Identification strategies for flame retardants employing time-of-flight mass spectrometric detectors along with spectral and spectra-less databases

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Abstract

In the past, the preferred strategy for the identification of unknown compounds was to search in an appropriate mass spectral database for spectra obtained using either electron ionisation (GC-MS analyses) or collision-induced dissociation (LC-MS/MS analyses). Recently, an increase has been seen in the use of accurate mass instruments and spectra-less databases, based on monoisotopic accurate mass alone. In this article, we describe a systematic workflow for the screening and identification of new flame retardants (NFRs). This approach utilises LC-(Q)TOF-MS and spectra-less databases based only on monoisotopic accurate mass for the identification of "unknowns". An in-house database was built and the input parameters used in the data analysis process were optimised for FR chemicals, so that it can be easily transferred to other laboratories. The procedure was successfully applied to dust, foam and textiles from car interiors and indoor consumer products. The developed method was demonstrated for the main NFR present in Antiblaze V6 and for three unreported reaction by-products/impurities present in the same technical mixture.

Keywords: Non-target screening, flame retardants, high resolution mass spectrometry, time-of-flight, Identification of unknowns, screening for halogenated analytes

1. Introduction

In the last decades, an increasing number of additives were added to consumer products to enhance certain material properties, such as plasticity or resistance to fire. A particular class of additives are flame retardants (FRs), typically added to materials in order to inhibit or slow down the spreading of fire. FRs are present in a multitude of consumer products ranging from electronics to curtains, carpets and furniture. During the lifetime of the products, FRs may leach out into the indoor environment,[1,2] where they can be potentially harmful to human health[3]. Some FRs, such as polybrominated diphenyl ethers (PBDEs), are persistent, bioaccumulative and toxic, and have a potential for long-range transport[4]. As a result, all PBDE technical mixtures have been banned or restricted,[5,6] which has accelerated the appearance of new FR chemicals (NFRs) on the market. However, since such chemicals are considered proprietary technology, the producing companies are not obliged to make the information available to the public. It is difficult to assess exactly what the long-term environmental impact of the use of these NFRs might be. Because of the increasing number of NFRs and their potentially harmful (long-term) effects on the environment and on human health, there is a need for the development of wider scope screening techniques to detect and identify these new compounds. This is the first step in formulating a realistic risk assessment.

In the past, gas chromatography-low resolution mass spectrometry (GC-MS) with either an electron ionisation (EI) or electron capture negative ionisation (ECNI) source, in combination with mass spectral databases, e.g., Wiley/NIST, was the main technique used for non-targeted screening for contaminants in environmental samples.[7,8,9,10] However, this technique suffers from low sensitivity for some halogenated FRs when using the EI source or low selectivity with the ECNI source due to generation of unspecific low molecular mass fragments (e.g. *m/z* 79/81 for Br^-). Many FRs with high molecular mass FRs are also not GC-amenable. On the other hand, high resolution (HR) MS detectors can provide superior sensitivity and selectivity for (halogenated) FRs by promoting the formation of the molecular ion or higher molecular mass fragments, when the detectors are equipped with a softer ionisation source, such as atmospheric pressure chemical ionisation (APCI) or electrospray ionisation (ESI). The higher resolution translates to superior resolving power, which makes it possible to distinguish between analytes with the same nominal mass[11] and greatly reduces interferences, thus increasing selectivity. All these factors make HR MS detectors, such as HR time-of flight (TOF), a suitable tool for the identification of unknowns.

Recently, HR TOF detectors have been successfully employed, coupled to either GC or LC systems, in applications such as non-targeted screening for contaminants in food,[12,13,14,15] water,[15,16,17], herbal preparations,[18,19] polar bear plasma,[20] metabolite discovery,[21,22,23] as well as other applications.[15] TOF detectors are also fast enough to allow coupling to two-dimensional chromatographic techniques (e.g. GC×GC or LC×LC), which have the advantage of providing "cleaner" mass spectra by increasing the chromatographic resolving power and by separating co-eluting analytes. A number of studies successfully employing GC×GC-TOF-MS have already been published.[24,25,26,27,28]

However, up to now, LC in combination with TOF-MS for the screening / non-target analysis of (halogenated) FRs has been scarcely exploited. LC is applicable to compounds with a wider polarity range, including the less volatile, non GC-amenable and highly hydrophobic FRs. Although the main providers of mass spectrometers offer software tools for this purpose, non-targeted screening is still a complex procedure for which instrumental and data processing parameters need to be carefully optimised for obtaining successful results in a reasonable amount of time.

