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Research Article

Identifying halogenated emerging contaminants in the human food chain by exploring the potential of sentinel animal species

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Abstract

The characterization of the human dietary exposure to emerging contaminants (ECs) typically relies on the analysis of food or is extrapolated from the analysis of human tissues. However, the detection of ECs in such matrices is limited by sensitivity and/or ethical considerations. To address these challenges, utilizing sentinel animal species offers a promising alternative since they can accumulate contaminants over time, providing an integrated and early indication of emerging chemical exposures in the environment and food chain that may pose risks to humans. This proof-of-concept study aimed to evaluate a sampling strategy based on the selection of a sentinel species for the early identification of Cl/Br-containing ECs in the human food chain. Gulls, pigeons and rats were selected as sentinel models because of their relative dependence on anthropogenic food sources. After preparation, the samples were analyzed by gas and liquid chromatography coupled to high-resolution mass spectrometry. Of the 126 Cl/Br-containing compounds detected, 73 were legacy persistent organic pollutants (POPs) while 53 were potential POP-like ECs. Further analysis revealed that 71 out of the 126 compounds were detected in foods of animal origin that form part of the human diet, mostly at statistically significant lower concentration levels and detection frequencies than in sentinel samples. The results support the relevance of using biological matrices from so-called sentinel animal species for the early detection and identification of POP-like ECs forming an integral part of the human dietary chemical exposome.

Key words: halogenated compounds; potential emerging contaminants; sentinel species; chemical exposome; non-targeted analysis; high-resolution mass spectrometry.

Introduction

The concept of exposome refers to all environmental exposures throughout life.[1] The chemical exposome encompasses the changing exposure to cocktails of chemical substances that vary for each individual throughout his or her life, depending on diet, lifestyle habits, residential and occupational environments.[2,3] This dynamic vision of exposures is a new dimension in the way we approach risk and consider the question of the link between these exposures and potential adverse health effects in humans. Persistent organic pollutants (POPs) are a group of well-known legacy contaminants whose risk has been characterized and that, despite being banned or restricted, continue to pose a threat due to their historical release and persistence in the environment.[4] The Stockholm Convention defines POPs according to four criteria: persistence, long-range transport, bioaccumulation and toxicity. Although polyhalogenation is not itself a defining criterion, all substances listed under the Convention, except for UV-328, are polyhalogenated (chlorine, bromine or fluorine). It is widely acknowledged that food, particularly food of animal origin (FOAO), represents a significant source of human exposure to POPs, accounting for over 90% of exposure for general population, all routes combined.[5-8] The regulatory pressure on POPs has resulted in the emergence of alternative substances that are not yet fully described and characterized.[9] It is anticipated that other unidentified persistent chemicals will be present in the environment and are therefore considered to be emerging contaminants (ECs). Some of these ECs can be released into the environment and enter the human food chain.

The search for ECs has been made more feasible by the advent of suspect screening and non-targeted analysis using high-resolution mass spectrometry (HRMS) coupled with chromatography.[10,11] However, two significant challenges hinder the emergence research as part of the human exposome when analyzing food or human matrices. The first relates to the analytical methodology. In order to explore a wide chemical space, sample preparation must be non-selective, while simultaneously eliminating as many interfering compounds from the matrix as possible. Such sample preparation can affect the analytical sensitivity, resulting in the non-detection of ECs present at relatively low concentrations in food and human fluids (eg, blood, urine). The second relates to ethical and logistical considerations. While human matrices reflect internal exposure and integrate all exposure sources, their use is limited by tissue availability (eg, adipose tissue, brain, liver) and is subject to a rigorous ethical

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framework. Approval and supervision for human samples are time-consuming and are carried out on a case-by-case basis.

Using sentinel animal species to characterize the human dietary exposome offers a practical and ethical alternative to direct sampling (food or humans). Liminal species are especially relevant in this respect. The term "liminal species" is used to describe wild and domestic animals that share their living spaces with humans and whose diet is based on anthropogenic sources.[12] These species can bioaccumulate POP-like ECs in liver and lipid-rich matrices (eg, adipose tissue, egg yolk) over time, providing integrated exposure data. Seeking POP-like ECs in these matrices may help identifying early-warning signals of potential human exposure, and support targeted risk assessments in the food chain. Rivière proposed to consider gull, pigeon and rat species, which are widely distributed worldwide,[13,14] as liminal sentinel species to monitor contamination to which humans are exposed.[15]

Gull is a high trophic level omnivorous species whose diet varies according to its living environment.[16-19] In areas with human activity, its diet is largely composed of food waste found in garbage bins, on refuse dumps or on the ground. Gull is mainly exposed to POPs through its diet and its eggs could concentrate these contaminants up to $\mu g.g^{-1}$ w.w.[20-25] In France, gulls are protected species.[26] However, a derogation may be granted to regulate gull populations by destroying the eggs.[27]

Pigeon is granivorous but, having adapted to urban environments, it has become omnivorous, feeding on seeds, bread and other food waste that are either found or distributed.[28,29] POPs have been quantified in the $ng.g^{-1}$ range in pigeon egg,[30] liver[31,32] and muscle[33] sampled in or near urbanized areas. The growth of pigeon population has resulted in them becoming a nuisance, hence the necessity for the population to be regulated. In France, the regulation techniques employed include the destruction of their eggs or euthanasia.

Brown rat is an opportunistic omnivore whose diet depends on food availability.[34,35] It is frequently used to assess the toxicity of chemicals under controlled experiments. However, wild individuals have also been used to monitor environmental contamination. POPs have been quantified in rat liver,[36-40] adipose tissue and muscle[39] at concentrations ranging $ng.g^{-1}$ to $\mu g.g^{-1}$. Like for pigeon, rat is considered a nuisance, and its population is regulated in France by frightening or rodenticides.

The public health issues raised by many POPs have led public authorities to regulate them internationally, from production to use, and also to set maximum limits in foodstuffs.[41] Many of these substances are known, but entire classes of chemical compounds remain undescribed because they result from unregistered manufacture, or from the degradation of historical molecules in the environment, or their metabolism by animal or plant organisms.[42,43]

The present study was designed to test the sentinel species concept within the context of the human food chain, as a preliminary step before implementing large-scale investigations. As a proof of concept, we assessed gull, pigeon and rat as potential bioconcentrators for the early identification of Cl/Br-containing ECs from the human food chain. A sentinel sample set, including species whose diet is at least partly based on human refuse, was compared to a FOAO sample set including selected food items representative of the French consumption habits. The comparison aimed at assessing which sample set appeared more effective in the early detection and identification of ECs. Sample sets were characterized by both gas and liquid chromatography (GC and LC)–HRMS couplings to identify and semi-quantify potential POP-like ECs. The two sampling strategies (sentinel species and FOAO) were assessed based on their signal prioritization efficiency, that is, high concentration and detection frequency.

Material and methods

Materials and chemicals

Toluene, acetone, cyclohexane, ethyl acetate (LV-GC SuperTrace grade) and dichloromethane (Dioxins, Pesti-S, Furans, PCB's Analysis grade) were purchased from Biosolve (Valkenswaard, The Netherlands). Water and acetonitrile LC-MS grade were obtained from VWR (Radnor, PA, USA) and Riedel-de-Haën (Seelze, Germany), respectively. Anhydrous sodium sulfate (Na_2SO_4) was purchased from Merck (Emsure® grade, Darmstadt, Germany). $^{13}C_{10}$-*anti* Dechlorane Plus (*anti*-DP) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). $^{13}C_{12}$-2,2',3,4,4',6-Hexabromodiphenyl ether (BDE-139), $^{13}C_{12}$-Tetrabromobisphenol A (TBBPA), $^{13}C_{12}$-γ- and $^{2}H_{18}$-β-hexabromocyclododecane (HBCDD) were obtained from Wellington Laboratories (Guelph, Ontario, Canada).

Sample collection

The sentinel sample set consisted of composite samples related to liminal species matrices with the potential to concentrate POP-like ECs. The set included gull egg yolk ($n = 16$), pigeon egg yolk ($n = 4$), adipose tissue ($n = 4$) and liver ($n = 4$), rat adipose tissue ($n = 1$) and liver ($n = 1$). The samples were collected in urban, suburban or peri-urban areas in France between 2015 and 2022 in an opportunistic way through collaborative efforts with research teams and municipal services regulating pest population (Figure 1). All gull eggs were collected under permits from the French "Ministère de la Transition Écologique et Solidaire", the "Ministère de l'Agriculture et de l'Alimentation" and local institutions. The Paris pigeon eggs were laid by individuals that had been captured in Paris and subsequently held in captivity with the approval of the French authorities (authorization APAFIS#17554- 129 201811161046635v2). The other samples relating to pigeon and rat were obtained from the pest control services in Tours and Montpellier municipalities prior to the rendering of the eggs and carcasses, with the approval of the respective mayors. The samples were pooled according to their species, matrices, year and site of collection and in the case of pigeon tissues according to their sex and age. Further details are presented in supporting information Text S1 with associated figures and table.

