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Nontargeted Comprehensive Two-Dimensional Gas Chromatography/Time-of-Flight Mass Spectrometry Method and Software for Inventorying Persistent and Bioaccumulative Contaminants in Marine Environments

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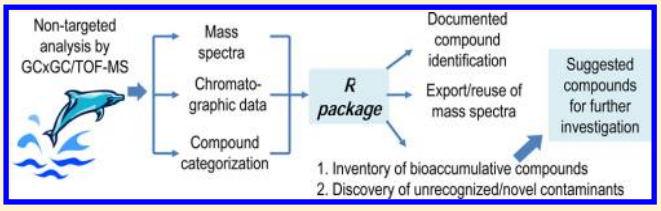
Supporting Information

ABSTRACT: Analytical methods for contaminant monitoring are generally targeted; i.e., they measure defined lists of compounds. Routine monitoring projects using targeted methods are not usually designed to screen for unrecognized or novel contaminants and therefore miss compounds within the region or population of study that cause, or have the potential to cause, adverse biological impacts. We describe a nontargeted analytical method utilizing direct sample introduction coupled to comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. To test the capabilities of this instrumental method within the context of marine contaminant surveys, we characterized a broad array of nonpolar, persistent, and bioaccumulative contaminants in Atlantic common dolphin (*Delphinus delphis*) blubber, including compounds that are not typically monitored. Compound identifications were made by searching a standard reference database, by contemporaneously analyzing mass spectra from reference standards, and by de novo interpretation. We identified a total of 271 compounds belonging to 24 classes; all compounds but 1 were halogenated. Anthropogenic contaminants and halogenated natural products were concurrently detected. A total of 86 compounds were anthropogenic contaminants that are not routinely targeted in environmental surveys, and 54 compounds were halogenated natural products. A total of 112 spectra were identified de novo, demonstrating that exclusive reliance on commercially available reference standards and mass spectral libraries may miss a significant fraction of identifiable compounds. We also cataloged 27 halogenated mass spectra that were not able to be identified. Due to the volume and complexity of the identification data, we developed custom software to organize and provide shared access to the identified mass spectra and related information. The nontargeted analytical method and data reporting system, in combination with the analysis of a high-trophic-level sentinel species, demonstrates a framework for creating an inventory of persistent and bioaccumulative contaminants in marine environments, with the future goal of suggesting new compounds for further investigation by targeted monitoring and risk assessment.



INTRODUCTION

Chemical contaminant monitoring programs are used to determine the magnitude and variability of contamination, determine the potential for adverse impacts, identify sources to the region or population of study, determine temporal trends, and/or assess the effectiveness of management actions. Contaminants are usually measured using targeted mass spectrometry or gas chromatography with electron capture detection (GC-ECD), and monitoring efforts are typically focused on legacy contaminants with a gradual introduction of new compounds once it has been shown by smaller scale studies that they are pervasive or otherwise of concern. However, this approach may exclude relevant contaminants.[1]

Targeted methods based on mass spectrometry use either single-quadrupole selected ion monitoring (SIM) or triple-quadrupole multiple-reaction monitoring (MRM) to quantify a defined list of at most a few hundred compounds within one

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method and may exclude contaminants with the potential to cause adverse biological impacts.[2,3] Unrecognized contaminants are those that have been previously identified, but are excluded from targeted monitoring due to economic or analytical limitations and therefore remain undetected. Unknown contaminants are those within the exposure universe that have not been previously identified or hypothesized to exist and have not been targeted for analysis and therefore remain undetected. The polybrominated diphenyl ether (PBDE)[4,5] and dechlorane plus[6,7] halogenated flame retardants are examples of previously unknown contaminants. They accumulated at detectable concentrations in the environment for decades before discovery that they were widespread in the environment. Ideally, contaminant monitoring programs should be capable of determining the occurrence of known emerging and legacy contaminants from the large number of possibilities and discovering novel contaminants. Nontargeted mass spectrometry addresses these goals to broaden the scope of analysis.

