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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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 non-targeted analytical method utilizing direct sample introduction (DSI) coupled to comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC/TOF-MS). To test the capabilities of this instrumental method within the context of marine contaminant surveys, we characterized a broad array of non-polar, 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, contemporaneously analyzing mass spectra from reference standards, and *de novo* interpretation. We identified a total of 271 compounds belonging to 24 classes; all compounds but one were halogenated. Anthropogenic contaminants and halogenated natural products were concurrently detected. Eighty-six compounds were anthropogenic contaminants that are not routinely targeted in environmental surveys, and 54 compounds were halogenated natural products. One hundred twelve 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

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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 non-targeted 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 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 (Muir *et al*. 2006).

Targeted methods based 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 method, and may exclude contaminants with the potential to cause adverse biological impacts (Daughton 2004, Howard *et al*. 2010). 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, have not been targeted for analysis, and therefore remain undetected. The polybrominated diphenyl ether (PBDE; Sellstrom *et al*. 1993, Hites 2004) and dechlorane plus (Hoh *et al*. 2006, Sverko *et al*. 2011) 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. Non-targeted mass spectrometry addresses these goals to broaden the scope of analysis.

Mass spectrometers for the non-targeted analysis of non-polar 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 (Daughton 2003). 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 co-eluting compounds (Stein 1993). 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 (Dallüge *et al*. 2002a,b). GC×GC/TOF-MS has previously been used to perform non-targeted analysis of specific classes of chemical contaminants; for example, halogenated methyl bipyrroles in marine mammals (Pangallo *et al*. 2008), and nonylphenols in technical mixtures (Eganhouse *et al*. 2009). It has also been used to identify multiple classes of contaminants in fish oil (Hoh *et al*. 2009a) and sediment (Skoczynska *et al*. 2008). This discussion excludes instrumentation such as liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q/TOF) for the non-targeted analysis of polar compounds, and GC/MS SIM screening methods for halogenated compounds within certain chemical classes (La Guardia *et al*. 2010, Rosenfelder *et al*. 2010).

Previously, we demonstrated that the non-targeted 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 (Skoczynska *et al*. 2008). 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 non-targeted analytical approach used different tactics than conventional targeted analyses for quantification, which require extensive sample cleanup and ideally symmetric and non-overloaded chromatographic peaks. We have applied this non-targeted GC×GC/TOF-MS method to characterize a complex mixture of non-polar, 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 non-polar contaminants (Kucklick *et al*. 2011), 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 (Nelson 2009, Reichman *et al*. 2011).

This non-targeted instrumental method and software reporting framework can be used to create a more complete contaminant inventory than targeted monitoring alone. Non-targeted mass spectrometry methods will likely not replace targeted methods because they are qualitative and lower-throughput; however, our results show non-targeted 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.

Methods

A list of defined acronyms and a description of the reference standards are available in the Supporting Information (SI) available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_06SI.pdf.

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 (Pangallo *et al*. 2008). 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 (Hoh *et al*. 2009a). Therefore, only the GPC cleanup was applied. One gram of the blubber oil was dissolved in 5 ml of 1:1 of ethylacetate:cyclohexane. The solution was injected into a gel permeation chromatography (GPC, J2 Scientific, Columbia, MO, USA) system to separate the lipids. The GPC method was optimized as described previously to ensure the recovery of several classes of halogenated compounds (Hoh *et al*. 2008). The GPC column, with a 2 cm id and 22.5 cm length, was packed with 24 g of BioBeads S-X3 in 1:1 ethylacetate:cyclohexane. The flow rate was 5 ml/minute and the mobile phase was 1:1 ethylacetate:cyclohexane. The eluent fraction between 12.5 and 22.5 minutes was collected and reduced to 1 ml using N_2 gas. The extract was brought to 5 ml with the mobile phase solvent and re-injected into 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. The instrumental parameters were previously optimized (Hoh *et al*. 2007, 2008, 2009). The DSI system is a programmable temperature vaporizer. It enables a relatively large volume injection and a high tolerance to matrix interference (Jing and Amirav 1997, Hoh and Mastovska 2008). For DSI, the sample was injected into a disposable micro-vial contained in a liner, 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 semi-volatile chemicals into the GC column. The micro-vial and liner was replaced between injections, which prevents non-volatile 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 minutes with a 50:1 split ratio, then ramped to 320°C at 12°C/second with a splitless period of 7.5 minutes, followed by a constant 50:1 split ratio for 16.5 minutes, and then the split flow ratio was reduced to 25:1 and the injector temperature was cooled to 250°C. The carrier gas flow rate was held at 1 ml/minute for 8 minutes, ramped to 2.5 ml/minute as a pressure pulse during the 7.5-minute splitless period, then reduced to 1 ml/minute for 33 minutes, and ramped to 1.5 ml/minute until the end of the analysis.

