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Contaminants of emerging concern in Antarctica

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Abstract

A holistic investigation of legacy persistent organic pollutants and contaminants of emerging concern was conducted for 14 biota samples collected from Antarctica between 2018 and 2020. The sample set included sea stars, sea urchins, macrophytes, fish muscle, seal muscle and placenta, and penguin muscle and eggs. The four Water Framework Directive heavy metals (lead, cadmium, nickel, and mercury) were present in all samples. Organophosphorus flame retardants and brominated flame retardants were detected sporadically at low concentration levels (below 0.7 ng/g ww). Isomers of Dechlorane Plus were not detected (< 0.01 ng/g ww). In contrast, dioxins, polybrominated diphenyl ethers (PBDEs) and polychlorinated bisphenols (PCBs) were frequently detected. The highest concentration was observed for PCBs, specifically PCB118 (up to 2,478 ng/g ww) and PCB105 (up to 977 ng/g ww). Wide-scope target screening of 2,236 compounds and suspect screening of 65,591

compounds were performed. Thirty-three contaminants from various chemical classes were detected through wide-scope target screening, of which 42% were pharmaceuticals and personal care products (PPCPs) and 30% were industrial chemicals (ICs) and their transformation products. An additional 55 compounds were identified through suspect screening, with PPCPs and ICs each accounting for 26 compounds. Most of the identified compounds are registered as REACH substances by the European Chemicals Agency, with some produced in very high volumes, exceeding 1,000,000 tonnes. Contaminant levels in Antarctic biota samples were lower than those reported in similar European studies, such as those conducted in the Danube River Basin.

Keywords: Contaminants of emerging concern, polar regions, Antarctica, target screening, suspect screening, environmental risk assessment

INTRODUCTION

The assessment of contaminants of emerging concern (CECs) in biota samples is significant in environmental risk assessment, particularly concerning substances that exhibit bioaccumulative (B) and persistent (P) potentials. These compounds meet two of the three PBT (persistent, bioaccumulative, and toxic) criteria outlined in the REACH legislation[1]. The presence of emerging and legacy pollutants, such as priority substances under the Water Framework Directive, present substantial threats to ecosystems and human health as they could cause adverse effects on the fertility and growth of fish[2-4] and bioaccumulate in the food chain[5,6]. Consequently, there is a growing interest in performing holistic chemical characterization of biota samples, especially the apex predators, to inform prioritization and support risk assessment of chemical pollution[7].

The polar regions, notably Antarctica, are often considered pristine and less susceptible to the pervasive reach of chemical pollutants[8]. Therefore, limited information on the chemical pollution status of the polar regions is available. With global warming and increased accessibility to polar areas, more pollution sources from anthropogenic activities could reach Antarctica[9]. Therefore, the systematic monitoring of chemicals in the polar regions is essential, aiming to assess the chemical burden of remote areas from anthropogenic activities.

Although several studies over the past two decades have reported the presence of persistent organic pollutants (POPs) such as polychlorinated bisphenols (PCBs), polybrominated diphenyl ethers (PBDEs), hexachlorocyclohexanes (HCHs), and dichlorodiphenyltrichloroethane (DDTs) in Antarctic biota, these investigations have often focused on targeted chemical groups and specific species, with limited application of broad-spectrum analytical methods[10-12]. Furthermore, much of the existing work has concentrated on traditional legacy contaminants, overlooking the occurrence of newer or less-regulated chemicals such as pharmaceuticals, industrial additives, and transformation products (TPs). Antarctica is uniquely vulnerable due to its role as both a global sink and a long-range receptor for pollutants transported through atmospheric and oceanic currents[13,14]. Seasonal processes, such as sea ice melting, remobilization from environmental reservoirs, and increased human activity near scientific stations, can contribute to the redistribution and accumulation of these substances in local ecosystems[15,16]. Climate change further compounds these pressures by altering environmental pathways, biological productivity, and the bioaccumulation behavior of contaminants[17,18]. This study addresses several critical gaps by applying a wide-scope and suspect screening approach to a diverse set of Antarctic biota, including both predator and lower trophic level species. In contrast to most previous studies, we employed high-resolution mass spectrometry to examine a chemical space exceeding 65,000 substances. This enables the detection of both regulated and emerging compounds, including many that have not previously been reported in Antarctic wildlife. By combining legacy contaminant analysis with HRMS-based techniques, our study represents one

of the most comprehensive chemical characterizations of Antarctic biota to date and provides a basis for refining future monitoring strategies in polar regions.