Mass spectrometry methods for the nontargeted analysis of nonpolar compounds include comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/TOF-MS) and quadrupole GC/MS operated in scan mode. The instruments ideally acquire a mass spectrum of every detectable compound eluting from the chromatography system. However, the analytical method only detects compounds within a range of physical-chemical properties and above the method detection limits, both of which are dependent on the specific extraction and instrumental method used.[8] Furthermore, the complexity of the sample limits the ability of the chromatography system to isolate all detectable compounds. In these instruments, the analytes are fragmented prior to entry into the mass analyzer, and spectral deconvolution software can isolate individual spectra from coeluting compounds.[9] GC×GC/TOF-MS, in contrast to full-scan quadrupole GC/MS, can utilize faster spectral acquisition rates and increased chromatographic resolution to increase the sensitivity of the analysis and "purity" of the mass spectra.[10,11] GC×GC/TOF-MS has previously been used to perform nontargeted analysis of specific classes of chemical contaminants, for example, halogenated methylbipyrroles in marine mammals[12] and nonylphenols in technical mixtures.[13] It has also been used to identify multiple classes of contaminants in fish oil[14] and sediment.[15] This discussion excludes instrumentation such as liquid chromatography−quadrupole time-of-flight (LC−Q/TOF) mass spectrometry for the nontargeted analysis of polar compounds and GC/MS SIM screening methods for halogenated compounds within certain chemical classes.[16,17]

Previously, we demonstrated that the nontargeted analysis of fish oil by direct sample introduction (DSI)−GC×GC/TOF-MS followed only by gel permeation chromatography (GPC) provided the ability to detect and identify multiple classes of contaminants.[14] The GPC cleanup maximized the number of detected compounds compared to more extensive cleanup procedures. Overloading of the final extract on the GC column increased the number of detectable compounds at low concentrations. Thus, the nontargeted analytical approach used tactics different from those of conventional targeted analyses for quantification, which require extensive sample cleanup and ideally symmetric and nonoverloaded chromatographic peaks. We have applied this nontargeted GC×GC/TOF-MS method to characterize a complex mixture of nonpolar, persistent, bioaccumulative, and potentially biomagnifying compounds in dolphin blubber. Dolphins and other apex predators are sentinels with the potential to accumulate relatively high concentrations of nonpolar contaminants,[18] which preferentially accumulate in fatty tissues and can biomagnify through the food web.

In addition to the instrumental method, data management was critical to developing the contaminant inventory and required custom software. We describe the development of open source software to organize the mass spectra and ancillary information, to ensure the identifications are reproducible, and to provide a mechanism for sharing the data (a mass spectral library) with other researchers using a standard data format. The progress of scientific work in data-intensive fields that do not address issues of data sharing and reproducibility may be hindered.[19,20]

This nontargeted instrumental method and software reporting framework can be used to create a more complete contaminant inventory than targeted monitoring alone. Nontargeted mass spectrometry methods will likely not replace targeted methods because they are qualitative and lower throughput; however, our results show nontargeted methods could supplement targeted methods through periodic screening to help prioritize the monitoring of contaminants of emerging concern and other locally relevant contaminants in marine ecosystems.

EXPERIMENTAL SECTION

A list of defined acronyms and a description of the reference standards are given in the Supporting Information.

Sample Preparation. The blubber of a common dolphin (*Delphinus delphis*) fatally stranded in January 2006 in Orleans, MA, was obtained from the Cape Cod Stranding Network (identification number CCSN06-013Dd). The male dolphin had a body length of 195.5 cm; the weight and age were not available. The blubber was homogenized in a blender and filtered through a glass fiber filter with a nominal pore size of 0.7 μm.[12] We previously found that removal of lipids by GPC only, as opposed to acidification only or GPC in combination with silica-gel chromatography, resulted in the maximum number of detected compounds.[14] Therefore, only the GPC cleanup was applied. A 1 g sample of the blubber oil was dissolved in 5 mL of 1:1 ethyl acetate/cyclohexane. The solution was injected into a GPC (J2 Scientific, Columbia, MO) system to separate the lipids. The GPC method was optimized as described previously to ensure the recovery of several classes of halogenated compounds.[21] The GPC column, with a 2 cm i.d. and length of 22.5 cm, was packed with 24 g of BioBeads S-X3 in 1:1 ethyl acetate/cyclohexane. The flow rate was 5 mL/min, and the mobile phase was 1:1 ethyl acetate/cyclohexane. The eluent fraction between 12.5 and 22.5 min was collected and reduced to 1 mL using N_2 gas. The extract was brought to 5 mL with the mobile-phase solvent and reinjected into the GPC system to remove residual lipids. The extract was then evaporated and solvent-exchanged to isooctane to a final volume of 100 μL.