The main aim of this study was to provide a novel systematic workflow for non-target screening and identification of halogenated chemicals that are potentially used as new FRs in consumer products. The NFRs tend to be less volatile than the FRs they had replaced and so GC analysis is no longer feasible. For example, the Penta-BDE mixture, used in polyurethane foam, was replaced by FRs, such as the Antiblaze V6 (alternatively abbreviated as BCMP-BBCP[29]). This chemical was first detected in 2011 in foam and textile baby care products,[30] in samples produced as early as 2003. Other examples are PBDPP (or RDP), BPA-BDPP (or BDP) and TTBP-TAZ, which are also used as replacements for Deca-BDE in the plastic components of electronics.[31,32] For these NFRs, liquid chromatography (LC)-based techniques are recommended for analysis and (Q)TOF-MS is a suitable tool for the identification of unknown NFRs. In this study, an LC-(Q)TOF methodology was employed together with "spectra-less" databases to allow detection of high molecular weight and non-volatile compounds and so, to keep up with the trend of the industry of employing ever heavier FRs.

2. Materials and methods

Information about the reagents and materials used in this study can be found in the Supporting Information of this article.

2.1. Instrumentation

The instruments employed in the present study were: 1) a microTOF II MS (Bruker Daltonics, Bremen, Germany), with a mass accuracy <2 ppm and resolution >16500 FWHM, equipped with an atmospheric pressure chemical ionization (APCI) source and coupled to an Agilent 1290 LC; and 2) an Agilent 6530 Q-TOF MS (Agilent Technologies, Palo Alto, CA, USA), with a mass accuracy <1 ppm and resolution >20000 FWHM, equipped with an Agilent JetStream electrospray ionization (AJS ESI) source and coupled to an Agilent 1290 Infinity LC.

On the Agilent instrument, the gas temperature for the source was 300°C (negative mode) and 350°C (positive mode), gas flow 10 L/min (negative mode) and 3.2 L/min (positive mode), nebuliser pressure 45 psig (negative mode) and 25 psig (positive mode), sheath gas temperature was 250°C (negative mode) and 400°C (positive mode) and the sheath gas flow was 11 L/min (negative mode) and 10 L/min (positive mode). A volume of 5 µL of extract was injected and separation was achieved using a Phenomenex Kinetex XB-C18 column (150 mm × 2.1 mm i.d., 2.6 µm particle size) with a flow rate of 0.3 mL/min and a linear gradient from 5% methanol to 99% methanol in 30 min, followed by a 5 min hold before returning to the original conditions and hold for 10 min.

For the Bruker instrument, the detector parameters were: capillary voltage 1000 V, end plate offset -1000 V (negative mode) and +500 V (positive mode), corona current -10000 nA (negative mode) and +6000 nA (positive mode), dry gas flow 4 L/min, nebuliser 3 bar, dry heater 285 °C and vaporiser temperature 285 °C. The injection volume employed was 5 µL and the column was a Kinetex Core-shell C_{18} column (100 mm × 2.1 mm i.d., 2.6 µm particle size), with a similar mobile phase program as above.

2.2. Extraction

Various samples (Supporting Information 1) were extracted using a combination of ultrasound assisted extraction (UAE) and solvent vortexing. A mixture of *n*-hexane:acetone (3:1) was used for the extraction of dust samples.[33] A mixture of DCM:acetone (1:1) was employed for the car interior samples, such as foam and textile materials,[34] while DCM was used for the (hard/soft plastic) consumer products. The samples did not undergo any conventional clean-up to avoid selective removal of possible analytes of interest. The final extracts were filtered through 0.22 µm centrifugal filters, diluted by a factor of 100-1000 and injected.

3. Results and discussions

3.1. Ionisation source selection

For non-targeted screening, it is important to efficiently ionise as many analytes as possible. An ion source which can simultaneously ionise analytes through multiple ionisation mechanisms, such as a multimode source, is a possible solution. Unfortunately, this source has a lower sensitivity compared to separate ESI and APCI sources. In this study, we have used separate ESI and APCI sources, the latter employed to cover the more apolar FRs. To further extend the range of chemicals, we selected an Agilent Jet Stream ESI source over classical ESI. This source has an added sheath (heated) gas flow, which increases sensitivity by a factor of 5 to 10 times compared to a classical ESI, and a nozzle voltage (electric potential difference applied between the sheath gas nozzle elements, providing a charging electrical field that further focuses the electrospray plume), which makes it possible to ionise less polar analytes and to diminish ion suppression.

We acquired and optimised the sources parameters for some representative analytes of each group of FRs (brominated, chlorinated and phosphorous-containing compounds). The preferred ionisation mode for all PFRs was ESI(+) giving $[M+H]^+$ as major ion. It has been previously reported that the sensitivity for all PFRs is better in standard ESI(+), except for the least polar chemicals, such as RBDPP and BPA-BDPP[32], better analysed by APCI(+). To achieve good sensitivity for all analytes and avoid having to use two ionisation sources, we tested the Jet Stream ESI source that provided the desired sensitivity for both the classical PFRs and RBDPP and BPA-BDPP. In negative mode though, this source can ionise only analytes with a polar functional group, such as TBBP-A, TCBP-A, TBBP-S, TBBPA-BHEE (Supporting Information 2). So, brominated and chlorinated phenols and in general any halogenated chemical with a hydroxyl group which is not too shielded by other functions can be easily detected by ESI(-) using the Jet Stream source showing $[M-H]^-$ as main ion. HBCDD is an exception, as it ionises well even in the absence of such functions. It is noteworthy to mention that this source is also very well-suited for metabolites of heavy, non-polar halogenated FRs, such as HO-PBDEs, sulphated HO-PBDEs, HO-HBCDDs, etc.