The FOAO sampling plan was described by Godéré et al.[44] Briefly, the FOAO sample set consisted of 51 composite samples (2 subsamples): fish and seafood ($n = 21$), meat and meat products ($n = 17$), milk and dairy products ($n = 7$), eggs ($n = 3$) and animal fats ($n = 3$). Further details are presented in Table S2. The food items were selected according to the third French individual and national food consumption survey (INCA3),[45] among (i) the most consumed and (ii) the likely high contaminant concentration expected. This selection covered 70.9% of the FOAO consumption for the French adult population group (18-64 years old). For the most consumed items, multiple samples were collected in order to consider various aspects including place of purchase, qualities, preparation type and geographical origin.

Sample preparation

The applied methodology combined common steps for both sample sets including sample preparation (Figure 2).

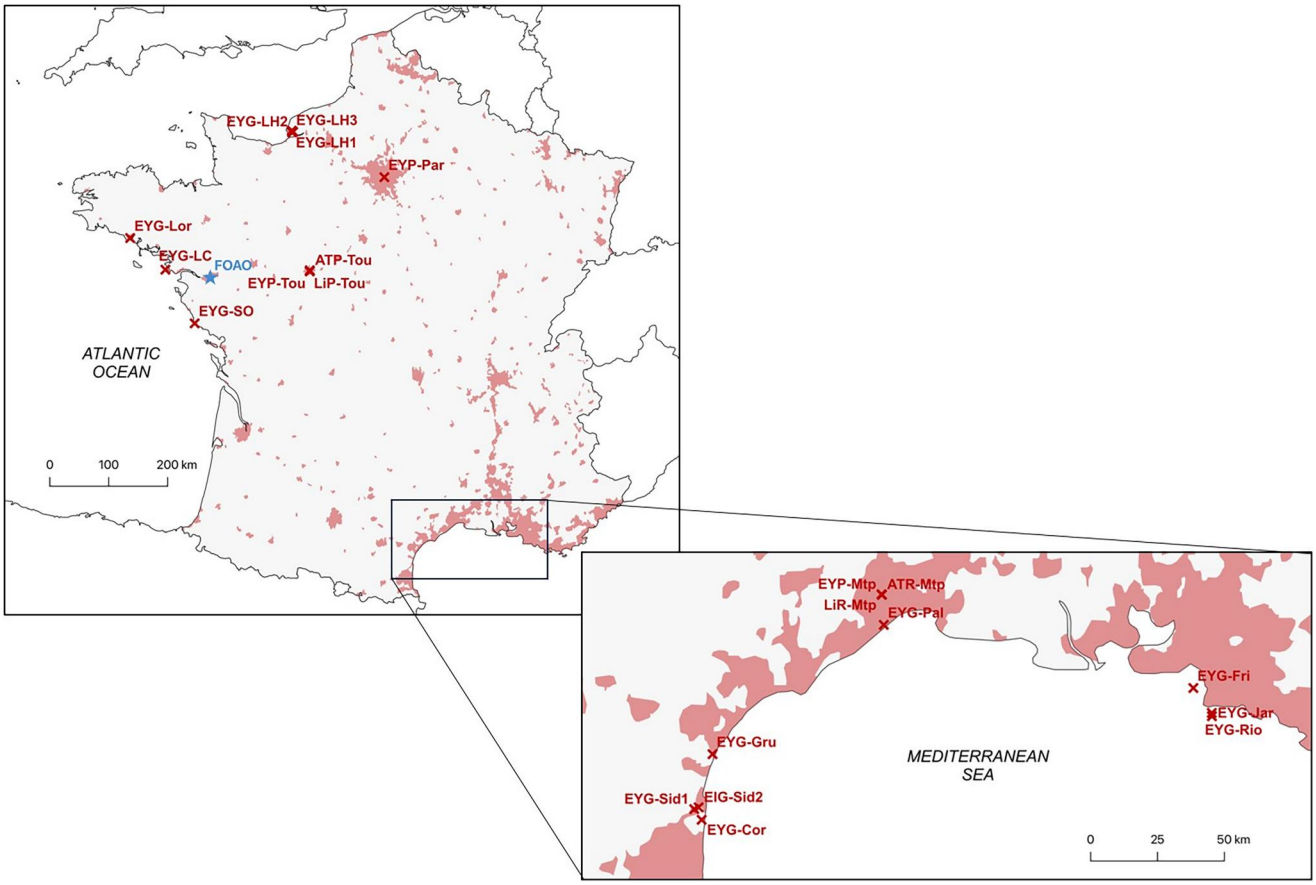

Figure 1. Map of France showing the sampling sites for the sentinel samples (crosses) and the FOAO samples (stars). Coloured zones represent the urbanized areas. EYG: gull egg yolks; EYP: pigeon egg yolk; ATP: pigeon adipose tissue; LiP: pigeon liver; ATR: rat adipose tissue; LiR: rat liver.

Samples were lyophilized and homogenized, with the exception of the butter samples. The dried content was determined gravimetrically. The extraction and clean-up method was described by Padioleau et al.[46] Briefly, contaminants and lipids (≈200 mg; min. = 148 mg; max. = 287 mg) were extracted with mixture of toluene/acetone (70:30, *v/v*) using microwave assisted extraction. The lipid content was determined gravimetrically. The lipids were subsequently eliminated by gel permeation chromatography (GPC). Prior to GPC, the extracts were fortified with $^{13}C_{12}$-γ-HBCDD and $^{13}C_{12}$-BDE-139 (5 ng each) as internal standards (IS). The IS were added after the extraction step, as the test mass was normalized in lipid weight in order to prevent discrimination of the samples based on their lipid content.

Butter subsamples were melted at 45°C for 15 min and allowed for phase separation between lipids and water. Lipid fractions were pooled and cleaned-up by GPC.

Extracts were dried under a gentle stream of nitrogen and reconstituted in 40 µL toluene with $^{13}C_{12}$-TBBPA (5 ng) and $^{13}C_{10}$-*anti*-DP (10 ng) as recovery standards (RS) for GC/HRMS analysis (GC fraction). A 20 µL aliquot of the GC fraction was transferred to a new vial, dried under a gentle stream of nitrogen and reconstituted in 20 µL acetonitrile with $^{2}H_{18}$-β-HBCDD (RS) for LC/HRMS analysis (LC fraction).

Data acquisition

Analyses were carried out separately for sentinel and FOAO samples. The various matrix types (egg, pigeon tissue, rat tissue, fish, meat, …) were grouped in the injection sequence. Chromatographic separations were performed according to the methods described by Padioleau et al.[46] The first analytical stage was identical for both sample sets (GC/ECNI-HRMS and LC/ESI-HRMS; soft ionizations), each set analyzed in distinct sequences. The second analytical stage, which aimed at identifying the structure of the POP-like ECs (GC/EI-HRMS and LC/ESI-MS/HRMS; fragmentations), was carried out on the sentinel sample set only (Figure 2).

GC/HRMS

Electron capture negative ionization (ECNI; soft ionization) or electron ionization (EI; energetic ionization) sources were fitted at the interface between the gas chromatograph and the high-resolution mass spectrometer (Trace 1310 and Q Exactive GC Orbitrap, Thermo Scientific San José, CA, USA). Briefly, the GC fraction (1 µL) was injected onto a DB-5MS column (30 m × 0.25 mm, 0.25 µm, Agilent, Palo Alto, CA, USA) and carried with helium (100°C to 325°C, 10°C.min^{-1}) to the mass spectrometer operated in full-scan mode. The mass spectrometer parameters are detailed in Table S3.

LC/HRMS

An electrospray ionization source (ESI), operated in positive and negative polarities during distinct analytical sequences, was fitted at the interface between the liquid chromatograph and the high-resolution mass spectrometer (Ultimate 3000 UHPLC and Q Exactive Orbitrap, Thermo Scientific). Briefly, the LC fraction (5 µL) was injected on a reverse phase C_{18}-like column (Hypersil