Instrumental Analysis. The sample was analyzed using a DSI system coupled to a GC×GC/TOF-MS system. The instrumental parameters were previously optimized.[14,21,22] The DSI system is a programmable temperature vaporizer. It enables a relatively large volume injection and a high tolerance to matrix interference.[23,24] For DSI, the sample was injected into a disposable microvial contained in a liner and then placed in the GC×GC inlet. The sample solvent was evaporated first at

a relatively low temperature, followed by rapid heating to introduce the semivolatile chemicals into the GC column. The microvial and liner were replaced between injections, which prevents nonvolatile compounds from transferring to the GC column and keeps the inlet and column clean. A 10 μL sample injection was conducted using a Combi-PAL autosampler (Leap Technologies, Carrboro, NC) and automated DSI accessory (Linex) in combination with an Optic 3 programmable temperature vaporizer system (Atas-GL International, Veldhoven, The Netherlands). The initial injector temperature was held at 70 °C for 8 min with a 50:1 split ratio and then ramped to 320 at 12 °C/s with a splitless period of 7.5 min, followed by a constant 50:1 split ratio for 16.5 min, and then the split flow ratio was reduced to 25:1 and the injector temperature cooled to 250 °C. The carrier gas flow rate was held at 1 mL/min for 8 min, ramped to 2.5 mL/min as a pressure pulse during the 7.5 min splitless period, then reduced to 1 mL/min until 33 min, and ramped to 1.5 mL until the end of the analysis.

A Pegasus 4D (LECO, St. Joseph, MI) GC×GC/TOF-MS was used with a Restek (Bellefonte, PA) Siltek deactivated column (5 m length, 0.25 mm i.d.) attached to the inlet as a guard column, a Restek Rtx5Sil-MS (15 m length, 0.25 mm i.d., 0.25 μm film thickness) as the first dimension (^{1}D) of separation, and a J&W Scientific (Folsom, CA) DB-17MS (2 m length, 0.18 mm, 0.18 μm thickness) as the second dimension (^{2}D) of separation. Ultra-high-purity helium (Airgas, Radnor, PA) was used as the carrier gas. The primary oven temperature was held at 60 °C for 7.5 min, then ramped at 10 °C/min to 300 °C, and held for 20 min. The secondary oven temperature was programmed to be 20 °C higher than the primary oven temperature. For GC×GC, the modulation period was set to 3.5 s, with a 0.9 s hot pulse duration and a 35 °C modulator temperature offset vs the primary oven temperature. The MS transfer line and the ion source temperatures were at 270 and 250 °C, respectively. The electron energy was −80 eV, and the detector voltage was 1850 V. The data acquisition rate was 100 spectra/s.

Quantification of PBDEs and Halogenated Natural Products (HNPs). We quantified a suite of PBDEs and HNPs, listed in Table S-1 (Supporting Information), for which standard reference compounds were available. We used a previously reported method for quantification.[25] Briefly, dolphin oil (0.5 g) was dissolved in 1:1 cyclohexane/ethyl acetate solvent and brought to 10 mL volume. Half (5 mL = 0.25 g) of the sample was injected into a GPC system for lipid removal and reduced to 100 μL, and then 10 μL (equivalent to 25 mg of sample) was injected into the GC×GC/TOF-MS instrument via DSI.

Compound Identification. All isolated chromatographic peaks were examined for identification of their corresponding mass spectra. Data analysis was conducted with LECO ChromaTOF software version 4.33. Data processing for nontargeted analysis included automatic peak finding using mass spectral deconvolution (embedded within ChromaTOF) and spectral searching vs the National Institute of Standards and Technology (NIST) 2008 mass spectral library and contemporaneously analyzed mass spectra from reference standards for confirmation. Identification through spectral searching was based on the common presence of characteristic identifiable fragment ions (often halogenated isotopic clusters) and the spectral similarity score.