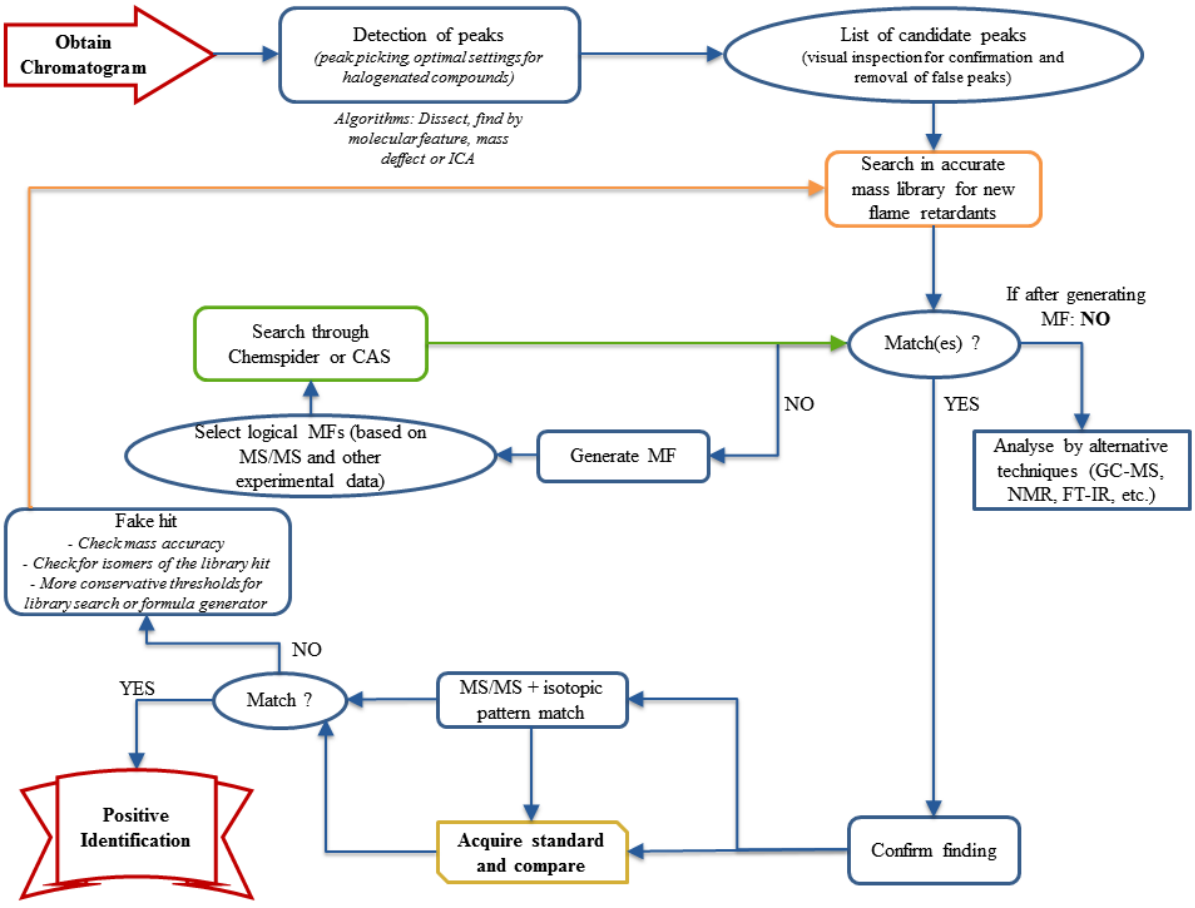
Nevertheless, since most current-use halogenated FRs are rather non-polar, the use of an APCI source in negative mode and thus stronger ionisation mechanisms due to the high corona voltage[35] is required. It is however more challenging to predict which ions will be generated. In general, $[M-H]^-$, $[M-Br+O]^-$ or $[M-Cl+O]^-$ are the major ions for most halogenated FRs in APCI(-). Heavy brominated FRs containing a triazine ring (e.g. TTBP-

TAZ) are an exception, since they can be detected in both APCI(-) and APCI(+), in the last case showing $[M+H]^+$ as major ion. To ensure that the vast majority of compounds were ionised, the APCI source parameters were similar to those used for the least polar analytes, such as the PBDEs.

3.2. Workflow of non-targeted screening

Once a chromatogram is obtained, it is ran through the screening procedure as described in Figure 1.