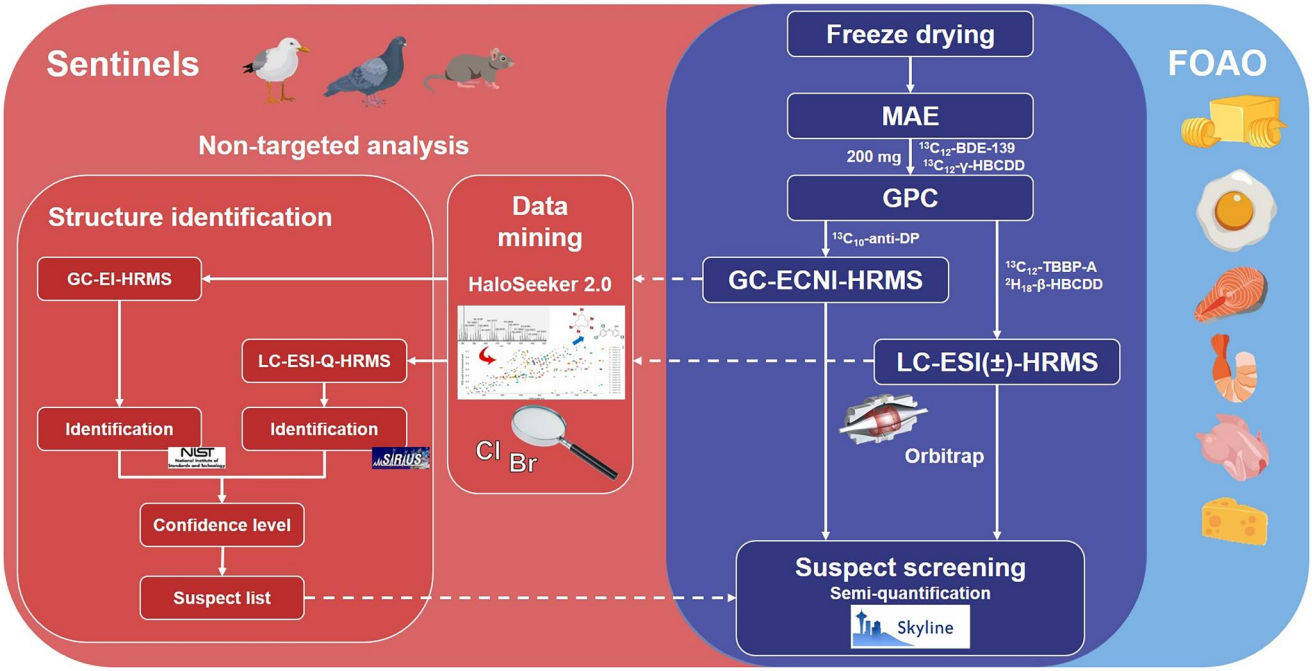

Figure 2. Schematic of the applied methodology for the sentinel and FOAO sample sets to identify and semi-quantify potential POP-like ECs. Middle area: steps applied to both the sentinel and FOAO sample sets; Left: steps applied to the sentinel sample set only; Dashed arrows: use of data from common steps to sentinel-only steps, and vice-versa.

Gold, 100 × 2.1 mm, 1.9 µm fitted with a Hypersil Gold guard column, 10 × 2.1 mm, 1.9 µm, Thermo Fisher Scientific) maintained at 45°C. The mobile phase consisted of water and acetonitrile containing 10 mM ammonium acetate with a flow rate set at 0.4 mL.min^{-1}. The mass spectrometer parameters are detailed in Table S4.

Data processing workflows

Seeking Cl/Br signatures

Data mining was performed on the full scan GC/ECNI-HRMS and LC/ESI-HRMS data for the sentinel sample set using HaloSeeker 2.0,[47] in order to pinpoint chlorine- and/or bromine-containing signals. The proprietary *raw* data were converted in the *mzXML* open format using MSConvert software (Proteowizard version 2.0.3.3) before being imported to HaloSeeker for deconvolution. The software then proceeded to peak picking, pairing—that is isotopic pattern (cluster) reconstitution according to the precise mass differences between naturally occurring C, Cl and Br isotopes—and the alignment of the clusters across the sequences. The data filtering was carried out using the F2+ filter, based on the triplet A (base peak), A–2, A + 2 and ion ratio rules, restricting to the polyhalogenated clusters. Furthermore, an additional filter related to the intensity differences of the aligned cluster between samples and procedural blanks was also applied. The deconvolution and filtering parameters are detailed in Table S5.

The filtered clusters were then reviewed in decreasing order of intensity. In order to discard false positives (non- or monohalogenated clusters), each cluster was manually reviewed using Xcalibur software (version 3.0.63, Thermo Scientific). The coelution, the precise mass difference and the isotopic pattern of the clustered features were subjected to examination.

Chemical formulas were manually annotated using HaloSeeker and Xcalibur in a double-blind procedure considering as first intention H, C, O, Cl, Br, N, P, S and Si up to 50, 30, 10, 10, 10, 10, 10, 5 and 5 elements, respectively. The computed ion formulas were deemed unsuitable if (i) the difference between the precise and the exact *m/z* (measured and theoretical *m/z*, respectively) was greater than ± 1 mDa, (ii) the computed isotopic pattern did not align with the observed one, or (iii) the double-bound equivalent was inconsistent (integer in the absence of adduct formation and float in the presence of adducts).

Structural identification

GC/EI-HRMS

The clusters identified from the GC/ECNI-HRMS data were sought in the GC/EI-HRMS data at the corresponding retention time (t_R) using HaloSeeker. The EI-HRMS mass spectra were cleaned from non-coeluted interfering ions, extracted using Xcalibur and imported into MS Search software (version 2.3, NIST). The EI spectra were then compared using the NIST and MONA low-resolution libraries (267,376 and 18,886 spectra, respectively) and Thermo Fisher high-resolution library (766 spectra). If the computed hits were not satisfying (absence of Cl/Br atoms, incompatible molecular formula, etc.), manual elucidation was performed.

LC/ESI-MS/HRMS

The mass spectra obtained by fragmenting the base peak isotopologue were cleaned from the non-coeluted interfering ions and extracted using Xcalibur. After conversion to *msp* format, fragmentation spectra were loaded to SIRIUS software (version 5.8.0) to compute a fragmentation tree, molecular fingerprints and for searching candidates in various databases.[48] If the computed hits were not satisfying, manual elucidation was performed.

Suspect list

After confidence levelling according to Schymanski et al.[49] identified Cl/Br-containing compounds were included in a suspect list.

Each compound in the suspect list was identified with the t_R, 1 quantifier *m/z* (most intense) and at least 1 confirmation *m/z* (second most intense) across the first analytical stage.

Semi-quantification

Semi-quantification was performed using the suspect list with Skyline software by integrating the peak areas of identified compounds (A_i) at $t_R \pm 0.1$ min.[50] Concentration in samples (C_i) was corrected by the procedural blank (Blk) and IS and expressed in wet weight according to the following equation:

$$C_i = \frac{(A_i - \bar{A}_{i,Blk}) \times Q_{IS} \times \%_{lip}}{A_{IS} \times m_{lip}}$$

Where Q_{IS} and A_{IS} stand for IS quantity (ng) and area, respectively, $\%_{lip}$ and m_{lip} stand for relative and absolute (g) lipid mass, respectively.

Quality assurance/quality control

To minimize procedural contamination, drastic precautions were taken. All glassware was cleaned by calcination (400°C, 4 h) while heat sensitive materials were rinsed with dichloromethane before use. Handling of samples and extracts was carried out in an overpressurized room.

Blanks were injected regularly throughout the analytical sequences. Procedural blanks, consisting of Na_2SO_4 solution (10% by mass, 15 g), undergone all sample preparation steps except the sampling process (field sampling and pooling). The Na_2SO_4 solution was selected to imitate a real wet sample since the lyophilization step. Instrument blanks consisted of IS and RS standard solutions at concentrations identical to those of sample extracts. Apparent clean-up recovery, t_R and *m/z* shifts were monitored using these procedural and instrument blanks as described in Text S2.

Results and discussion

Quality assurance/quality control

The quality assurance and quality control procedures were performed for the GC/ECNI-HRMS, GC/EI-HRMS and LC/ESI(−)-HRMS acquisitions in full scan mode as none of the labelled standards were ionized using ESI(+).

For the sentinel and FOAO sample sets, retention time deviations remained below 0.05 min for GC/HRMS, ensuring proper alignment of detected clusters. However, for LC/HRMS, retention time shifts were observed, particularly in FOAO samples due to column conditioning to the matrices. Mass deviations mostly remained within ± 1 ppm, except for some LC/HRMS samples where deviations of up to ± 10 ppm occurred due to lock mass activation. The signal intensities of RS exhibited variation, likely related to matrix effects or source clogging, especially in GC/ECNI-HRMS. Despite these fluctuations, the apparent recoveries were generally satisfactory, though lower in some matrices like liver and seafood likely due to ion suppression. The observed results of the quality assurance and quality control were not considered to present a limitation for the identification and semi-quantification for the purpose of this pilot study. Further details are presented and discussed in Text S3 sections and associated figure and tables.

Structural identification in sentinel animal samples

The first analytical stage data of the sentinel sample set were reviewed independently and without any preference. Overall, 81.1% (of which 18.0% were false positives), 91.1% (of which 57.8% were false positives) and 45.5% (of which 88.5% were false positives) of the total cumulative area of the filtered clusters were manually reviewed before being identified, for GC/ECNI-HRMS, LC/ESI(−)-HRMS and LC/ESI(+)-HRMS data respectively. The lower proportion reviewed for LC/ESI(+)-HRMS data was due to the higher number of false positives and the lower relative intensity of the clusters. After manual review, 102, 21 and 3 unique Cl/Br-containing compounds were identified from GC/ECNI-HRMS, LC/ESI(−)-HRMS and LC/ESI(+)-HRMS, respectively. The complementarity of the analytical techniques enabled comprehensive coverage of the chemical space since the identified compounds were each detected by only one technique. Legacy contaminants, which are easier to identify, were investigated first. Then, the focus shifted towards identifying POP-like ECs, which are more challenging.

Legacy contaminants

Among the 126 identified Cl/Br-containing compounds, 73 were identified as legacy known and characterized contaminants in environment and food. They were grouped in 3 congener series (#1 to #3) and 3 individual compounds, and were all detected by GC/HRMS.

Further details on their identification are provided Figures S4–S11 and Tables S9–S12. The molecular formula and/or the match with libraries indicated with little doubt the proposed structures. Considering that the aim of this study was not to identify legacy POPs congeners at the highest confidence level, upgrading the identification to level 1 was not deemed a valuable use of resources.

