MULTI-ANALYTE METHODOLOGY FOR POPs INCLUDING BROMINATED DIOXINS AND FURANS (PBDD/Fs), DIPHENYL ETHERS (PBDEs) AND BIPHENYLS (PBBs), ALONG WITH CHLORINATED DIOXINS AND FURANS (PCDD/Fs), BIPHENYLS (PCBs) AND CHLORINATED NAPHTHALENES (PCNs) IN FOOD

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Introduction

PCDD/Fs and PCBs

Methods for the analysis of polychlorinated dioxins and furans (PCDDs, and PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) based on modified silica and carbon clean-up are well established and comprehensively validated for the analysis of food, animal feed and other matrices. Standard methods as applied in many control laboratories achieve the analytical requirements of EU protocols (2002/69/EC and 2002/70/EC)[1,2] that are used to determine the compliance of food and animal feed with maximum permissible levels of chlorinated dioxins and biphenyls in these commodities.

PBDEs (inc deca-BDE):

Methods for other POPs fall into a variety of categories in terms of the amount of attention that they have received by analysts, toxicologists and regulators over the years, and also according to the complexity of analysis. Over the last decade, interest in brominated flame retardants (BFRs) such as the polybrominated diphenyl ethers (PBDEs), has grown year on year, partly because of the environmental impact associated with their use (still current or recent), and also because analysis is less complex than for some POPs such as PCDD/Fs.

PBBs:

Another class of brominated compounds, the polybrominated biphenyls (PBBs) were used up until the 1970s, until the Michigan disaster of 1973. Information available on the congener specific analyses of these compounds is meagre. Only recently have results of congener specific analysis of PBBs in marine organisms been reported,[3] which has lead to some extent to the re-emergence of interest in exposure to these compounds.

PBDD/Fs and PXDD/Fs:

Continued use of brominated chemicals (such as the BFRs) may inevitably result in the formation of brominated dioxins and furans (PBDD/Fs). These compounds have been shown to exhibit dioxin-like toxicity at comparable or even greater toxicity compared with their chlorinated analogues, although the toxicity seems to be associated with compounds with a lower degree of halogenation (tri- to hexa- congeners). There is also a likelihood of the formation of mixed halogenated dioxins and furans (PXDD/Fs) which have comparable predicted toxicology. Only limited data exists for these compounds and only few laboratories have analytical capability.

PCNs (and larger chlorinated PAHs):

The polychlorinated naphthalenes (PCNs) were used before the PCBs for similar applications. Although manufacture ceased only around a decade before the PCBs, and it is known that they are persistent in the environment, they have been relatively little studied. It has been shown that other chlorinated PAHs are also present in the environment.

Analytical approach for mixtures:
Since chemicals in food and the environment are always present as complex mixtures, the way forward is to gather datasets that are as comprehensive as possible. A single analytical protocol involving a single extraction and clean-up resulting in fractions for multiple run GC-MS and GC-HRMS analysis covering all of the above classes of compounds has many advantages. It will result in considerable efficiency savings and demonstrates a considerable advance in analytical methodology and potential cost savings when compared to the traditional approach using separate methods for each class of contaminant. Such a method has been developed based on the standard analytical approach used for PCDD/Fs in many laboratories and this has been validated with data comparable to that for standard PCDD/F methodology.[4]

Methodology

The method summarised here (Figure 1) uses acid hydrolysis and activated carbon chromatography to purify and fractionate very similar contaminant molecules based on their chemical stability and molecular configuration. This approach provides, from a single analysis, WHO–TEQ data for dioxins and PCBs as well as individual concentrations for toxic PCDD/F congeners, >50 commonly occurring PCBs, individual tri- to hexa- brominated dioxins (PBDDs and PBDFs), polybrominated biphenyls (PBBs), polybrominated diphenylethers including deca-BDE (PBDEs), and polychlorinated naphthalenes (PCNs) and some larger chlorinated PAHs. A wide range of ^{13}Carbon-labelled surrogates (currently 17 PCDD/Fs, 17 PCBs, 7 PBDEs, 2 PBBs, 6 PBDD/Fs and 4 PCNs) allow accurate internal standardisation. Measurements are carried out using high resolution GC coupled mostly to high resolution mass spectrometry (HRMS). The methodology has been validated with the frequent use of reference materials, and successful participation in international inter-comparison exercises. The full range of different food types that have been analysed for these compounds using this methodology over several years, demonstrates the robustness of the approach. Typical congener profiles for various food matrices, and validation data for all compounds has been collated.