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

Quantification of PBDEs and HNPs

We quantified a suite of PBDEs and HNPs, listed in Table SI-1, for which standard reference compounds were available. We used a previously reported method for quantification (Hoh *et al*. 2009b). Briefly, dolphin oil (0.5 g) was dissolved in 1:1 cyclohexane:ethylacetate 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 sample) was injected on the GC×GC/TOF-MS 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 non-targeted 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. The experimental mass spectrum and retention times were matched to those of a reference standard analyzed under the same conditions [Authentic MS RT]. The experimental spectrum, but not the retention times, was matched to a reference standard, indicating the experimental spectrum is an isomer [Authentic MS]. The experimental spectrum was matched to one within the NIST Electron Ionization Mass Spectral library [Reference Database MS]. The experimental spectrum was matched to one found in the literature [Literature MS]. The experimental spectrum was identified as potentially belonging to a class of congeners based on its mass spectrum in comparison to that of a reference standard within the same class of congeners [Manual-Congener Group]. A presumptive identification was made by manual interpretation of the experimental spectrum [Manual]. The experimental spectrum was identified as halogenated, but its chemical structure could not be further identified [Unknown].

Data Management and Sharing

The software is an extension package for the R statistical computing language (R Development Core Team 2011; Figure 1). It records the evidence for each identification and 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

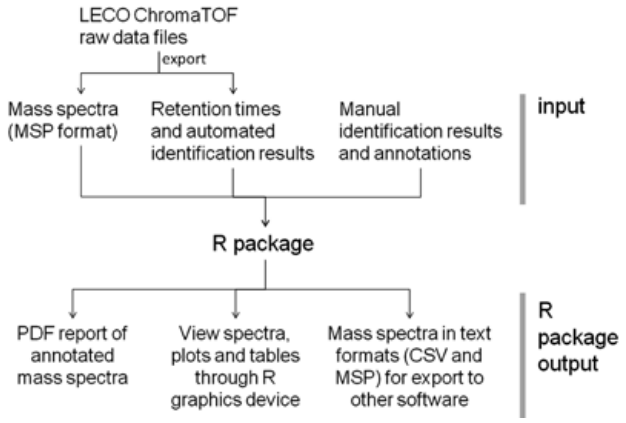

Figure 1. Input and output of the mass spectral library software.

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 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 website; 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 Methods section. The SI contains 1) the PDF report of the dolphin blubber mass spectral library containing all identified compounds and unknowns, with the exception of PCB congeners, and 2) a detailed description of the *de novo* identification of the uncommon contaminants, their relative peak intensities, and sources.

Retention times provided additional evidence for identifications, as compounds within a class tended to cluster together. Figure 2 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 based on the previous study that optimized the ^{1}D separation while enhancing the ^{2}D peak height via GC×GC (Hoh *et al*. 2008). This modulation setup resulted in ^{2}D GC wrapping at later ^{1}D GC elution times. In this case, the modulation time (3.5 seconds) was added to the ^{2}D retention time.

The ^{2}D column separated compounds that would otherwise co-elute in ^{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 2. This figure also 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 SI.

Implications of This Study

A majority of the identified compounds are not typically monitored in marine contaminant surveys.

Table 1. Compounds identified in the Atlantic common dolphin (*Delphinus delphis*) blubber.