Series #1, including 35 congeners, was annotated $C_{12}H_{10-x}Cl_x$ ($5 \leq x \leq 10$) and was then related to polychlorinated biphenyls (PCBs) at level 2a (MS Search library match) (Figure S4, Tables S9 and S10).

Series #2, including 17 congeners, was annotated $C_{12}H_{10-x}OBr_x$ ($4 \leq x \leq 9$) and was then related to polybrominated diphenyl ether (PBDEs) at level 2a (MS Search library match) or level 2 b (diagnostic evidence) (Figure S5, Tables S11 and S12).

Series #3 was annotated $C_{18}H_{14-x}Cl_x$ ($7 \leq x \leq 10$) and tentatively related to polychlorinated terphenyls (PCTs) at level 3 (tentative candidate) due to a lack of sensitivity using the EI source (Figures S6–S8). Because of the exceptionally high signal content resulting from the vast number of theoretically possible congeners for this family of compounds ($n = 8557$), only 18 congeners, for which there was no ambiguity in chlorine number considering in source fragmentation, were included in the suspect list.

The 3 individual compounds were identified as POPs organochlorine pesticides and their transformation product. The first one was annotated C_6Cl_6 ($m/z_{monoisotopic}$ = 281.814, score = 93.9%, $\Delta m/z$ = –0.05 mDa) and was further confirmed as hexachlorobenzene at level 1 (injection of the analytical standard, Figure S9). The second one was annotated $C_{10}H_4OCl_6$ ($m/z_{monoisotopic}$ = 349.840, score = 91.9%, $\Delta m/z$ = –0.04 mDa) and was then identified as heptachlor epoxide at level 2a (Figure S10). The last one was annotated $C_{12}H_8OCl_6$ ($m/z_{monoisotopic}$ = 377.871, score = 93.21%, $\Delta m/z$ = 0.0 mDa) and was further identified as dieldrin (level 2a) since the endrin analytical standard did not match retention time criteria (Figure S11).

The identification of legacy POPs and POP-like contaminants, which are usually quantified in human diet and plasma in France, demonstrated that this chemical space can be explored by the sampled sentinel matrices.[51-53] This observation indicated that sentinel matrices could be suitable for the identification of POP-like ECs.

Potential POP-like ECs

In contrast with the legacy contaminants previously identified, all the analytical techniques enabled identifying potential POP-like ECs, some of which were part of 6 congener series (#4 to #9). Further details on their identification are provided Figures S12–S21 and Tables S13–S15.

A unique compound, detected by LC/ESI(–)-HRMS, was annotated $[C_{24}H_{12}N_2Cl_4 - H]^-$ ($m/z_{monoisotopic}$ = 466.969) and showed a nitrate adduct (Figures S12–S13). A fragment was then annotated $[C_{12}H_6NCl_2]^-$ from the MS^2 data, indicating a symmetric structure for the parent ion (Figure S14). Among the 19 candidates within PubChem, only two compounds, related to dichlorocarbazole dimers, showed structures that could fragment as observed (Figure 3A). These isomeric dichlorocarbazole dimers differing by the binding sites between the monomer units were proposed as tentative candidates (level 3). To the best of our knowledge, the presence of dichlorocarbazole dimers in the environment or in human matrices has not been reported yet, but at least one has been synthesized by chemical engineering.[54-56] Polyhalogenated carbazoles are POP-like ECs: they exhibit dioxin-like activity, are persistent in soil, are biomagnified in the aquatic food web, and are widely distributed.[57-60] The origin of these substances remains unclear. Some studies suggest an anthropogenic origin while others suggest a natural biotransformation process. Despite the growing interest in polyhalogenated carbazoles over the past decade, no study has yet assessed their risk to human health. The identified dichlorocarbazole dimer may exhibit properties similar to those of polyhalogenated carbazoles, as the present study may demonstrate. The toxicity of the dimer may be different due to its non-planar structure.

Series #4, including 4 hexahalogenated congeners detected by LC/ESI(–)-HRMS, was annotated $[C_{16}H_6N_2Br_{6-x}Cl_x - H]^-$ ($0 \leq x \leq 2$) (Figures S15–S17). The elution pattern, that was attributed to –Br/+Cl substitution, and the close t_R resulted in the classification of these compounds as congeners. Unfortunately, the MS^2 data showed only HBr or HCl successive losses. The origin of the samples provided a clue to the tentative identification of this series, as congeners were only detected in gull egg yolk, which are closely related to the marine environment. The molecular formula $C_{16}H_6N_2Br_6$ was attributed to potential candidates from PubChem: 3 bromobiindole congeners and 2 diynyl-bis bromoaniline congeners. While no information was found on the origin of diynyl-bis bromoaniline, bromobiindoles are halogenated natural products that were extracted from marine cyanobacteria and algae.[61,62] According to Vetter (2006), mixed Cl/Br-containing compounds are rarely produced from anthropogenic sources, except through incineration. They are rather more frequently originated from natural sources, such as marine organisms.[63] This tends to identify this series as hexahalogenated biindole congeners (BIs) at confidence level 3 as MS^2 data could not indicate a potential structure (Figure 3B). Recently, the lipophilic cyanobacterial neurotoxin aetokthonotoxin (AETX), a pentabrominated biindole congener ($C_{17}H_6Br_5N_3$), was identified as responsible of a bald eagle mass mortality.[64] The tentatively identified BIs in the present study may exhibit similar bioactivity and be at risk to the animal and human populations.

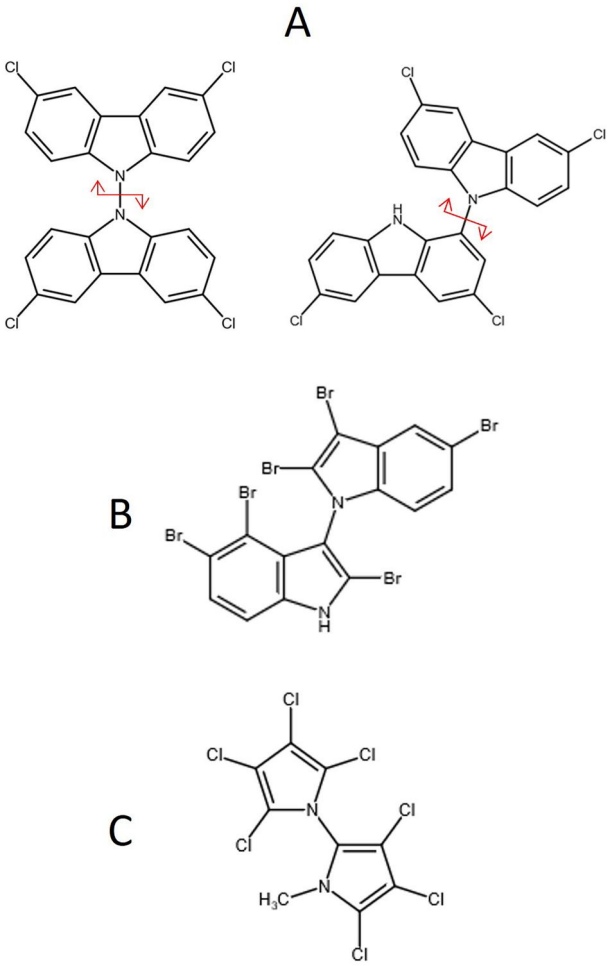

Figure 3. Candidate structures for selected potential POP-like ECs. A: dichlorocarbazole dimers; B: hexabromobiindole; C: 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Cl_7-MBP); Arrow: fragmentation pattern consistent with the MS2 experimental data.

Series #5, including 7 heptahalogenated congeners detected by GC/HRMS, was annotated $C_9H_3N_2Cl_{7-x}Br_x$ ($0 \leq x \leq 2$) and was further identified as heptahalogenated methylbipyrroles (MBPs) (Figure S18–S21). The relative retention times were found to be in accordance with the –Cl/+Br substitution, which resulted in the formation of less volatile compounds. The EI mass spectra of the heptachlorinated congener matched with 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Cl_7-MBP), thus upgrading the confidence to level 2a for this congener (Figure 3C). The mixed Cl/Br congeners were identified as mixed-MBPs at level 2b. The –Cl/+Br substitution was previously reported for MBPs.[65,66] MBPs are suspected to be halogenated natural products due to their identification in samples originating from marine ecosystems across various locations.[65,67] While the biomagnification of MBPs in the marine food web and human exposure has been reported,[68-71] no toxicological studies have been carried out, to the best of our knowledge and despite growing interest.