Figure 1: Structure elucidation flowchart. The round symbols indicate decision processes and processes in which the user is more extensively involved. Abbreviations: molecular formulae (MF)



The first stage in the identification process is peak detection (or "peak picking"), in which compounds are extracted using the "Dissect peaks" and "Molecular Features" tools (Bruker Data Analysis) or "Find by molecular feature" tool (Agilent MassHunter). The

software examines the chromatogram by using complex fuzzy-logic algorithms and separates co-eluting peaks without the need for user interaction or any prior information. The parameter input into the software has a big impact on what compounds are extracted. Adding adequate filters is extremely important in diminishing the amount of data through which the analyst needs to sieve.[36] Critical parameter values were optimised for the screening for FRs: *compound detection (peak height) filters*: higher values of this parameter (10-20) are recommended for compounds expected to be present at high levels in the sample. The lower this parameter, the more compounds are found by the software. A signal-to-noise value under 3 is not recommended. *Ion species/adducts*: it is recommended to monitor $[M+Na]^+$ and $[M+K]^+$ as well, as Na and K ions can originate from the LC system (e.g. from the solvent bottles) and any other adducts (e.g. with $NH4^+$) with cations or anions added to the mobile phases used previously. In APCI sources, the formation of complex ions (e.g. $[M-Br]^-$, $[M-Br+O]^-$ or $[M+O_2]^-$) has been described for brominated and chlorinated compounds[35] and therefore these ions were also included for formulae generation. If the employed software features different extraction algorithms, it is advisable to choose a general *small molecule algorithm* and avoid the ones designed for larger molecules such as peptides. Also, if available, it is best to avoid isotope models that favour the extraction of molecular features only for typical organic molecules containing C, H, N, O and S, such as peptides or glycans.

If there is a need to restrict the non-target screening to FRs that are known to be in use, the mass of the fragments extracted can be restricted to the range of 200 to 1400 Da. It is also important to select the appropriate parameters for the complexity of the matrix at hand. For example, for a complex matrix, if the data processing software allows to specify what the desired chromatographic resolving power or maximum number of overlapping compounds can be, it is best to select values closer to the high end of the numeric range for these parameters. Other vendor-specific critical instrumental parameters can be found in the Supporting Information 1.

The analyst must then manually review the results looking for the characteristic patterns such as those of chlorine or bromine clusters, with 3 or more atoms, as contained in most current-use halogenated FRs. Next, the fragments of interest can be searched in an in-house accurate mass library (if available). If the software indicates matches, the analyst must verify their validity, so the next step is to confirm the tentative identification by MS/MS experiments. A useful tool for this purpose is the Agilent Molecular Structure Correlator (MSC), which can run the unknown MS/MS spectrum against multiple candidate structures generated using a molecular formula generator (MFG) algorithm, which allows elemental composition

parameters to be defined. The correlation scores for each of the candidate structures are automatically calculated, based on mass accuracy and individual scores for each fragment ion signal, and the overall percentage of fragment ion intensity that can be plausibly explained with substructures. This is accomplished by attempting to explain each observed fragment ion into the proposed structure using a "systematic bond-breaking" approach[37]. There is also the possibility to retrieve all possible structures for the most likely formula from local compound or web-based databases (e.g. ChemSpider). The SmartFormula 3D tool included in the Bruker Data Analysis software package has similar functionality.

The final confirmation is to acquire the presumed chemical standard and to compare the retention times and two mass spectra.[38] However, in the event that they do not match, we are most likely dealing with a false positive hit. In this case, the analyst needs to explore the possibility that the unknown analyte is an isomer of the compound that generated a hit in the library. If this is not the case, then some of the search parameters need to be adjusted, such as setting more conservative thresholds for the library searches or formula generator tools, or to double-check the mass accuracy of the instrument for that particular analysis.

In the event that no match is found in the in-house accurate mass library, the elemental formulas can be generated using formula generator tools such as the Bruker Smart Formula or Agilent Generate Formula. A number of critical parameters were further optimised for this step to aid in the screening for FRs: *elemental composition expected*: this parameter was optimised for each class of FRs (Table 1); *adducts*: it is recommended to take into account all possible adducts, as detailed above; *ion electron state/configuration*: it is recommended to allow configurations with both odd and even electron states; *double bond equivalent*: in screening for the known FRs, this parameter varies in between -1 and 23. For non-targeted screening, a maximal value > 23 is recommended. However, this parameter is only a rough estimation of the degree of unsaturation in an organic molecule and it often produces erroneous values, especially for analytes containing multiple halogens, together with S, N and P[39]; *maximum MS mass error / Tolerance*: for a properly calibrated detector with a resolution greater than 15000 FWHM, a mass error as low as 5 ppm can be used.

The analyst must select the most likely molecular formulas based on all the available information. The candidate molecular formulas can then be searched through services like the Chemical Abstracts Service (CAS) Registry (>70 million substances) and ChemSpider (>28 million entries).