Identifications fell within the following categories, with the category names in brackets: (1) The experimental mass spectrum and retention times were matched to those of a reference standard analyzed under the same conditions [authentic MS RT]. (2) The experimental spectrum, but not the retention times, was matched to a reference standard, indicating the experimental spectrum is that of an isomer [authentic MS]. (3) The experimental spectrum was matched to one within the NIST Electron Ionization Mass Spectral Library [reference database MS]. (4) The experimental spectrum was matched to one found in the literature [literature MS]. (5) The experimental mass spectrum was identified as potentially belonging to a class of congeners on the basis of comparison to that of a reference standard within the same class of congeners [manual-congener group]. (6) A presumptive identification was made by manual interpretation of the experimental spectrum [manual]. (7) The experimental spectrum was identified as belonging to a halogenated compound, but the chemical structure could not be further identified [unknown].

Data Management and Sharing. The software is an extension package for the R statistical computing language;[26] see Figure 1. It records the evidence for each identification and

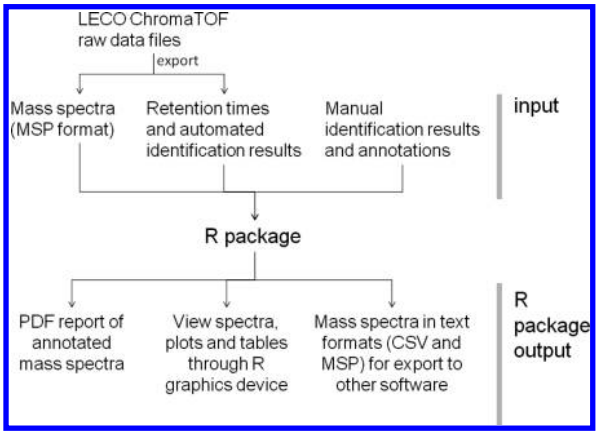

Figure 1. Input and output of the mass spectral library software.

ancillary information on the compound: mass spectrum, molecular and fragment ion identifications, GC×GC retention times, elemental formula, chemical structure, instrument acquisition parameters, similarity scores (when matched to a standard reference spectrum), and literature references. The largest GC×GC modulation slice was used to determine the reported retention times. Note that due to the peak slicing there is no established method for the calculation of relative retention times from GC×GC chromatographic data. The spectra are organized by user-defined categories, including the compound class (e.g., chlordane), source (e.g., anthropogenic), method of identification (e.g., authentic MS RT), and halogen type. The intention is a flexible system for storing identification evidence, in particular that obtained by the manual interpretation of mass spectra, and organization of the spectra into categories. This is different from standard reference databases and software focused on automated spectral identification.

In addition to viewing spectra within R, the package allows data to be viewed and shared by two other mechanisms. First, a portable document format (PDF) report of the mass spectra can be generated. This is intended as the primary mechanism

Table 1. Compounds Identified in Atlantic Common Dolphin (*D. delphis*) Blubber[a]

chemical class	no. of compds	source	no. of compds identified de novo	no. of bromines	no. of chlorines
polychlorinated biphenyls (PCBs)	55	anthropogenic	0	0	4, 5, 6, 7, 8, 9, 10
chlordane-related compds	20	anthropogenic	13	0	6, 7, 8, 9, 10
heptachlor-related compds	8	anthropogenic	4	0	6, 7, 8, 9
DDT-related compds	14	anthropogenic	3	0	2, 3, 4, 5, 6, 7
toxaphene	12	anthropogenic	3	0	6, 7, 8, 9
mirex	4	anthropogenic	3	0	10, 11, 12
other legacy chlorinated pesticides	6	anthropogenic	1	0	5, 6
polybrominated diphenyl ethers (PBDEs)	16	anthropogenic	0	3, 4, 5, 6, 7	0
polybrominated biphenyls (PBBs)	10	anthropogenic	4	4, 5, 6	0
polychlorinated diphenyl ethers (PCDEs)	15	anthropogenic	15	0	5, 6, 7, 8
methoxy polychlorinated diphenyl ethers (MeO-CDEs)	3	anthropogenic	3	0	7, 8
polychlorinated styrenes	16	likely anthropogenic	5	3, 4, 5, 6, 7, 8	0
tribromophenol	1	natural/ anthropogenic	0	3, 4	0
tribromoanisole	1	natural	0	3	0
brominated indoles	3	natural	0	1, 2	0
methoxy polybrominated/chlorinated diphenyl ethers (MeO-B/CDEs)	9	natural	6	3, 4, 5	0, 1
dimethoxy tetrabromobiphenyl (2MeO-BB)	1	natural	0	4	0
methylbipyrroles (MBPs)	28	natural	15	3, 4, 5, 6, 7	1, 2, 6, 7
dimethtylbipyrroles (DMBPs)	10	natural	7	2, 3, 4, 5, 6	1, 2, 4
polybrominated hexahydroxanthene (PBHDs)	2	natural	0	3, 4	0
mixed bromo/chlorodiphenyl ethers	7	unknown	7	3, 4, 5	1
polychlorinated ethylbenzene	2	unknown	1	0	5
unknowns (containing Br/Cl)[b]	27	unknown	22	0, 3, 4, 5, 7	1, 2, 3, 5, 8
tetraphenyltin (nonhalogenated)	1	anthropogenic	0	0	0