Chemical Class	Number of Compounds	Source	Number of Compounds Identified *de novo*	Number of Bromines	Number of Chlorines
Polychlorinated biphenyls (PCBs)	55	Anthropogenic	0	0	4, 5, 6, 7, 8, 9, 10
Chlordane related compounds	20	Anthropogenic	13	0	6, 7, 8, 9, 10
Heptachlor related compounds	8	Anthropogenic	4	0	6, 7, 8, 9
DDT related compounds	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 tetrabromo biphenyl (2MeO-BB)	1	Natural	0	4	0
Methyl bipyrroles (MBPs)	28	Natural	15	3, 4, 5, 6, 7	1, 2, 6, 7
Dimethtyl bipyrroles (DMBPs)	10	Natural	7	2, 3, 4, 5, 6	1, 2, 4
Polybrominated hexahydroxanthene (PBHDs)	2	Natural	0	3, 4	0
Mixed Br/Cl diphenyl ethers	7	Unknown	7	3, 4, 5	1
Polychlorinated ethylbenzene	2	Unknown	1	0	5
Unknowns (containing Br/Cl)	27	Unknown	22	0, 3, 4, 5, 7	1, 2, 3, 5, 8
Tetraphenyltin (non-halogenated)	1	Anthropogenic	0	0	0

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 contaminants 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 (PCS), tribromophenol, and tetraphenyltin. As discussed in the SI and summarized in Table 2, based on 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 (Rajapakse *et al*. 2002, Yordy *et al*. 2010).

Halogenated natural products (HNPs) were detected concurrently with the anthropogenic contaminants. Both have a similar molecular weight range, contain halogens, and are non-polar, 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 (Tittlemier et al. 2002, Blust and Covaci 2009, Pangallo and Reddy 2010). 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 (Unson *et al*. 1994; Haglund *et al*. 1997; Faulkner 2001, 2002; Vetter *et al*. 2002; Teuten and Reddy 2007; Pangallo *et al*. 2012), and their environmental

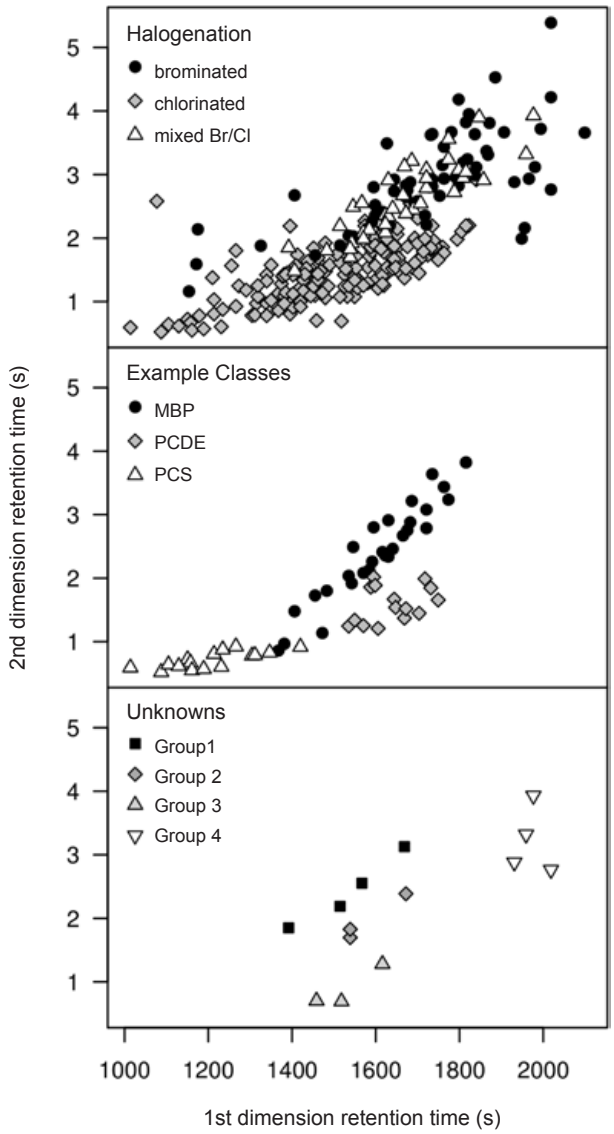

Figure 2. GC×GC plots illustrating retention time clustering of structurally similar compounds: all identified compounds grouped by halogenation, example compound classes, and four unknown classes, described further in the SI.

occurrence and toxicological relevance is unclear. Table 3 shows the concentrations of HNPs, for which reference standards were available, were similar to that 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 non-targeted 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 than that used for quantitative analysis. Lehotay *et al*. (2008) and the Metabolomics Standard Initiative (Sumner *et al*. 2007) 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, based on 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 (NRC 2001). The potential chemical exposure universe is immense, possibly comprising millions of substances after accounting for all anthropogenic compounds, their degradation products, and naturally occurring xenobiotics (Daughton 2001). Non-polar, 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 (Howard and Muir 2006). 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

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. 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 SI, where additional information on the compound is documented.