Table 1: Typical elemental composition per flame retardant class

Element	PFRs		CFRs		BFRs		Mixed Cl/Br FRs	
	Min	Max	Min	Max	Min	Max	Min	Max
C	6	39*	5	18*	4	25*	4	39*
H	9	51*	0	36*	0	50*	0	51*
N	0	0	0	0	0	3	0	0*
O	4**	8	0	8	0	6	0	0*
S	0	0	0	0	0	1	0	0*
P	1	2	0	0	0	0	0	0*
Cl	0	12	4	12*	0	0	1	6*
Br	0	9	0	0	3	14*	1	5*

For the CFRs, the values do not take the chlorinated paraffins into account
*Value suited for screening for FRs; higher values are recommended for true non-targeted screening
**The only exception: 9,10-Dihydro-9-oxa-10-phosphaphenanthrene 10-oxide (DOPO) only contains 2 oxygen atoms

These searches can be automatically performed with Bruker Compound Crawler in a large range of internet databases, including ChemSpider. Alternatively, accurate mass searches can be done directly on the ChemSpider website or even in the NIST Chemistry Webbook. If the generated formula does not produce any hits in the databases, then the analyst must use another technique, such as NMR or FT-IR. Such complementary analysis techniques can be valuable tools in narrowing down the number of candidate formulas to just one.[40]

3.3. Steps for automation

As the data processing is the most time-consuming and work-intensive part of non-targeted screening, any operation or resource that can save the analyst's time is invaluable. For instance, the Agilent Qualitative Analysis offers two analysis templates: "Identify Chromatogram Peaks", better suited for simpler matrices and "Find targets by molecular feature extraction (MFE) + Database Search + molecular formula generation (MFG)", which proves more useful with complex chromatograms containing multiple coeluting compounds. Similarly, the Bruker Automation Engine can be used for a wide array of operations by employing Visual Basic scripts.

3.4. In-house databases

A valuable knowledge base on the screening for FRs is the work of Bergman et al. (2012)[29], where many of the current-use FRs are listed and categorised. To complement this database, a

systematic search was performed for NFRs that might be used in the indoor environment and the results were added to an in-house prepared database (Supporting Information 2) to aid in the screening for new/rarely used FRs. Special emphasis was put on the list of restricted / controlled halogenated FRs of big corporations that manufacture electronics, as they are often the main source of FR contamination in an indoor environment.[41] This database is meant to complete the information provided in (Bergman et al., 2012)[29] and is meant to be used alongside it. The database searches were done on the basis of type of ions generated with our instruments. On the Agilent 6530 QTOF MS with the JetStream ESI source, all PFRs ionised mostly as $[M+H]^+$, with the optimised experimental parameters. PFRs behaved similarly on the APCI(+) source. Brominated and chlorinated FRs are better ionised by APCI, as described above.

3.5. Considerations for non-targeted screening: the "known-unknown" approach

A major issue in conducting non-target screening experiments is the efficient handling of the sheer amount of generated data. One solution would be to set stricter filters, but relevant data can be lost. Our solution was to set filters more "directed" to FRs. Many chemicals are used as FRs, ranging from inorganic chemicals (hydrated aluminium, magnesium oxides, aluminium diethylphosphinate, etc.), nitrogen FRs (melamine polyphosphate, melamine cyanurate, etc.) to PFRs (ammonium polyphosphate, organophosphate esters, etc.), CFRs (chlorinated organophosphate esters, etc.) and BFRs (PBDEs, TBBPA, HBCDs, etc.). Since halogenated FRs pose the greatest risk of being persistent, bioaccumulative and toxic (PBT) chemicals,[24] we focus our study on these chemicals which are most likely to be harmful to humans and to the environment. This has been done by selecting the compounds with at least 3 halogen atoms during the manual review of the results from the "peak picking" step. This process can be simplified by using software tweaks, which are discussed in the following subsection.

Another way of obtaining more directed information is to set targeted values when generating formulas for the number of atoms that an unknown may contain, according to the main FR classes (Table 1). For example, most PFRs typically contain between 4 and 8 O atoms, 1-2 P atoms, additionally up to 9 Br atoms and up to 12 Cl atoms.

3.6. Tweaks to facilitate the detection of halogenated FRs

3.6.1. Mass defect filtering – Agilent Qualitative Analysis

As most elements commonly encountered in organic molecules (C, H and N) have mass defect close to or slightly above zero, most organic molecules have a positive mass defect. However,

halogens have fairly large negative mass defects. Based on this property, halogen-containing molecules can be differentiated from other compounds in complex samples. The exceptions here would be molecules which contain other atoms with negative mass defects (O, S, etc.) in high numbers. The filtering by mass defect is part of the "Find by Molecular Feature" tool of the Agilent Qualitative Analysis software. Among halogenated FRs, one of the mass defects closest to zero is the one of TCEP, with a value of -0.0461 Da, while 1,2,4,5-tetrabromo-3,6-bis(2,3,4,5,6-pentabromophenoxy)-benzene has one of the largest negative mass defects (-1.1534).