Data from 42 clusters ($n = 23$ for GC/HRMS, Table S13; $n = 16$ for LC/ESI(–)-HRMS, Table S14; $n = 3$ for LC/ESI(+)-HRMS, Table S15) did not meet the criteria for identification at a confidence level lower than 3 for various reasons. For some clusters identified using the ECNI source, it was not possible to carry out comparison with spectral libraries due to a lack of sensitivity using

the EI source. For some other clusters detected using these two ionization sources, the matching with spectral libraries was not satisfying. The LC/ESI-MS/HRMS data were sometimes meaningless due to consecutive loss of halogen atoms, which provided no structural information. Besides, the MS^2 data may have provided information that was not consistent with the annotation. Furthermore, structural elucidation using SIRIUS was often a failure due to the confusion between the monoisotopic and the most intense signals. The identification of Cl/Br-containing compounds assisted with bioinformatics software has been identified as an issue.[72] The development of dedicated software that can take into account the full isotopic pattern is required. Among these clusters, 4 series were established from GC/HRMS acquisitions. Series #6, including 2 congeners, was annotated $C_8H_2Cl_6$ without tentative structure(s). Series #7, including 2 congeners, was annotated $C_{10}H_5Cl_9$ with a proposed structure analogous to nonachlor. Series #8, including 4 congeners, suggested a pentachlorinated isotopic pattern but annotation remained equivocal. The annotation of series #9, including 4 congeners, remained equivocal as well, although the isotopic pattern suggested hexachlorinated congeners. Series #8 and #9 may be related to each other due to a $\Delta m/z = 33.961$ Da, corresponding to the –H/+Cl substitution. The relative retention times supported this hypothesis. Despite the low confidence level associated with these 42 compounds, they were included in the suspect list and semi-quantified in both sample sets. They will be denominated from their feature information [$m/z_{monoisotopic}$@t_R] in the following paragraphs.

Suspect screening and semi-quantification

The suspect list contained 126 Cl/Br-containing compounds, identified from the sentinel sample set as outlined in the previous paragraphs. Due to the inherent uncertainty associated with the performed semi-quantification, which was dependent on the relative response of the analyte signal in comparison to the selected labelled standard, the concentrations discussed in this paragraph are expressed in orders of magnitude. Semi-quantification results and further details about the suspect screening results are provided in Table S16. In the following paragraphs, semi-quantification results are discussed to verify consistency with previous studies before comparing the sample sets. These results may reveal potential trends across the samples within each sample set.

Sentinel sample set

PCBs, PBDEs, PCTs and the 3 organochlorine pesticides were quantified in the sentinel matrices with orders of magnitude ranging from pg.g^{-1} to µg.g^{-1} w.w., which appeared consistent with the literature.[20-25,30,31,33,36,73] For many of these POPs, the highest concentrations were observed in gull egg yolks. This could be explained by an easier access to fish and seafood, which are known to be a major source of POPs exposure.[5-8] In pigeon, adipose tissue seemed to concentrate much more PBDEs than liver and egg yolk, a finding that has also been reported for herons.[74]

The dichlorocarbazole dimer was identified in almost all the samples at concentrations in the range of ng.g^{-1} w.w. The concentrations in gull egg yolks did not demonstrate local trend. On the other hand, the pigeon egg yolks from Montpellier, a town located a few kilometers from the coast, evidenced higher concentrations than the samples obtained in Paris and Tours. Estimated concentrations for pigeon adipose tissue could indicate bioaccumulation of the dimer, considering that the adults concentrated more than the young did.

BI and MBP congeners were only identified in gull egg yolks at concentrations ranging from a dozen of pg.g^{-1} w.w. to hundreds of ng.g^{-1} w.w. Their identification in gull egg yolks exclusively may support the natural marine origin, as previously suggested.

The other compound concentrations ranged from pg.g^{-1} w.w. to ng.g^{-1} w.w. The highest concentration was estimated for [297@17.9], with more than 200 ng.g^{-1} w.w. in 2 pigeon adipose tissue samples. Apart from a few exceptions, these other compounds were present in all gull samples, regardless of the colony's location, suggesting a wide spatial distribution across the French littoral.

FOAO sample set

The suspect screening resulted in the identification of 71 out of the 126 Cl/Br-containing compounds in the FOAO sample set. Among them, 23 PCBs, 7 PBDEs and the 3 organochlorine pesticides were identified with estimated concentrations ranging from pg.g^{-1} w.w. to ng.g^{-1} w.w. The highest estimated concentrations were observed for seafood products. The orders of magnitude of concentrations and contamination patterns were consistent with those reported in the literature,[5-8] suggesting a reliable semi-quantification.

The dichlorocarbazole dimer was identified in 4 meat and meat products at lower level than those observed in the sentinel samples (<0.1 ng.g^{-1} w.w.).

Some BI and MBP congeners were identified in the FOAO sample set. While 4 BIs were included in the suspect list, Br_6-BI was the only identified congener in 6 seafood products with estimated concentrations that did not exceed a few ng.g^{-1} w.w. for the Atlantic mussel. These concentrations were in the same range as in gull egg yolks, but below the most contaminated samples. The 7 MBPs included in the suspect list were all observed in seafood products. In addition, Cl_7-MBP was also identified in terrestrial foodstuffs but with lower concentrations. The estimated concentrations ranged from pg.g^{-1} w.w. to hundreds of ng.g^{-1} w.w. The highest estimated concentration was observed for great scallops at one order of magnitude above the most contaminated gull egg yolk. Once more, the detection of BIs and MBPs mostly in seafood products supports the identification of halogenated natural products of marine origin.

Among the 42 compounds identified at confidence level 3 and above, 28 were detected in the FOAO sample set. The estimated concentrations were mostly below a dozen ng.g^{-1} w.w. although samples reached concentrations above hundreds ng.g^{-1} w.w for [297@17.9]. While this latter compound was quantified in 2 sentinel samples, it was semi-quantified in 51% of the FOAO samples, including almost all meat and meat products at the higher concentrations. This observation supports the hypothesis of a ubiquitous contaminant.

Furthermore, the cross exploration of the data acquired for the 2 sample sets provided a basis for formulating of new proposals regarding the origin of potential POP-like ECs. The recurrent detection of [354@22.3], [318@7.5], [454@14.1], [542@15.4] and [542@18.8] in seafood products, as well as in gull eggs, suggested that these compounds are of marine origin, or at the very least, that the marine environment is highly contaminated by them. Conversely, the detection in multiple FOAO categories indicates the ubiquitous presence of potential POP-like ECs, including [297@17.9], [160@7.7], [627@28.5] and [406@19.7], as well as series #6.

Sample sets comparison

The aim of comparing the results between the sentinel and FOAO sample sets was to determine whether the early identification of new POPs such as ECs is effective when either of these sampling strategies is employed. In the present study, a signal prioritization to assess this efficacy was proposed based on two criteria:

- detection frequency, which highlights the prevalence of a compound across the sample set;
- estimated concentration levels, which provide an indication of the signal intensity.

A statistical test was carried out on the estimated concentrations to identify significant differences between the two sample sets, ensuring robustness in comparing contaminant profiles. Detection frequency was then considered a complementary criterion to mitigate potential biases introduced by elevated concentrations in a limited number of samples, enabling a balanced and representative assessment of the overall contamination patterns.

The results of Shapiro-Wilk tests indicated that the concentrations of each compound were not normally distributed in both sample sets (P-value < 0.05). Consequently, Mann-Whitney U tests were performed to compare the estimated concentrations between the two sample sets.

The tests demonstrated statistically significant concentration differences between the sample sets for 23 out of 31 compounds or congener series (P-value < 0.05) (Table 1 and Figure S22). Series #5 and [297@17.9] concentrations were significantly higher in the FOAO sample set with detection frequencies greater than 25%. In this case, the application of a sentinel sampling strategy did not appear relevant to effectively prioritize these potential POP-like ECs. In contrast, the concentrations of the remaining 21 compounds or series were significantly higher in the sentinel sample set. In addition, 17 of them were detected in the FOAO sample set with a frequency lower than 25%, including BIs and

Table 1. Identification, detection frequency (%) and mean estimated concentration (ng.g^{-1} w.w.) for the Cl/Br-containing compounds included in the suspect list.