Compound	Relative Peak Area (%)	Ion (*m/z*)	Comparison Ion	Page
Chlordane related 1	120	238	to *m/z* 373 of γ-chlordane	4
Chlordane related 6	61	373		10
Chlordane related 10	230	339		15
Chlordane related 13	65	373		22
Heptachlor related 2	58	337	to *m/z* 263 of heptachlor epoxide	27
Heptachlor related 3	96	272		29
PBDE 6Br isomer 2	92	484	to *m/z* 484 of BDE-153	76
PBDE 7Br isomer 1	350	562	to *m/z* 562 of BDE-183	80
PBB 4Br isomer 1	223	310	to *m/z* 468 of BB-153	83
PBB 4Br isomer 2	167	310		84
PBB 5Br isomer 2	80	388		86
Heptachlorostyrene 1	246	344	to *m/z* 380 of octachlorostyrene	120
Heptachlorostyrene 2	62	344		121
Heptachlorostyrene 3	107	344		122

Table 3. Concentrations (ng/g lipid weight) of selected halogenated natural products compared to the six major PBDE congeners.

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

to GC/MS analysis. Similar approaches and results were described by Brown and Wania (2008), Arnot and Mackay (2008), and Mitchell *et al*. (2002).

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, by-products, and unpredicted transformation products are not included in the databases; and 3) chemicals with small production volumes that may be important within a specific geographic region may not be included in the databases. Therefore, non-targeted analysis complements the predictive approaches.

Based on their bioavailability, biomagnification, toxicity, and abundance in the geographical region of study, different contaminants will have varying potential for biological impacts. The non-targeted

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.

Literature Cited

Arnot J.A. and D. Mackay. 2008. Policies for chemical hazard and risk priority setting: can persistence, bioaccumulation, toxicity, and quantity information be combined? *Environmental Science & Technology* 42:4648-4654.

Brown, T.N. and F. Wania. Screening chemicals for the potential to be persistent organic pollutants: a case study of Arctic contaminants. *Environmental Science & Technology* 42:5202-5209.

Dallüge, J., M. van Rijn, J. Beens, R.J. Vreuls and U.A. Brinkman. 2002a. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection applied to the determination of pesticides in food extracts. *Journal Chromatography A* 965:207-217.

Dallüge, J., L.L. van Stee, X.B. Xu, J. Williams, J. Beens, R.J. Vreuls and U.A. Brinkman. 2002b. Unraveling the composition of very complex samples by comprehensive gas chromatography coupled to time-of-flight mass spectrometry: cigarette smoke. *Journal Chromatography A* 974:169-184.

Daughton, C.G. 2001. Emerging pollutants, and communicating the science of environmental chemistry and mass spectrometry: pharmaceuticals in the environment. *Journal of American Society for Mass Spectrometry* 12:1067-1076.

Daughton, C.G. 2003. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. *Environmental Health Perspectives* 111:757-774.

Daughton, C.G. 2004. Non-regulated water contaminants: emerging research. *Environmental Impact Assessment* 24:711-732.

Eganhouse, R.P., J. Pontolillo, R.B. Gaines, G.S. Frysinger, F.L. Gabriel, H-P.E. Kohler, W. Giger and L.B. Barber. 2009. Isomer-specific determination of 4-monylphenols using comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry. *Environmental Science & Technology* 43:9306-9313.

Faulkner, J.D. 2001. Marine natural products. *National Product Reports* 18:1-49.

Faulkner, J.D. 2002. Marine natural products. *National Product Reports* 19:1-48.

Haglund, P.S., D.R. Zook, H.R. Buser and J. Hu. 1997. Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. *Environmental Science & Technology* 31:3281-3287.

Hites, R.A. 2004. Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. *Environmental Science & Technology* 38:945-956.

Hoh, E. and K. Mastovska. 2008. Large volume injection techniques in capillary gas chromatography. *Journal Chromatography A* 1186:2-15.

Hoh, E., K. Mastovska and S.J. Lehotay. 2007. Optimization of separation and detection conditions for comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Journal Chromatography A* 1145:210-221.