[a]Compounds identified de novo are those belonging to the manual-congener group, manual, and unknown categories. [b]Unknowns were counted as identified de novo if the number of halogens could be determined.

for viewing sets of spectra and is a method for sharing spectra independent of specialized software. Second, spectra can be exported in the NIST MSP format, a text-based format for storing centroid m/z values and the corresponding intensities. These spectra can be imported into NIST MS Search (or other compatible software) as a custom library and used by that program to automatically search other experimental spectra against the library. The R package containing the dolphin blubber library, SpecLibDolphin2011, is dependent on OrgMassSpecR, a general package for mass spectrometry data analysis. Both are available at http://orgmassspecr.r-forge.r-project.org/. Documentation is available within the package and on the Web site; the source code and data can be anonymously accessed through a version control system.

RESULTS AND DISCUSSION

We identified a total of 271 compounds belonging to 24 classes. Table 1 summarizes the 271 compounds in terms of class, source, degree of halogenation, and identification category. Except for tetraphenyltin, all identified compounds contain either bromine or chlorine, or both. The spectra for 112 compounds were identified de novo due to the lack of authentic standards and reference mass spectra and encompass the "manual-congener group", "manual", and "unknown" categories defined in the Experimental Section. The Supporting Information contains (1) the PDF report of the dolphin blubber mass spectral library containing all identified compounds and unknowns, with the exception of polychlorinated biphenyl (PCB) congeners, and (2) a detailed description of the de novo identification of the uncommon contaminants and their relative peak intensities and sources.

Retention times provided additional evidence for identifications, as compounds within a class tended to cluster together. Figure 2A shows the GC×GC separation of the 270 compounds containing halogens. The x and y axes represent the ^{1}D and ^{2}D retention times, respectively. The ^{1}D and ^{2}D retention times are defined as the most intense modulation slice for each compound. The modulation period was selected on the basis of the previous study that optimized the ^{1}D separation while enhancing the ^{2}D peak height via GC×GC.[21] This modulation setup resulted in ^{2}D GC wrapping at later ^{1}D GC elution times. In this case, the modulation time (3.5 s) was added to the ^{2}D retention time.

The ^{2}D column separated compounds that would otherwise coelute in the ^{1}D mode. On the ^{1}D column, chlorinated compounds tended to elute earlier than the brominated compounds due to their smaller molecular weights and lower vapor pressures. On the ^{2}D column, brominated compounds tended to elute later than chlorinated compounds due to their higher boiling point and greater polarity. Additional clustering due to structural similarity takes place within compound classes, as shown for three example classes in Figure 2B. Figure 2C shows that, in addition to sharing common fragment ions, chromatographic clustering of unknown groups provides additional evidence of structural similarity. This is further discussed in the Supporting Information.