					Sentinel		FOAO		
Analysis	**Name**	**Molecular formula**	**Structure**	**Confidence level**	**$F_{detection}$**	**Mean conc.**	**$F_{detection}$**	**Mean conc.**	**p-value**
GC/ECNI	Series #1	$C_{12}H_{10-x}Cl_x$	PCBs	2a	77	517	37	0.9	<0.001
	Series #2	$C_{12}H_{10-x}OBr_x$	PBDEs	2a/2b	100	382	61	4	<0.001
	Series #3	$C_{18}H_{14-x}Cl_x$	PCTs	2b	83	4	ND	ND	
	[281@12.1]	C_6Cl_6	Hexachlorobenzene	1	90	19	76	2	<0.001
	[349@15.6]	$C_{10}H_5OCl_7$	Heptachlor epoxide	2a	57	0.6	6	<0.01	<0.001
	[377@16.7]	$C_{12}H_8Cl_6O$	Dieldrin	2a	73	32	41	0.2	<0.001
	Series #5	$C_9H_3N_2Cl_{7-x}Br_x$	Heptahalogenated methylbipyrrolles (MBPs)	2a/2b	53	19	29	26	0.036
	[327@11.4]	$C_6H_3OBr_3$	Tribromophenol	3	60	0.3	14	0.01	<0.001
	Series #6	$C_8H_2Cl_6$		4	53	0.4	20	0.04	<0.001
	Series #7	$C_{10}H_5Cl_9$	Nonachlor	3	53	2	8	0.03	<0.001
	[365@16.9]	$C_{10}HCl_7$	Heptachloronaphtalene	3	53	0.1	2	<0.01	<0.001
	[297@17.9]	$C_{13}H_5O_2Cl_3$	Trichloroxanthone	3	7	16	51	70	<0.001
	[373@18.5]	$C_{12}H_4OCl_6$	Hexachlorodiphenyl ether	3	63	0.6	10	<0.01	<0.001
	Series #8			5	80	9	8	0.02	<0.001
	[451@21.4]	$C_{14}H_4Cl_8$		4	70	2	ND	ND	
	[422@22.0]			5	30	2	6	<0.01	0.002
	[354@22.3]	$C_{20}H_{12}O_2Cl_2$		4	53	6	14	0.2	<0.001
	Series #9			5	70	3	8	0.01	<0.001
	[491@24.9]			5	17	0.2	ND	ND	
	[611@25.1]			5	53	0.02	ND	ND	
	[469@25.7]	$C_{13}H_2O_2Cl_8$		4	27	0.1	ND	ND	
LC/ESI(−)	[467@24.8]	$C_{24}H_{12}N_2Cl_4$	Dichlorocarbazole dimer	3	93	2	8	<0.01	<0.001
	Series #4	$C_{16}H_6N_2Br_{6-x}Cl_x$	Hexahalogenated biindoles (BIs)	3	33	3	12	0.07	0.013
	[273@2.0]	$C_7H_3NOBr_2$	Bromoxynil	3	13	0.9	ND	ND	
	[220@5.6]	$C_7H_4O_4Cl_2$	Dichlorodihydroxybenzoic acid	3	37	0.4	ND	ND	
	[202@6.3]	$C_8H_6O_2Cl_2$	Dichlorohydroxyphenylethanone	3	3	0.01	6	0.02	0.630
	[262@7.5]	C_6HOCl_5	Pentachlorophenol	3	67	3	18	0.01	<0.001
	[318@7.5]	$C_{10}H_{10}O_2Br_2$	Dibromophenylbutanoic acid	3	17	0.02	16	0.09	0.927
	[312@7.7]	$C_{14}H_{13}NO_3Cl_2$		4	57	0.2	6	<0.01	<0.001
	[160@7.7]	$C_6H_4OCl_2$	Dichlorophenol	3	90	4	55	0.2	<0.001
	[408@9.7]			5	33	0.7	ND	ND	
	[758@10.6]			5	10	0.4	12	0.02	0.879
	[578@11.2]	$C_{16}H_{10}N_4O_7Cl_6$		4	20	0.05	6	<0.01	0.037
	[592@13.8]			5	27	0.07	8	<0.01	0.012
	[454@14.1]	$C_{12}H_{17}O_2Br_2Cl_3$		4	27	0.2	29	0.6	0.792
	[542@15.4]			5	13	0.1	6	<0.01	0.210
	[542@18.8]			5	27	0.03	27	0.08	0.965
	[620@21.0]			5	23	0.1	ND	ND	
	[627@28.5]			5	37	5	43	0.01	0.435
LC/ESI(+)	[406@19.7]			5	30	0.1	35	0.02	0.551
	[553@28.6]			5	7	0.04	ND	ND	
	[597@28.7]			5	7	0.04	ND	ND	

ND, not detected.

dichlorocarbazole dimer. The identification of these 17 potential POP-like ECs could be prioritized and identified more efficiently using a sentinel sampling strategy based on liminal species.

The concentrations of 8 compounds were not statistically different (P-value > 0.05). The detection frequencies for these 8 compounds were low in both sample set, which can explain these results using a Mann-Whitney U test.

Furthermore, PCTs and 8 compounds were not identified in the FOAO sample set. This does not indicate the absence in the samples but suggest at least that the detection limit of the method may be greater than the actual concentrations. The detection of these compounds with more sensitive analytical methods could support a more efficient signal prioritization through the analysis of sentinel samples.

It is important to note that the sentinel samples analyzed in the present study were derived from multiple pooled aliquots, which introduces a dilution effect and prevents any inter-individual investigation. Consequently, the concentration levels in some individual samples may be significantly higher than those reported here.

Although pigeon and rat were promising sentinel species due to their high dependence on human refuse, concentrations and detection frequencies in the sampled matrices were often lower than those observed in gull egg. The preference of pigeons and rats for seed-based food could explain the lower concentrations. In addition, low concentrations in pigeon egg could be explained by the high reproductive rate, up to five clutches per year,[28] whereas gull lays only once a year, allowing higher bioaccumulation until the contaminant discharge to the egg yolk. Overall, the statistical power remains limited for these two species to draw any conclusion, especially for rat.

Despite limited statistical power to compare sentinel species between each other regarding differences in the number of samples, gull appears to be relevant for exploring the chemical contaminants present in human food. However, since this species can feed on food rejected by humans as well as seafood because of its living environment, it could be argued that contamination linked to the consumption of seafood would be considered more as environmental contamination, even if certain seafood products enter the human food chain. Gastric content analysis could provide valuable insight on the dietary habits of the gull. Yet, such analysis can only be carried out by sampling dead animals, and therefore only reflects the last consumed foodstuffs. Anyhow, analysis of the stable isotope ratios of carbon and nitrogen in eggs could provide information on food sources and trophic level, respectively.[75]

Conclusions

This study assumed that liminal species could serve as an early warning for prioritizing and identifying ECs conveyed through to the human food chain. This potential is supported by their diet based on human refuse and their capacity to bioconcentrate these ECs. The applied HRMS-based methodology was successful in identifying 126 Cl/Br-containing compounds including 73 legacy contaminants and 53 potential POP-like ECs, some of which could be grouped into 9 congener series. A limited number of potential POP-like ECs were identified with a candidate structure (level 2), including dichlorocarbazole dimer and MBPs. The human exposure to these compounds is not fully characterized, if at all. Considering its presence at relatively higher concentrations in many FOAO items, it appears particularly important to focus efforts on the identification confirmation of [297@17.9], tentatively identified as trichloroxanthone. In addition, the combined analysis of samples from aquatic and terrestrial environments may confirm or discard some candidates based on their suggested origin. The identification of potential ECs with the highest confidence levels remains challenging, particularly when Cl/Br-containing compounds must be identified due to the lack of bioinformatics software designed to compute the entire isotopic pattern.

The suspect screening strategy resulted in the identification of 71 Cl/Br-containing compounds across both sentinel and FOAO sample sets, with most compounds exhibiting significantly higher concentrations and detection frequencies in the sentinel sample. These findings underscore the promise of signal prioritization when using a sentinel sampling strategy based on liminal species. The use of liminal sentinel species demonstrates strong potential for the early detection of ECs within the human food chain, providing a valuable tool for advancing the characterization of the human dietary exposome. Developing such a strategy will require developing sampling networks. Future research should also focus on quantifying human internal exposure, in the studied areas, to these POP-like ECs to better assess associated risks.

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Author contributions

Antonin Padioleau (Conceptualization [equal], Data curation [equal], Methodology [equal], Writing—original draft [equal]), Bruno Le Bizec (Conceptualization [equal], Funding acquisition [equal], Resources [equal], Writing—review & editing [equal]), and Gaud Dervilly (Conceptualization [equal], Methodology [equal], Supervision [equal], Writing—review & editing [equal]), Ronan Cariou (Conceptualization [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—review & editing [equal])

Supplementary material

Supplementary material is available at *Exposome* online.

The following files are available free of charge. Detailed description of the sentinel and FOAO samples, additional analytical and QA/QC elements, Figures and Tables relatives to the annotation and the identification of the Cl/Br-containing compounds (mass defect plots, chromatograms and mass spectra) (PDF). Detailed concentrations for the Cl/Br-containing compounds in the sentinel and FOAO samples (XLSX).