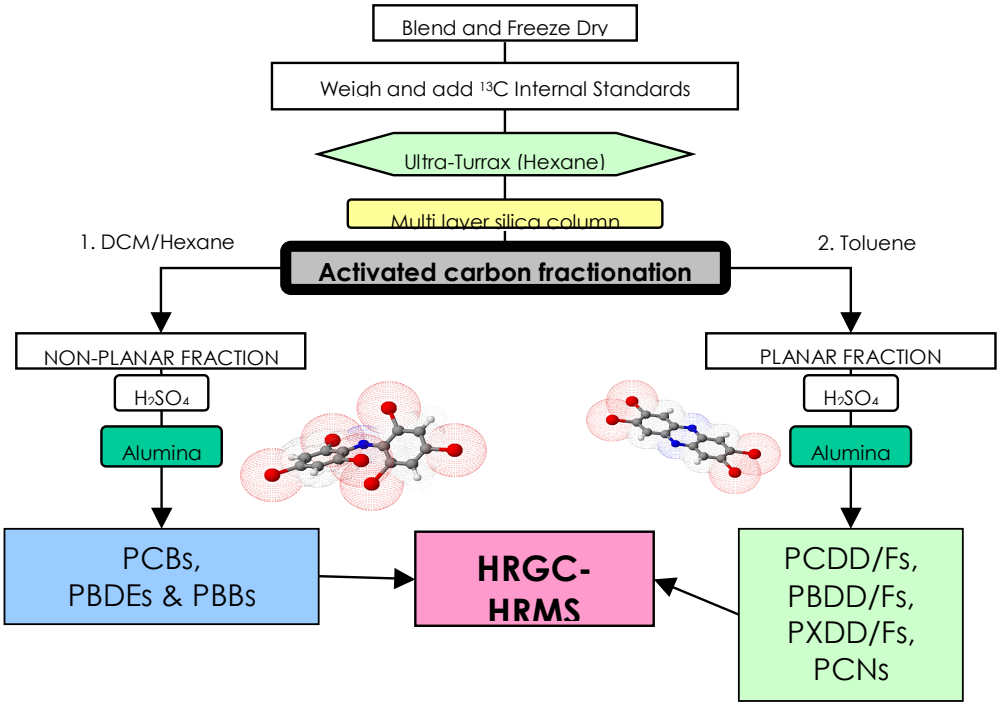

Figure 1. Schematic of analytical procedure for multi-class extraction and clean-up method for POPs

Results
All classes above have been successfully validated with results similar to those found for PCDD/Fs (see Table 1). An example of a chromatogram of an extract for PCNs in 4 different food types is shown in Figure 2.

Table 1: Summary of some method performance characteristics

	LOD (fat wt, $ng.kg^{-1}$)	Linearity (range, ng)	Precision (CV, %)	Recovery (%)	MU (%)[5]
PCDD/Fs	0.01	0.00001-5.0	8	60-110	25 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)
dl-PCBs	0.01	0.00001-5.0	5	60-100	25 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)
Non-dl-PCBs	10	0.0005-5.0	10	60-110	17 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)
PBDD/Fs	0.01-3.0	0.0001-0.1	22	50-75	25 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)
PBBs	10	0.005-50	14	50-100	17 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)
PBDEs (inc. deca)	10	0.0001-4.0	11	50-100	17 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)
PCNs (and other Cl-PAHs)	100	0.0005-1.0	10	40-90	25 (at 0.5 ng kg^{-1} fat) – 200 (at 0.02 ng kg^{-1} fat)

Not included
Other classes of emerging contaminants are not amenable to this combined analytical approach; typically this will be for compounds that are thermally labile during GC and therefore more amenable to an LCMS approach. The underlying principle however of using internal standardisation with ^{13}C labelled surrogates and specific MS measurement remains the same. Such compounds include other BFRs such as Tetrabromobisphenol A (TBBA) and hexabromocyclododecane (HBCD) diasterioisomers, and the organofluorine compounds (PFOS and related compounds). For these it is more normal to adopt a solid phase extraction clean-up including acid hydrolysis where appropriate followed by LC-MSMS analysis[6] and as such it will be difficult to encompass such compounds into a single combined methodological approrach for POPs.