Implications of This Study. A majority of the identified compounds are not typically monitored in marine contaminant surveys. This includes 86 of the anthropogenic contaminants, 54 of the halogenated natural products (HNPs), and the 36 compounds with unknown sources (including unidentified compounds) listed in Table 1. The anthropogenic contami-

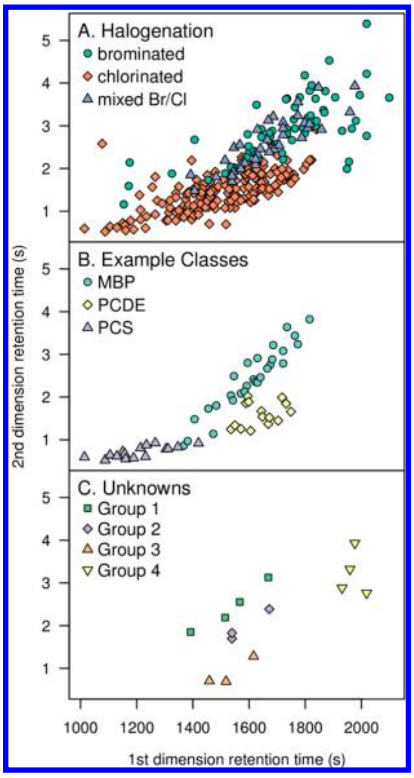

Figure 2. GC×GC plots illustrating retention time clustering of structurally similar compounds. Clustering enabled confirmation of identifications within a class. (A) All identified compounds, grouped by halogenation. (B) Example clustering of three compound classes. (C) Clustering of four unknown classes, described further in the Supporting Information.

nants that are not typically monitored are 15 chlordane-related compounds, 6 heptachlor-related compounds, 8 DDT related compounds, 3 Mirex-related compounds, 9 PBDEs, 9 polybrominated biphenyls (PBBs), 15 polychlorinated diphenyl ethers (PCDEs), 3 methoxylated polychlorinated diphenyl ethers (MeO-CDEs), 15 polychlorinated styrenes (PCSs), tribromophenol, and tetraphenyltin. As discussed in the Supporting Information and summarized in Table 2, on the basis of relative peak intensities, some of the contaminants that are not typically monitored had similar or larger chromatographic peak intensities compared to typically monitored contaminants. Therefore, a targeted survey of only typical/regulated compounds would measure only a subset of the total inventory of contaminants or body burden. A more complete inventory may better inform risk assessment. For example, mixtures of contaminants with similar modes of action may exert a combined effect resulting in toxicity, even if the individual concentrations are below no-observed-effect concentrations.[27,28]

HNPs were detected concurrently with the anthropogenic contaminants. Both have a similar molecular weight range, contain halogens, and are nonpolar, indicating they likely have similar physical-chemical properties. Some highly halogenated natural products, such as the MBPs, DMBPs, and MeO-BDEs, biomagnify in marine food webs.[29−31] Several HNPs have been found in samples dated prior to the large-scale industrial production of halogenated compounds and may be produced by marine algae and/or sponges, or perhaps by symbiotic microbes,[32−39] and their environmental occurrence and toxicological relevance are unclear. Table 3 shows the

Table 2. Comparison of the Relative Peak Areas of Selected Contaminants That Are Not Typically Monitored with a Typically Monitored Contaminant from the Same Group[a]

compd	rel peak area (%)	ion (*m/z*)	comparison ion	page
chlordane-related 1	120	238	to *m/z* 373 of γ-chlordane	5
chlordane-related 6	61	373		11
chlordane-related 10	230	339		16
chlordane-related 13	65	373		23
heptachlor-related 2	58	337	to *m/z* 263 of heptachlor epoxide	28
heptachlor-related 3	96	272		30
PBDE 6Br isomer 2	92	484	to *m/z* 484 of BDE-153	77
PBDE 7Br isomer 1	350	562	to *m/z* 562 of BDE-183	81
PBB 4Br isomer 1	223	310	to *m/z* 468 of BB-153	84
PBB 4Br isomer 2	167	310		85
PBB 5Br isomer 2	80	388		87
heptachlorostyrene 1	246	344	to *m/z* 380 of octachlorostyrene	121
heptachlorostyrene 2	62	344		122
heptachlorostyrene 3	107	344		123

[a]The "ion" and "comparison ion" columns refer to the selected ions used to determine the peak areas for the not typically monitored and typically monitored contaminants, respectively. The "page" column refers to the page number in the Mass Spectral Library Report, within the Supporting Information, where additional information on the compound is documented.