3.6.2. Isotope cluster analysis – Bruker Data Analysis

This particular analysis allows searching for chemicals having the same number of a certain atom that shows a noticeable isotopic pattern, such as Cl or Br. There are two main parameters that need to be defined to obtain an Isotope Cluster Analysis Chromatogram: *1) m/z*: the mass difference between the two isotopes. Between ^{35}Cl and ^{37}Cl, there is a difference of 1.99705 Da, and between ^{79}Br and ^{81}Br, the difference is 1.99795 Da. Therefore, the value set was 2, with a tolerance of up to 0.1; 2) Intensity: this parameter is the ratio between the intensity of two fragments containing different halogen isotopes. It is advisable to select the two most abundant isotope peaks from the cluster (table SI-1).

One critical parameter is the tolerance of the intensity ratio, because this can be affected by other atoms with A+2 stable isotopes, such as ^{34}S (4.3%) and ^{18}O (0.2%). To check the impact of these atoms on the theoretical halogen isotope cluster ratios, we have calculated the values for clusters of 3 to 14 halogen atoms, which is the maximum we have encountered for any FR (table SI-1), and compared with the values for several FRs (table SI-2). The deviation from the theoretical halogen cluster was <5%, so this is a good value for the tolerance of this parameter for general screening of FRs. However, if instrumental variations are expected, we recommend a value of <15%.

3.7. Exemplification of the described workflow

We analysed dust, car interiors and consumer products to screen for new FRs and check the applicability of the proposed workflow to real samples. For more information about the samples, see the Supporting Information 1.

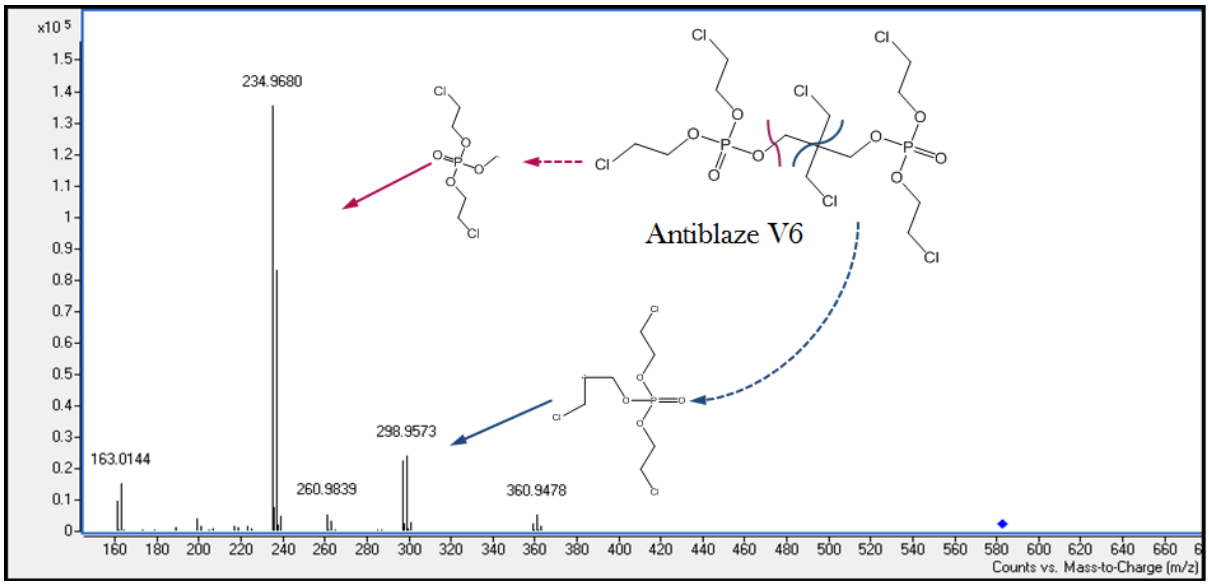
After the chromatograms and corresponding mass spectra were obtained, they were analysed through the procedure described in the workflow section. The analytes detected in multiple samples were logged and the mass spectra were manually reviewed for specific

molecular features. On the identification of new targets, we focused on analytes displaying pattern characteristics of more than 3 Cl or Br atoms.

TBBP-A, BDE-209 were detected in 67% of the Thailand dust samples and TCEP and BTBPE in 50% and, respectively, 17% of the samples. As for the US dust, TCPP and TDCPP were present in 80% of the samples, while TCEP and penta BDEs in 60% and 20%, respectively. One particular hexachlorinated compound was detected in 40% of the dust samples and in 63% of the car interior samples. A search in our accurate mass database yielded one possible match with a mass error of 3.95 ppm: 2,2-Bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate]. This chemical is used as FR under the trade names Amgard V6, Antiblaze AB100, Antiblaze V6 or Phosgard 2XC20. Its main uses are in polyether-type polymers, high-resilience and moulded foams, with main applications in automotive furnishings and in upholstery and foam used in furniture.