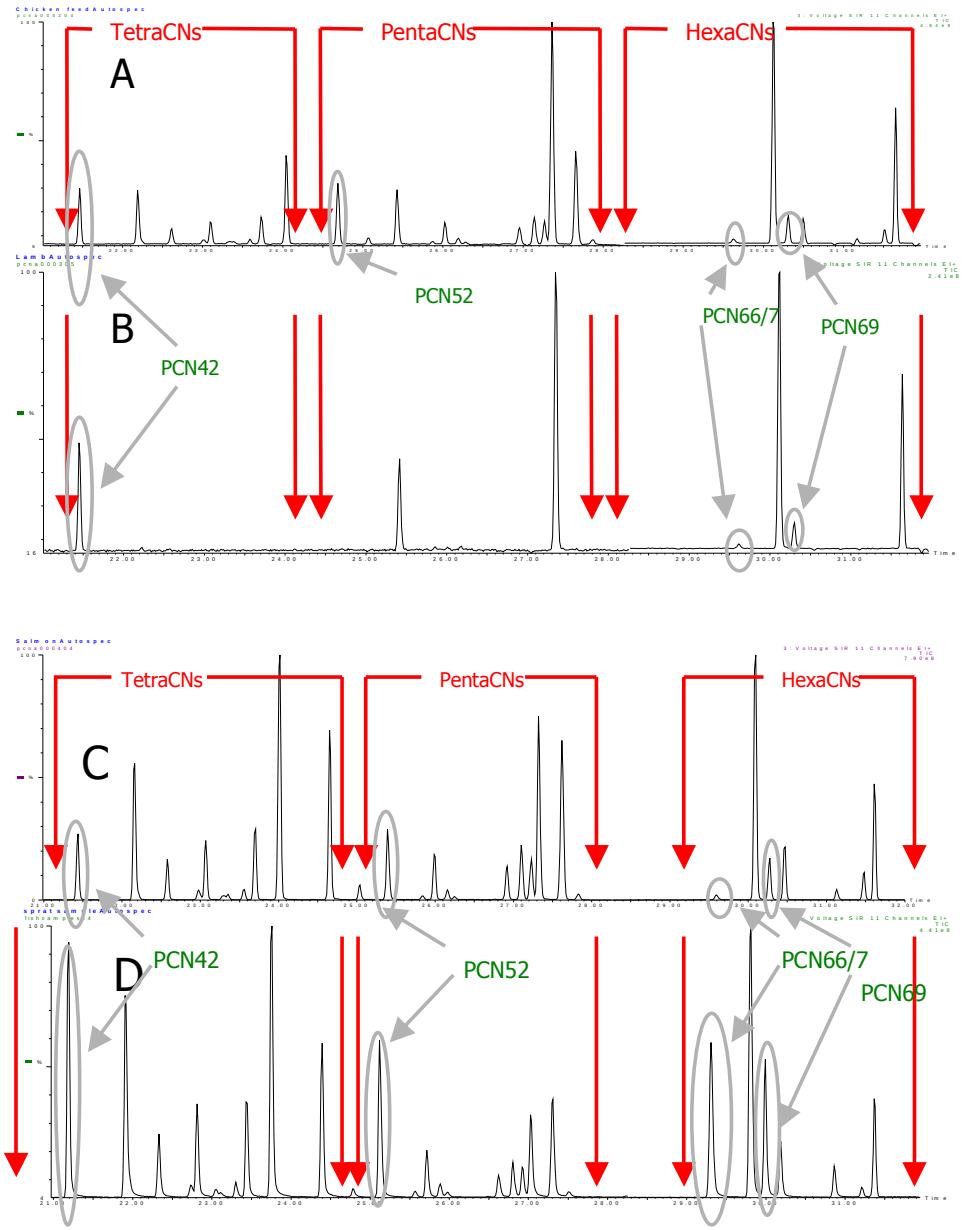

Figure 2: Example of chromatogram for PCNs in: A chicken feed; B lamb; C salmon; and D sprats

Conclusion

A single analytical protocol involving a single extraction and clean-up resulting in fractions for multiple run GC-MS and GC-HRMS analysis covering all of the above classes of compounds has many advantages. It will result in considerable efficiency savings and demonstrates a considerable advance in analytical methodology and potential cost savings when compared to the traditional approach using separate methods for each class of contaminant. Such a method has been developed based on the standard analytical approach used for PCDD/Fs in many laboratories and the method has been shown to produce validation data for other analyte classes comparable to that for standard PCDD/F methodology.

References

1. Commission Directive 2002/69/EC of 26/07/2002 establishing requirements for the methods of analysis of dioxins and dioxin-like PCBs in food stuffs, *Official Journal of the European Communities*, L 209, 2002, p5-14
2. Council Directive 2002/70/EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs. *Official Journal of the European Communities*, L 209/15, 2002, p15-21
3. Alwyn Fernandes, Pamela Dicks, David Mortimer, Martin Gem, Frankie Smith, Malcolm Driffield, Shaun White and Martin Rose. (2008) Brominated and Chlorinated Dioxins and Brominated Flame Retardants in Scottish Shellfish: Methodology, Occurrence and Human Dietary Exposure. *Molecular Nutrition and Food Research* 52 (2) 238-249.
4. Alwyn Fernandes, Shaun White, Kyle D'Silva and Martin Rose. (2004) Simultaneous determination of PCDDs, PCDFs, PCBs and PBDEs in food. *Talanta* 63 (5) 1147-1155
5. S. Ellison, M. Rosslein and A. Williams, Quantifying Uncertainty in Analytical Measurement. Eurachem/CITAC Guide, 2000.
6. Nick Harmer, Malcolm Driffield, Alwyn Fernandes, Emma Bradley and Martin Rose. (2008) The determination of brominated flame retardants in food by liquid chromatography with tandem mass spectrometric detection: diastereoisomer specific hexabromocyclododecane and tetrabromobisphenol A. In press, *Food Additives and Contaminants*