Hoh, E., S.J. Lehotay, K. Mastovska and J.K. Huwe. 2008. Evaluation of automated direct sample introduction with comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for the screening analysis of dioxins in fish oil. *Journal Chromatography A* 1201:69-77.

Hoh, E., S.J. Lehotay, K. Mastovska, H.L. Ngo, W. Vetter, K.C. Pangallo and C.M. Reddy. 2009a. Capabilities of direct sample introduction-comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry to analyze organic chemicals of interest in fish oils. *Environmental Science & Technology* 43:3240-3247.

Hoh, E., S.J. Lehotay, K.C. Pangallo, K. Mastovska, H.L. Ngo, C.M. Reddy and W. Vetter. 2009b. Simultaneous quantitation of multiple classes of organohalogen compounds in fish oils with direct

sample introduction comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry* 57:2653-2660.

Hoh, E., L.Y. Zhu and R.A. Hites. 2006. Dechlorane plus, a chlorinated flame retardant, in the Great Lakes. *Environmental Science & Technology* 40:1184-1189.

Howard, P.H. and D.C. Muir. 2010. Identifying new persistent and bioaccumulative organics among chemicals in commerce. *Environmental Science & Technology* 44:2277-2285.

Jing, H. and A. Amirav. 1997. Pesticide analysis with the pulsed-flame photometer detector and a direct sample introduction device. *Analytical Chemistry* 69:1426-1435.

Kucklick, J., L. Schwacke, R. Wells, A. Hohn, A. Guichard, J. Yordy, L. Hansen, E. Zolman, R. Wilson, J. Litz, D. Nowacek, T. Rowles, R. Pugh, B. Balmer, C. Sinclair and P. Rosel. 2011. Bottlenose dolphins as indicators of persistent organic pollutants in the western North Atlantic Ocean and northern Gulf of Mexico. *Environmental Science & Technology* 45:4270-4277.

La Guardia, M.J., R.C. Hale, E. Harvey and D. Chen. 2010. Flame-retardants and other organohalogens detected in sewage sludge by electron capture negative ion mass spectrometry. *Environmental Science & Technology* 44:4658-4664.

Lehotay, S.J., K. Mastovska, A. Amirav, A.B. Fialkov, T. Alon, P.A. Martos, A. de Kok and A.R. Fernandez-Alba. 2008. Identification and confirmation of chemical residues in food by chromatography-mass spectrometry and other techniques. *TrAC-Trends in Analytical Chemistry* 27:1070-1090.

Mitchell, R.R., C.L. Summer, S.A. Blonde, D.M. Bush, G.K. Hurlburt, E.M. Snyder and J.P. Giesy. 2002. SCRAM: A scoring and ranking system for persistent, bioaccumulative, and toxic substances for the North American Great Lakes-resulting chemical scores and rankings. *Human and Ecological Risk Assessment* 8:537-557.

Muir, D.C. and P.H. Howard. 2006. Are there other persistent organic pollutants? A challenge for environmental chemicals. *Environmental Science & Technology* 40:7157-7166.

National Research Council (NRC). 2001. Grand Challenges in Environmental Sciences. Committee on Grand Challenges in Environmental Sciences, Oversight Commission for the Committee on Grant Challenges in Environmental Sciences. National Academy Press. Washington, DC.

Nelson, B. 2009. Data sharing: Empty archives. *Nature News* 461:160-163.

Pangallo, K.C. and C.M. Reddy. 2010. Marine natural products, the halogenated 1'-methyl-1,2'-bipyrroles, biomagnify in a northwestern Atlantic food web. *Environmental Science & Technology* 44:5741-5747.

Pangallo, K., R.K. Nelson, E.L. Teuten, B.E. Pedler and C.M. Reddy. 2008. Expanding the range of halogenated 1'-methy-1,2'-bipyrroles (MBPs) using GC/ECNI-MS and GC×GC/TOF-MS. *Chemosphere* 71:1557-1565.

Pangallo, K.C., M.C. Reddy, M. Poyton, J. Bolotin and T.B. Hofstetter. 2012. $\delta^{15}N$ enrichment suggests possible source for halogenated 1′-methyl-1,2′-bipyrroles (MBPs). *Environmental Science & Technology* 46:2064-2070.