For confirmatory purposes, the first step was to perform MS/MS experiments and investigate if the obtained product ions match of V6 the possible fragmentation pathways. The two main fragments obtained at *m/z* 234.9680 and 298.9573 corresponded to expected fragmentation pathways (Fig 2).

Figure 2: MS/MS spectrum for V6 indicating the corresponding product ions.



The final confirmation step was done by acquiring the analytical reference standard and comparing the spectra from the sample and the standard, as well as the retention time. Since the retention time and two ion clusters matched in both accurate mass and isotopic pattern (Fig

SI-1), and also considering the positive outcome of our MS/MS confirmation experiment, we were confident about having positively identified the FR Antiblaze V6.

3.8. Identification of reaction by-products by mass defect filtering: case study for the technical V6 mixture

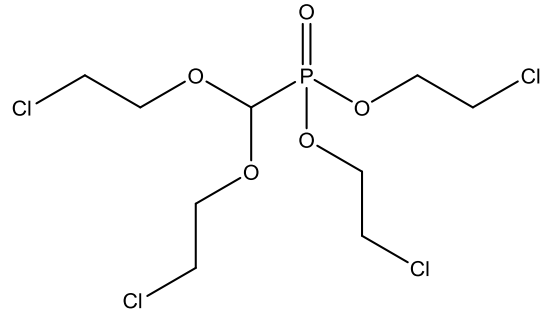
We used our approach to identify the by-products in the technical V6 mixture. We used the MFE step to filter the peaks according to the mass defect of the compounds, which are the reactants (in this case TCEP) and the reaction products (V6). A critical parameter in this process is the *mass defect tolerance*, which we recommend to keep at ±0.01 Da. As a result, the extracted compounds have a high degree of similarity to either TCEP or V6. Next formulas are generated with targeted element limits: 2-16 O, 1-12 Cl and 1-4 P atoms. The upper element limits were doubled as compared to V6, to ensure that any possible dimers are also detected. The upper threshold for the MFG was set to 80 (out of 100). A score of 90-100 represents a very good match, which can directly be searched in in-house and online databases, while matches with a score of 80-90 should be reviewed carefully. More details about how the Agilent Qualitative Analysis software calculates these scores are given in the Supporting Information 1.

Following the procedure described in fig. 1, with the amendments above, the compounds with a very good match score were searched in Chemspider and Google and three compounds were tentatively identified (fig.3). The most abundant was *Bis(2-chloroethyl) (bis(2-chloroethoxy)methyl)phosphonate*, with a mass error of -2.9 ppm and an overall match score of 98.65 (out of 100). Its abundance was close to the one of TCEP, which constitutes 10% of the technical V6 mixture[30]. A peak roughly half the size of the smaller TCEP isomer was tentatively identified as *1-chloropropan-2-yl (2,2-dichlorovinyl) ethyl phosphate*, with a mass error of -1.4 ppm and an overall match score of 98.72. And the third and least abundant compound identified is *Bis(2-chloroethyl) hydrogen phosphate* (BCEP).

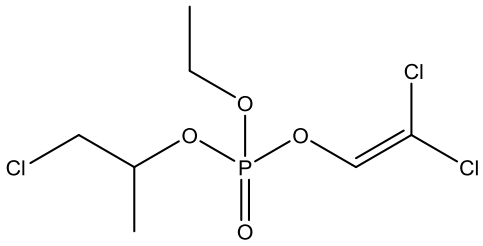
A number of other by-products/impurities for which formulas with very good match score were generated are available in table SI-3.

We find that this case study is of special environmental significance, because even if the new FRs, which come into use, are safer, some of the reaction by-products and impurities can be significantly more toxic than the main component of the mixture[42].

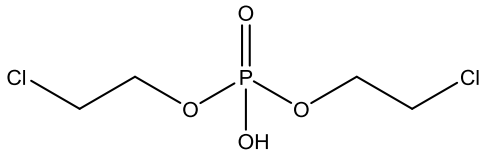
Figure 3: Reaction by-products and impurities tentatively identified from the Antiblaze V6 technical mixture.