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

1. Wild CP. The exposome: from concept to utility. *Int J Epidemiol*. 2012;41:24–32. https://doi.org/10.1093/ije/dyr236
2. Pruvost-Couvreur M, Le Bizec B, Béchaux C, Rivière G. A method to assess lifetime dietary risk: Example of cadmium exposure. *Food Chem Toxicol*. 2020;137:111130. https://doi.org/10.1016/j.fct.2020.111130
3. Gastellu T, Le Bizec B, Rivière G. Integrating the lifelong exposure dimension of a chemical mixture into the risk assessment process. Application to trace elements. *Food Chem Toxicol*. 2025;195:115111. https://doi.org/10.1016/j.fct.2024.115111
4. UNEP. *Final Act of the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic*. United Nations Environment Programme; 2001. https://chm.pops.int/Portals/0/docs/from_old_website/documents/meetings/dipcon/25june2001/conf4_finalact/en/FINALACT-English.PDF
5. Baars AJ, Bakker MI, Baumann RA, *et al*. Dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs: occurrence and dietary intake in The Netherlands. *Toxicol Lett*. 2004;151:51–61. https://doi.org/10.1016/j.toxlet.2004.01.028
6. Darnerud PO, Atuma S, Aune M, *et al*. Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. *Food Chem Toxicol*. 2006;44:1597–1606. https://doi.org/10.1016/j.fct.2006.03.011
7. Polder A, Savinova TN, Tkachev A, Løken KB, Odland JO, Skaare JU. Levels and patterns of Persistent Organic Pollutants (POPs) in selected food items from Northwest Russia (1998–2002) and implications for dietary exposure. *Sci Total Environ*. 2010;408:5352–5361. https://doi.org/10.1016/j.scitotenv.2010.07.036
8. Fernandes AR, Mortimer D, Rose M, Smith F, Steel Z, Panton S. Recently listed Stockholm convention POPs: Analytical methodology, occurrence in food and dietary exposure. *Sci Total Environ*. 2019;678:793–800. https://doi.org/10.1016/j.scitotenv.2019.04.433
9. Muir DCG, Howard PH. Are there other persistent organic pollutants? A challenge for environmental chemists. *Environ Sci Technol*. 2006;40:7157–7166. https://doi.org/10.1021/es061677a
10. Hollender J, Schymanski EL, Singer HP, Ferguson PL. Nontarget screening with high resolution mass spectrometry in the environment: ready to go? *Environ Sci Technol*. 2017;51:11505–11512. https://doi.org/10.1021/acs.est.7b02184
11. Simonnet-Laprade C, Bayen S, McGoldrick D, *et al*. Evidence of complementarity between targeted and non-targeted analysis based on liquid and gas-phase chromatography coupled to mass spectrometry for screening halogenated persistent organic pollutants in environmental matrices. *Chemosphere* 2022;293:133615. https://doi.org/10.1016/j.chemosphere.2022.133615
12. O'Connor T. *Animals as Neighbors: The Past and Present of Commensal Animals*. Michigan State University Press; 2013.
13. Winkler DW, Billerman SM, Lovette IJ. Gulls, Terns, and Skimmers (Laridae), version 1.0. *Birds World*. Published online 2020. https://doi.org/10.2173/bow.larida1.01species_shared.bow.project_name
14. Winkler DW, Billerman SM, Lovette IJ. Pigeons and Doves (Columbidae), version 1.0. *Birds World*. Published online 2020. https://doi.org/10.2173/bow.columb2.01species_shared.bow.project_name
15. Rivière JL. Les animaux sentinelles. *Courr Environ INRA* 1993;59–67.
16. Duhem C, Vidal E, Legrand J, Tatoni T. Opportunistic feeding responses of the Yellow-legged Gull Larus michahellis to accessibility of refuse dumps. *Bird Study*. 2003;50:61–67. https://doi.org/10.1080/00063650309461291
17. Bond AL. Diet changes in breeding Herring Gulls (Larus argentatus) in Witless Bay, Newfoundland and Labrador, Canada, over 40 Years. *Waterbirds*. 2016;39(sp1):152–158. https://doi.org/10.1675/063.039.sp115
18. Ramos R, Ramírez F, Sanpera C, Jover L, Ruiz X. Diet of Yellow-legged Gull (Larus michahellis) chicks along the Spanish Western Mediterranean coast: the relevance of refuse dumps. *J Ornithol*. 2009;150:265–272. https://doi.org/10.1007/s10336-8–0346-2
19. Marinao C, Kasinsky T, Suárez N, Yorio P. Contribution of recreational fisheries to the diet of the opportunistic Kelp Gull. *Austral Ecol*. 2018;43:861–875. https://doi.org/10.1111/aec.12627
20. Roscales JL, Vicente A, Muñoz-Arnanz J, *et al*. Influence of trophic ecology on the accumulation of dioxins and furans (PCDD/Fs), non-ortho polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in Mediterranean gulls (Larus michahellis and L. audouinii): A three-isotope approach. *Environ Pollut*. 2016;212:307–315. https://doi.org/10.1016/j.envpol.2016.01.078
21. Kocagöz R, Onmuş O, Onat İ, Çağdaş B, Sıkı M, Orhan H. Environmental and biological monitoring of persistent organic pollutants in waterbirds by non-invasive versus invasive sampling. *Toxicol Lett*. 2014;230:208–217. https://doi.org/10.1016/j.toxlet.2014.01.044
22. Morales L, Martrat MG, Olmos J, *et al*. Persistent organic pollutants in gull eggs of two species (Larus michahellis and Larus audouinii) from the Ebro delta Natural Park. *Chemosphere*. 2012;88:1306–1316. https://doi.org/10.1016/j.chemosphere.2012.03.106
23. de Solla SR, Weseloh DVC, Hughes KD, Moore DJ. Forty-year decline of organic contaminants in eggs of Herring Gulls (Larus argentatus) from the Great Lakes, 1974 to 2013. *Waterbirds* 2016;39(sp1):166–179. https://doi.org/10.1675/063.039.sp117
24. Jang M, Shim WJ, Han GM, *et al*. Spatial distribution and temporal trends of classical and emerging persistent organic pollutants (POPs) in black-tailed gull (Larus crassirostris) eggs from Korea. *Sci Total Environ*. 2022;845:157244. https://doi.org/10.1016/j.scitotenv.2022.157244
25. Knudtzon NC, Thorstensen H, Ruus A, *et al*. Maternal transfer and occurrence of siloxanes, chlorinated paraffins, metals, PFAS and legacy POPs in herring gulls (Larus argentatus) of different urban influence. *Environ Int*. 2021;152:106478. https://doi.org/10.1016/j.envint.2021.106478
26. Arrêté Du 29 Octobre 2009 Fixant La Liste Des Oiseaux Protégés Sur l'ensemble Du Territoire et Les Modalités de Leur Protection; 2009. Accessed October 18, 2023. https://www.legifrance.gouv.fr/loda/id/JORFTEXT000021384277
27. Article L411-2 - Code de l'environnement; 2021. Accessed October 27, 2023. https://www.legifrance.gouv.fr/codes/article_lc/LEGIARTI000044192443