Table 3. Concentrations (ng/g of lipid mass) of Selected Halogenated Natural Products Compared to the Six Major PBDE Congeners

compd	concn	compd	concn
MBP-Cl_7	85	2,2′-diMeO-BB-80	12.8
MBP-HBr_5Cl	1110	PBHD (3Br)	18
MBP-HBR_6	478	PBHD (4Br)	246
MBP-Br_6Cl	1.62	BDE-28	8.6
MBP-Br_7	0.504	BDE-47	727
DMBP-Br_4Cl_2	124	BDE-100	241
DMBP-Br_5Cl	16.5	BDE-99	123
DMBP-Br_6	30.9	BDE-154	103
2′-MeO-BDE68	47	BDE-153	51.3
6-MeO-BDE47	103		

concentrations of HNPs, for which reference standards were available, were similar to those of anthropogenic PBDE congeners. It is important to identify, quantify, and understand the potential for biological impacts of HNPs in marine environments. HNPs may confound the study of toxicological impacts of anthropogenic contaminants and cannot be restricted or regulated like anthropogenic contaminants.

De novo interpretation is critical in nontargeted contaminant analysis. The number of spectra identified de novo (41% of the total) demonstrates that exclusive reliance on commercially available reference standards or on automated identification by searching standard spectral libraries is limiting. The confidence in qualitative mass spectral identifications varies, and a description of the uncertainty will be different from that used for quantitative analysis. Lehotay et al.[40] and the Metabolomics Standard Initiative[41] both describe similar approaches to expressing the uncertainty in an identification and caution

against over-reliance on arbitrary criteria. They propose placing the identifications into categories with varying levels of confidence, on the basis of the method by which the identification was made. We have followed this approach. The confidence in an identification is generally [authentic MS RT] > [authentic MS] > [reference database MS] > [literature MS] > [manual-congener group] ≥ [manual]. Fragment ion identifications are the primary evidence for those spectra identified de novo and, along with the ancillary information, are reported to ensure reproducibility and assist data reuse by other researchers. Compounds not identified by comparison to reference standards are considered presumptively identified.

Limitations in the current targeted contaminant monitoring framework were recognized previously. New compounds are constantly being incorporated into modern technology and may enter the environment.[42] The potential chemical exposure universe is immense, possibly comprising millions of substances after accounting for all anthropogenic compounds and their degradation products and naturally occurring xenobiotics.[43] Nonpolar, persistent, and bioaccumulative compounds are a subset of this "chemical sea" to which the environment is exposed. In an effort to identify new persistent and bioaccumulative contaminants, Howard and Muir reviewed the Canadian Domestic Substance List (~3000 substances) and the U.S. Environment Protection Agency Toxic Substance Control Act Inventory Update Rule database (~22000 chemicals) for chemicals with the potential for persistence and bioaccumulation based on theoretical calculations.[3] This review yielded approximately 600 potentially persistent and bioaccumulative compounds. As expected, the legacy pollutants and brominated flame retardants (BFRs) were in this group; however, these compounds were just 20% of the total. Approximately 500 of the chemicals are neglected by targeted contaminant surveys, and two-thirds of them would be amenable to GC/MS analysis. Similar approaches and results were described by Brown and Wania,[44] Arnot and Mackay,[45] and Mitchell et al.[46]

These lists of potential persistent and bioaccumulative contaminants of emerging concern provide an important starting point for researchers to test the hypothesis that they exist in the environment and are of concern. However, this predictive approach may have limitations: (1) It is economically difficult to create targeted analytical methods for the hundreds of potential bioaccumulative compounds identified by these studies. (2) Impurities, byproducts, and unpredicted transformation products are not included in the databases. (3) Chemicals with small production volumes that may be important within a specific geographic region may not be included in the databases. Therefore, nontargeted analysis complements the predictive approaches.

On the basis of their bioavailability, biomagnification, toxicity, and abundance in the geographical region of study, different contaminants will have varying potential for biological impacts. The nontargeted analytical method and data reporting system described here demonstrate a framework for creating an inventory of persistent and bioaccumulative compounds in a marine environment. Prior to further investigation of compounds of interest, it may be necessary to verify their identity by matching the retention times and spectra to those of standard compounds. Combined with an adequate survey design, the resulting inventory for a region and sample matrix may complement ongoing targeted contaminant surveys by suggesting new compounds for more extensive monitoring and risk assessment.

■ ASSOCIATED CONTENT

Supporting Information

Glossary of acronyms, description of reference standards, description of the de novo identification of the uncommon contaminants, and a PDF mass spectral library report are provided. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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