In that field, the leading ideas are 1) fast, 2) screening and 3) low cost.

As potential tools for screening method, biological assays (based on antibodies or cells response) attracted lot of attention during last few years with the emergence of a battery of bio and immuno-assays presenting advantages and drawbacks between them [6]. The mean appeal of these assays is not only the low cost (generally 5 times lower than classical HRMS) but also the possibility of parallel processing of samples. However, since the assays can also be activated by other chemicals present in the mixture in often higher concentration than analytes of interest, the sample preparation steps are still required to reduce the risks of false positives and they become the bottleneck of the procedure. These steps can often require several days of tedious work including delicate solvents exchanges due to the need of performing the assays in aqueous-type media [7]. In addition, depending on the considered assay, cross-reactivities (based on 2,3,7,8-TCDD) can be significantly different from a given TEF scale. Knowing that final TEQ estimation for many matrices mainly rest on the relative contribution of few congeners, these disparities regarding to the TEF can introduce uncertainties on the estimation.

On the side of the development of these biological methods, advances in physico-chemical analysis tools have also reach an interesting level [8]. This is worthwhile to evaluate their capabilities in term of screening for dioxins on a selected congeners basis [9].

Discussion

From reviewing recent available literature concerning congeners distribution in food and human matrices, some tendencies can be outlined. It appears that for the hundreds of computed samples, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF are the major contributors to the WHO-TEQ (Table 1). These relative contributions are quite constant over matrices types.

Table 1 : Relative contributions (%) of selected penta congeners.

	Mean 1,2,3,7,8-PeCDD	Mean 2,3,4,7,8-PeCDF	Sum	SD	Range	References
Food						
Beef	26	45	71	3	[68-74]	10-12
Veal	39	35	74	n.a.	n.a.	10
Pork	39	36	75	11	[68-83]	10-12
Lamb	47	34	77	9	[71-84]	10,12
Horse	29	36	65	9	[56-73]	10-12
Chicken	21	46	67	2	[65-69]	10-12
eggs	22	43	65	n.a.	n.a.	11
cheese	23	50	73	n.a.	n.a.	11
Creme	62	16	78	n.a.	n.a.	12
Butter	19	59	78	1	[77-78]	11,12
Milk	25	43	68	10	[51-79]	11-18
Prawn	13	42	55	n.a.	n.a.	12
Trout	16	41	57	n.a.	n.a.	12
Salmon	21	41	62	n.a.	n.a.	19
Mackerel	13	30	43	1	[42-44]	11,15
Herring	28	46	74	n.a.	n.a.	15
Plaice	24	49	73	n.a.	n.a.	15
Rice	26	11	37	n.a.	n.a.	20
Badley	28	19	47	n.a.	n.a.	20
Bean	32	8	40	n.a.	n.a.	20
Spinach	36	22	58	6	[54-62]	21
Human						
Blood	29	26	55	10	[37-70]	11,22-37
Breast milk	32	31	63	11	[45-78]	13,23,24,31,32,38

n.a. : not applicable due to the limited sets of data available for congeners distribution.

The standard deviations are relatively low for all types of matrices, indicating that these contributions are representative of the general situation in good proportions. This observation has different consequences, 1) it is of prime necessity for a screening tool to be able to consider these two congeners as accurately as possible, 2) these two congeners can be use as "screening congeners" for first sorting out of samples before relevant HRMS analysis. Since biological tools sometimes have relative response factors (especially for these two congeners) which does not match with the WHO-TEF values [39] and that these assays only give a global response, another tool would be appropriate. Knowing that even for assays, relevant clean-up methodology is required, it is quite conceivable to use GC to separate the congeners of interest and MS sensitive detector for screening. Recent improvement in the quadrupole ion storage tandem mass spectrometers (QISTMS) sensitivity present them as valuable detector [40,41,42]. They are easy to operate, their cost is acceptable, they have low picogram detection limits in isotopic dilution mode and they permit recovery rates calculations without any standards compatibility problems. Using adequate parameters and after time compression of the GC run, analyses can be carried out very rapidly. Fig. 1 illustrates an example of fast run (cycle time of less than 10 min) obtained with a benchtop ion trap mass spectrometer coupled to a classical GC system (A:1,2,3,7,8-PeCDF, B:2,3,4,7,8-PeCDF, C:1,2,3,7,8-PeCDD).

In order to avoid false-negative production, a safe screening condition is to use the mean value for the sum of the two PeCDD/Fs as representative value for each type of matrix with a confidence of 20 %. Calculations from the obtained quantities would then allow the evaluation of the total WHO-TEQ and eventually the complementary analysis on HRMS. A crucial point here is that the samples which need further injection in HRMS are

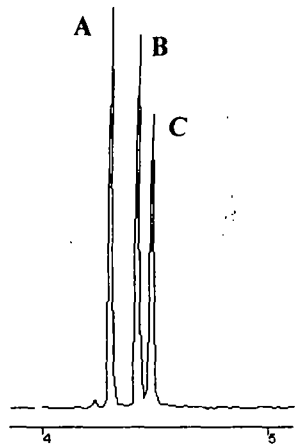

Fig. 1 : PeCDD/Fs (min)

already available and does not require any additional preparation before injection, greatly improving the overall speed and cost of the process. Considering the global control of the screening approach, as in the case of assays [43], certain amount (10%) of declared negative samples can systematically be confirmed by HRMS. This approach is currently under investigations to evaluate its robustness and it's already clear that such a strategy would not yields to the production of greater amounts of false negatives than other screening processes.

In addition to a fast GC-MS analysis time, automated clean-up allows the preparation of large number of samples in parallel in a short amount of time. The global process considering milk samples for example can be as fast as few hours for batches of 10 samples, also allowing isolation of the PCBs and persistent pesticides fractions.

This clean-up step is of course the key step on which efforts have to be made since most of the screening cost results of that. A promising alternative being for us the use of disposable solid phase extraction (SPE) pre-packed cartridges that can easily be combined to produce clean extracts [44]. The optimization as well as the transposition of this to automated systems using the new 96-well SPE technology for high sample throughput preparation would then also really be adapted to micro-plates bio-assays screening capabilities for the analysis of many samples in parallel.

Conclusions

Screening capability is one of the most wanted criteria for large number samples analysis in order to reduce the time spent to process samples containing negligible analyte levels. However, since the analysis of trace levels of dioxins require complex clean-up procedure, high sample throughput biological tools are currently not exploited at their optimum level. The production of such a number of samples has to include simple and/or automated processes which then produce extracts presenting levels of cleanness compatible with the GC. The approach suggested here rests on the screening out of negative samples, before expensive GC-HRMS analysis, using quantification of selected representative congeners isolated by automated clean-up and analyzed by FGC-QISTMS. This method is versatile, the "screening congeners" are still representative in different contamination types (TCDD can be added if necessary) and the correlation between their concentration and the TEQ is easier than in the case of marker PCBs analysis for dioxins levels evaluation.

This strategy can be seen as a cost effective "dioxin-dedicated" physico-chemical screening method complementary to a powerful biological tool capable of estimating the total toxicity of complex mixtures of large numbers of different halogenated aromatic hydrocarbons contained in samples.

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