Bis(2-chloroethyl) (bis(2-chloroethoxy)methyl)phosphonate



1-chloropropan-2-yl (2,2-dichlorovinyl) ethyl phosphate



Bis(2-chloroethyl) hydrogen phosphate

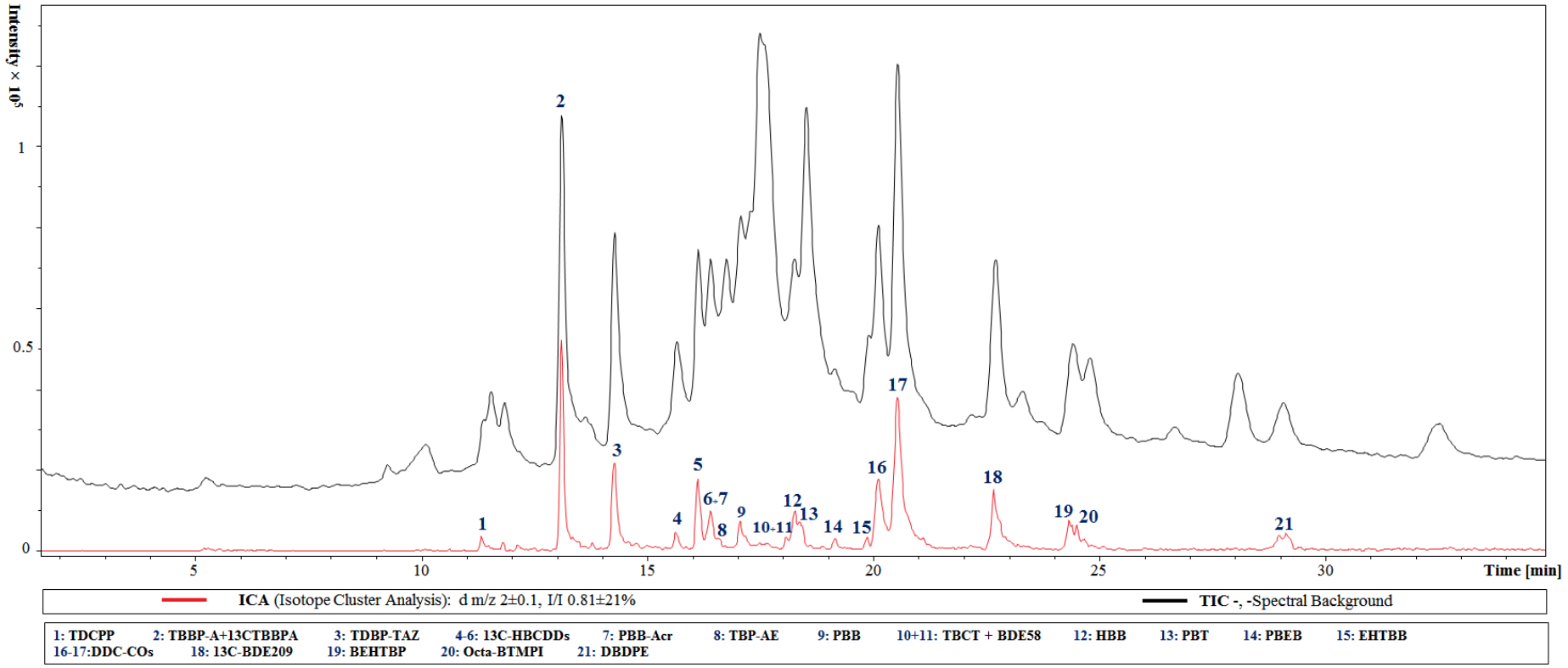
3.9. Identification of halogenated analytes by Isotope Cluster Analysis (ICA)

For the unspecific detection of chlorinated and brominated FRs in a very complex sample, ICA can be ran, with an intensity ratio of 0.81 and a tolerance of 21%. This puts the intensity with the range of 0.64 to 0.98, which encompasses all isotopic clusters of Cl and Br, from 3 to 14 atoms (Table SI-1). The result will be an ICA chromatogram containing just the peaks of analytes containing Cl and Br (Fig. 4), thus simplifying the screening/identification process.

4. Conclusions

A new systematic non-target screening procedure for the identification of halogenated FRs was developed, meant to facilitate the challenging process of unambiguously identifying a true unknown compound via its accurate mass MS and MS/MS spectra alone. This comprehensive study offers new strategies for the identification of FRs, as a set of optimal parameters and recommendations for the use of typical MS software in the non-target screening of new FRs and an in-house database. This method can be used to selectively screen FRs in environmental samples, as well as for other relevant applications such as identifying reaction by-products of

Figure 4: Isotope cluster analysis (ICA) chromatogram of a complex mixture of standards comprised of both halogenated and non-halogenated FRs.



halogenated chemicals or their metabolites, which are becoming the object of an increasing amount of studies in recent years.

5. Acknowledgements

ACI and AMBG acknowledge the funding of a PhD scholarship and a post-doctoral fellowship from the European Union Seventh Framework Program (*FP7/2007-2013*) under grant agreement no 264600 (INFLAME project). AMBG also acknowledges funding by the Netherlands Organization for Scientific Research (NWO) (VENI2014-722.014.003). Dr. Stefan Voorspoels and the Flemish Institute for Technological Research (VITO) are acknowledged for kindly providing the car interior samples. Dr. Dudsadee Muenhor and Prof. Dr. Stuart Harrad are acknowledged for the dust samples from Thai e-waste storage, while Dr. Robin Dodson and Ms. Ruthann Rudel are acknowledged for the house dust samples from California. Sicco Brandsma (IVM) is acknowledged for collecting the electronic product samples. Ms. Nele Van den Eede, Dr. Eszter Simon and Dr. Svetlana Malysheva are acknowledged for valuable discussions.

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