Rajapakse, N., E. Silva and A. Kortenkamp. 2002. Combining xenoestrogens at levels below individual no-observed-effect concentrations dramatically enhances steroid hormone action. *Environmental Health Perspectives* 110:917-921.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN: 3-900051-07-0. http://www.R-project.org

Reddy, C.M., L. Xu, G.W. O'Neil, R.K. Nelson, T.I. Eglinton, D.J. Faulkner, R. Norstrom, P.S. Ross and S.A. Tittlemier. 2004. Radiocarbon evidence for a naturally produced, bioaccumulating halogenated organic compound. *Environmental Science & Technology* 38:1992-1997.

Reichman, O.J., M.B. Jones and M.P. Schildhauer. 2011. Challenges and opportunities of open data in ecology. *Science* 331:703-705.

Rosenfelder, N., N.J. Van Zee, J.F. Mueller, C. Gaus and W. Vetter. 2010. Gas chromatography/electron ionization-mass spectrometry-selected ion monitoring screening method for a thorough investigation of polyhalogenated compounds in passive sampler

extracts with quadrupole systems. *Analytical Chemistry* 82:9835-9842.

Sellstrom, U., B. Jansson, A. Kierkegaard, C. Dewit, T. Odsjo and M. Olsson. 1993. Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. *Chemosphere* 26:1703-1718.

Skoczynska, E., P. Korytar and J. De Boer. 2008. Maximizing chromatographic information from environmental extracts by GC×GC/ToF-MS. *Environmental Science & Technology* 42:6611-6618.

Stein, S.E. 1999. An integrated method for spectrum extraction and compound identification from gas chromatography/mass spectrometry data. *Journal of American Society for Mass Spectrometry* 10:770-781.

Sumner, L.W., A. Amberg, D. Barrett, M.H. Beale, R. Beger, C.A. Daykin, T.W.-M. Fan, O. Fiehn, R. Goodacre, J.L. Griffin, T. Hankemieier, N. Hardy, J. Harnly, R. Higashi, J. Kopka, A.N. Lane, J.C. Lindon, P. Marriott, A.W. Nicholls, M.D. Reily, J.J. Thaden and M.R. Viant. 2007. Proposed minimum reporting standards for Chemical Analysis Working Group (CAWG) Metabolomics Standard Initiative (MSI). *Metabolomics* 3:211-221.

Sverko, E., G.T. Tomy, E.J. Reiner, B.E. McCarry, J.A. Arnot, R.J. Law and R.A. Hites. 2011. Dechlorane plus and related compounds in the environment: a review. *Environmental Science & Technology* 45:5088-5098.

Teuten, E.L. and C.M. Reddy. 2007. Halogenated organic compounds in archived whale oil: A pre-industrial record. *Environmental Pollution* 145:668-671.

Tittlemier, S.A., A.T. Fisk, K.A. Hobson and R.J. Norstrom. 2002. Examination of the bioaccumulation of halogenated dimethyl bipyrroles in an arctic marine food web using stable nitrogen isotope analysis. *Environmental Pollution* 116:85-93.

Unson, M.D., N.D. Holland and D.J. Faulkner. 1994. A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. *Marine Biology* 119:1-11.

Vetter, W., E. Stoll, M.J. Garson, S. Fahey, C. Gaus and J.F. Muller. 2002. Sponge halogenated natural products found at parts-per-million levels in marine mammals. *Environmental Toxicology and Chemistry* 21:2014-2019.

Weijs, L., S. Losada, K. Das, L. Roosens, P.J. Reijnders, J.F. Santos, H. Neels, R. Blust and A. Covaci. 2009. Biomagnification of naturally-produced methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in harbor seals and harbor porpoises from the southern North Sea. *Environment International* 35:893-899.

Yordy, J.E., M.A. Mollenhauer, R.M. Wilson, R.S. Wells, A. Hohn, J. Sweeney, L.H. Schwacke, T.K. Rowles, J.R. Kucklick and M.M. Peden-Adams. 2010. Complex contaminant exposure in cetaceans: a comprarative E-Screen analysis of bottlenose dolphin blubber and mixtures of four persistent organic pollutants. *Environmental Toxicology & Chemistry* 29:2143-2153.

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Supplemental Information

Supplemental Information is available at ftp://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2012AnnualReport/ar12_06SI.pdf