28. Johnston RF, Janiga M. *Feral Pigeons*. Oxford University Press; 1995.
29. Sol D, Santos DM, Garcia J, Cuadrado M. Competition for food in urban pigeons: the cost of being juvenile. *Condor*. 1998;100:298–304. https://doi.org/10.2307/1370270
30. Pandelova M, Schramm KW. Human and environmental biomonitoring of polychlorinated biphenyls and hexachlorobenzene in Saxony, Germany based on the German Environmental Specimen Bank. *Int J Hyg Environ Health*. 2012;215:220–223. https://doi.org/10.1016/j.ijheh.2011.11.005
31. Merino R, Bordajandi LR, Abad E, Rivera J, Jiménez B. Evaluation of organochlorine compounds in peregrine falcon (falco peregrinus) and their main prey (columba livia) inhabiting central Spain. *Environ Toxicol Chem*. 2005;24:2088–2093. https://doi.org/10.1897/04-018R.1
32. Hong SH, Reiner JL, Jang M, *et al.* Levels and profiles of perfluorinated alkyl acids in liver tissues of birds with different habitat types and trophic levels from an urbanized coastal region of South Korea. *Sci Total Environ*. 2022;806(Pt 3):151263. https://doi.org/10.1016/j.scitotenv.2021.151263
33. Hong SH, Shim WJ, Han GM, *et al.* Levels and profiles of persistent organic pollutants in resident and migratory birds from an urbanized coastal region of South Korea. *Sci Total Environ*. 2014;471:1463–1470. https://doi.org/10.1016/j.scitotenv.2013.07.089
34. Traweger D, Slotta-Bachmayr L. Introducing GIS-modelling into the management of a brown rat (Rattus norvegicus Berk.) (Mamm. Rodentia Muridae) population in an urban habitat. *J Pest Sci*. 2005;78:17–24. https://doi.org/10.1007/s10340-4–0062-5
35. Clapperton B. *A Review of the Current Knowledge of Rodent Behaviour in Relation to Control Devices. Science for Conservation 263*. Science & Technical Publishing; 2006:1–56.
36. Takasuga T, Senthilkumar K, Ishizuka M, Fujita S, Tanikawa R. Accumulation of chlorinated and brominated persistent toxic substances (PTS) and their relationship to testosterone suppression in Norway rats from Japan. Published online September 15, 2004. Accessed November 7, 2023. https://www.osti.gov/etdeweb/biblio/20828298
37. Yohannes YB, Ikenaka Y, Ito G, *et al.* Assessment of DDT contamination in house rat as a possible bioindicator in DDT-sprayed areas from Ethiopia and South Africa. *Environ Sci Pollut Res Int*. 2017;24:23763–23770. https://doi.org/10.1007/s11356-7–9911-7
38. Motohira K, Ikenaka Y, Yohannes YB, *et al.* Dichlorodiphenyltrichloroethane (DDT) levels in rat livers collected from a malaria vector control region. *J Vet Med Sci*. 2019;81:1575–1579. https://doi.org/10.1292/jvms.9–0168
39. Kuvarega AT, Taru P. Accumulation of Endosulfan in Wild Rat, Rattus norvegious as a Result of Application to Soya Bean in Mazoe (Zimbabwe). *Environ Monit Assess*. 2007;125:333–345. https://doi.org/10.1007/s10661-6–9526-9
40. Taniyasu S, Senthilkumar K, Yamazaki E, *et al.* Perfluoroalkyl substances in the blood of wild rats and mice from 47 prefectures in Japan: use of samples from Nationwide Specimen Bank. *Arch Environ Contam Toxicol*. 2013;65:149–170. https://doi.org/10.1007/s00244-3–9878-4
41. Commission Regulation (EU) 2023/915 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006. Vol 119; 2023. Accessed August 18, 2025. http://data.europa.eu/eli/reg/2023/915/oj/eng
42. Salvito D, Fernandez M, Jenner K, *et al.* Improving the environmental risk assessment of substances of unknown or variable composition, complex reaction products, or biological materials. *Environ Toxicol Chem*. 2020;39:2097–2108. https://doi.org/10.1002/etc4846
43. Cwiertny DM, Snyder SA, Schlenk D, Kolodziej EP. Environmental Designer Drugs: When Transformation May Not Eliminate Risk. *Environ Sci Technol*. 2014;48:11737–11745. https://doi.org/10.1021/es503425w
44. Godéré M, Cariou R, Padioleau A, *et al.* Polychlorinated naphthalenes in foods from the French market: Occurrence, dietary exposure, and evaluation of relative contributions to dioxin-like contaminants. *Environ Sci Technol*. 2024;58:1721–1730. https://doi.org/10.1021/acs.est.3c07838
45. Anses. *Étude Individuelle Nationale Des Consommations Alimentaires 3 (INCA 3) - Avis de L'Anses—Rapport D'expertise Collective*. Anses; 2017.
46. Padioleau A, Cariou R, Guiffard I, *et al.* Non-targeted analysis of lipidic extracts by high-resolution mass spectrometry to characterise the chemical exposome: comparison of four clean-up strategies applied to egg. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2024;1232:123963. https://doi.org/10.1016/j.jchromb.2023.123963
47. Léon A, Cariou R, Hutinet S, *et al.* HaloSeeker 1.0: a user-friendly software to highlight halogenated chemicals in nontargeted high-resolution mass spectrometry data sets. *Anal Chem*. 2019;91:3500–3507. https://doi.org/10.1021/acs.analchem.8b05103
48. Dührkop K, Fleischauer M, Ludwig M, *et al.* SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information. *Nat Methods*. 2019;16:299–302. https://doi.org/10.1038/s41592-9–0344-8
49. Schymanski EL, Jeon J, Gulde R, *et al.* Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Env Sci Technol*. 2014;48.
50. Adams KJ, Pratt B, Bose N, *et al.*; Alzheimer's Disease Metabolomics Consortium. Skyline for small molecules: a unifying software package for quantitative metabolomics. *J Proteome Res*. 2020;19:1447–1458. https://doi.org/10.1021/acs.jproteome.9b00640
51. Frenoy P, Cano-Sancho G, Antignac JP, *et al.* Blood levels of persistent organic pollutants among women in France in the 90's: Main profiles and individual determinants. *Environ Res*. 2024;258:119468. https://doi.org/10.1016/j.envres.2024.119468
52. Anses. *Etude de L'alimentation Totale Française 2 - Tome 1 : Contaminants Inorganiques, Minéraux, Polluants Organiques Persistants, Mycotoxines, Phyto-Estrogènes*. Agence Nationale de Sécurité Sanitaire Alimentation, Environnement, Travail; 2011.
53. Anses. *Etude de L'alimentation Totale Française 2 - Tome 2 : Résidus de Pesticides, Additifs, Acrylamide, Hydrocarbures Aroatiques Polycycliques*. Agence Nationale de Sécurité Sanitaire Alimentation, Environnement, Travail; 2011.
54. Balakrishna B, Mossin S, Kramer S. Photo-induced metal-free dehydrogenative N–N homo-coupling. *Chem Commun (Camb)*. 2022;58:10977–10980. https://doi.org/10.1039/D2CC04204E
55. Yin D, Jin J. Transition-metal-free dehydrogenative N–N coupling of secondary amines with KI/KIO4. *Eur J Org Chem*. 2019;2019:5646–5649. https://doi.org/10.1002/ejoc.201900763
56. Balsells RE, Frasca AR. Photochemical reaction of carbazole and some derivatives in dichloromethane. *Tetrahedron Lett*. 1984;25:5363–5366. https://doi.org/10.1016/S0040-4039(01)91284-5
57. Mumbo J, Lenoir D, Henkelmann B, Schramm KW. Enzymatic synthesis of bromo- and chlorocarbazoles and elucidation of their structures by molecular modeling. *Environ Sci Pollut Res Int*. 2013;20:8996–9005. https://doi.org/10.1007/s11356-3–1823-6
58. Chen Y, Lin K, Chen D, *et al.* Formation of environmentally relevant polyhalogenated carbazoles from chloroperoxidase-

catalyzed halogenation of carbazole. *Environ Pollut*. 2018;232:264–273. https://doi.org/10.1016/j.envpol.2017.09.045

59. Ma L, Li Y, Zhang X, Zhang Y, Niu Z. Pollution characteristics, distribution, and source analysis of carbazole and polyhalogenated carbazoles in coastal areas of Bohai Bay, China. *Environ Pollut*. 2023;333:122103. https://doi.org/10.1016/j.envpol.2023.122103
60. Deng J, Gao L, Liu W, *et al.* Environmental behavior and risk of the emerging organic contaminants halogenated carbazoles in chemical industrial park clusters. *Sci Total Environ*. 2024;956:177253. https://doi.org/10.1016/j.scitotenv.2024.177253
61. Norton RS, Wells RJ. A series of chiral polybrominated biindoles from the marine blue-green alga Rivularia firma. Application of carbon-13 NMR spin-lattice relaxation data and carbon-13-proton coupling constants to structure elucidation. *J Am Chem Soc*. 1982;104:3628–3635. https://doi.org/10.1021/ja00377a014
62. Hodder AR, Capon RJ. A new brominated biindole from an Australian Cyanobacterium, Rivularia firma. *J Nat Prod*. 1991;54:1661–1663. https://doi.org/10.1021/np50078a028
63. Vetter W, *et al.* Marine halogenated natural products of environmental relevance. In: Ware GW, Whitacre DM, Albert LA, eds. *Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews*. Springer; 2006:1–57. https://doi.org/10.1007/978-0-7–32964-2_1
64. Breinlinger S, Phillips TJ, Haram BN, *et al.* Hunting the eagle killer: A cyanobacterial neurotoxin causes vacuolar myelinopathy. *Science* 2021;371:eaax9050. https://doi.org/10.1126/science.aax9050
65. Cariou R, Méndez-Fernandez P, Hutinet S, *et al.* Nontargeted LC/ESI-HRMS detection of polyhalogenated compounds in marine mammals stranded on French Atlantic coasts. *ACS EST Water*. 2021;1:309–318. https://doi.org/10.1021/acsestwater.0c00091
66. Vetter W, Gaul S, Olbrich D, Gaus C. Monobromo and higher brominated congeners of the marine halogenated natural product 2,3,3′,4,4′,5,5′-heptachloro-1′-methyl-1,2′-bipyrrole (Q1). *Chemosphere* 2007;66:2011–2018. https://doi.org/10.1016/j.chemosphere.2006.07.054
67. Vetter W, Stoll E, Garson MJ, Fahey SJ, Gaus C, Müller JF. Sponge halogenated natural products found at parts-per-million levels in marine mammals. *Environ Toxicol Chem*. 2002;21:2014–2019. https://doi.org/10.1002/etc5620211002
68. Vetter W, Alder L, Kallenborn R, Schlabach M. Determination of Q1, an unknown organochlorine contaminant, in human milk, Antarctic air, and further environmental samples. *Environ Pollut*. 2000;110:401–409. https://doi.org/10.1016/S0269-7491(99)00320-6
69. Bendig P, Hägele F, Vetter W. Widespread occurrence of polyhalogenated compounds in fat from kitchen hoods. *Anal Bioanal Chem*. 2013;405:7485–7496. https://doi.org/10.1007/s00216-3–7194-5
70. Fujii Y, Harada KH, Kato Y, Kimura O, Endo T, Haraguchi K. Dietary exposure to dicofol, endosulfan, and naturally produced halogenated bipyrroles: 24-hr food duplicate surveys from five prefectures in Japan. *Expo Health*. 2023;16:989–999. https://doi.org/10.1007/s12403-023-00604-6
71. Vetter W, Stoll E. Qualitative determination of bioaccumulative halogenated natural products in food and novel food. *Eur Food Res Technol*. 2002;215:523–528. https://doi.org/10.1007/s00217-2–0593-z
72. Jonkers TJH, Meijer J, Vlaanderen JJ, *et al.* High-performance data processing workflow incorporating effect-directed analysis for feature prioritization in suspect and nontarget screening. *Environ Sci Technol*. 2022;56:1639–1651. https://doi.org/10.1021/acs.est.1c04168
73. González-Gómez X, Simal-Gándara J, Fidalgo Alvarez LE, López-Beceiro AM, Pérez-López M, Martínez-Carballo E. Non-invasive biomonitoring of organic pollutants using feather samples in feral pigeons (Columba livia domestica). *Environ Pollut*. 2020;267:115672. https://doi.org/10.1016/j.envpol.2020.115672
74. D'Silva K, Thompson H, Fernandes A, Duff M. PBDEs in Heron Adipose Tissue and Eggs from the United Kingdom. In: *Proceedings of the Third International Workshop on Brominated Flame Retardants BFR2004*, Toronto, Canada, June 6–9, 2004. 2004, 195–198.
75. Sánchez Barranco V, Van der Meer MTJ, Kagami M, *et al.* Trophic position, elemental ratios and nitrogen transfer in a planktonic host–parasite–consumer food chain including a fungal parasite. *Oecologia* 2020;194:541–554. https://doi.org/10.1007/s00442-0–04721-w

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