The objective of this work is to assess the occurrence of Water Framework Directive heavy metals (lead, cadmium, nickel and mercury)[19], organophosphorus and brominated flame retardants (BFRs), dioxins, PCBs, PBDEs, Dechlorane Plus, and hexachlorobenzene (HCB) by gas chromatography-mass spectrometry (GC-MS) and unregulated emerging organic contaminants utilizing liquid chromatography-high-resolution mass spectrometry (LC-HRMS) through wide-scope target screening of 2,236 substances and suspect screening of 65,591 substances. These extensive screening efforts enhance the understanding of pollutant prevalence in Antarctica and can support the prioritization of pollutants of environmental concern. Moreover, the importance of such investigation is acknowledged by regulatory bodies such as the European Union and other marine conventions.

EXPERIMENTAL

Sampling

In the framework of the present study, 14 biota samples were collected and analyzed using several MS-based instrumental techniques. Samples were collected in the Wilhelm Archipelago, West Antarctic Peninsula. Tier I, consisting of four biota samples representing lower trophic organisms, was collected in April 2018 from the waters adjacent to Galindez Island, where the Ukrainian Antarctic research station Akademik Vernadsky is situated, at depths up to 25 m, using scuba diving equipment and a fishing rod. Tier II, consisting of ten samples from predator species, was collected between July 2019 and March 2020. The sample code (project name-year of sample arrival at the laboratory-identification number), the species, the matrices, the date of sampling, and the tier number of the 14 samples are listed in Table 1.

Gentoo penguin eggs were collected at Galindez Island, and Adélie penguin eggs were collected at Yalour Island. Marine mammal and bird tissue samples were collected at Galindez Island and nearby islands. Samples of invertebrates and fish were collected alive. Placentas of Weddell seals were collected right after birth, and tissue samples of crabeater seals and gentoo penguins were collected from recently deceased individuals before decomposition began. We collected penguin eggs during the early incubation stages (stages a-c, according to Hays & LeCroy 1971[20]) for analysis. Sample collection was conducted under the Ministry of Education and Science of Ukraine permits Series AP No. 070/2-19 and 079-20/2-18. The samples were dispatched to the laboratory in two batches: Tier I samples were dispatched to the laboratory in December 2019 and Tier II were dispatched in December 2020. Transportation was made at -20 °C.

Sample preparation

Pre-treatment of penguin eggs samples

The penguin egg samples (detailed list in Supplementary Table 1) were delivered to Fraunhofer IME laboratory for preparation of composite samples. At Fraunhofer IME, samples were kept at temperatures below -135 °C. However, to remove the eggshells, the eggs had to be thawed for about 45 min to allow the frozen egg tissue to separate from the shells. Each egg was weighed after the separation of the shells and cryo-milled[21]. After the removal of the eggshells, it was observed that some of the eggs were not fresh but contained embryos of different development stages [Supplementary Figure 1]. To obtain homogeneous composite samples, only eggs without recognizable embryos were considered. After cryo-milling, aliquots of each egg were removed for mercury analyses of the egg samples. These data may help assess the variability in contaminant patterns among individual eggs. The cryo-milled eggs were mixed to form four composite samples. Initially, the plan was to prepare separate composite samples of P. papua eggs collected from Marina Point and Penguin Point (two breeding sites on Galindez lsland located approximately 600 m apart).

Table 1. List of Antarctica Tier I and Tier II samples

Sample code	Species	Matrix of analysis	Date of sampling	Decimal coordinates (latitude, longitude)	Tier
Ant-2019-1	Sea stars (*Odontaster validus*)	Muscles	01.04.2018	-65.24681,-64.26625	Tier I
Ant-2019-2	Sea urchin (*Sterechinus neumayeri*)	Muscles	01.04.2018	-65.24681,-64.26625	Tier I
Ant-2019-3	Macrophytes (species: Desmarestia anceps)	Macrophytes	01.04.2018	-65.24681,-64.26625	Tier I
Ant-2019-4	Fish black rockcod (*Notothenia coriiceps*)	Muscles	01.04.2018	-65.24703,-64.24051	Tier I
Ant-2020-1	Weddell seal (*Leptonychotes weddellii*)	Placenta	30.09.2019	-65.249512,-64.274999	Tier II
Ant-2020-2	Weddell seal (*Leptonychotes weddellii*)	Placenta	01.10.2019	-65.253207,-64.27258	Tier II
Ant-2020-3	Gentoo penguin (*Pygoscelis papua*)	Muscles	13.03.2020	-65.255648,-64.238267	Tier II
Ant-2020-4	Gentoo penguin (*Pygoscelis papua*)	Muscles	06.03.2020	-65.219645,-64.234852	Tier II
Ant-2020-5	Crabeater seal (*Lobodon carcinophaga*)	Muscles	30.07.2019	-65.217015,-64.273557	Tier II
Ant-2020-6	Crabeater seal (*Lobodon carcinophaga*)	Muscles	30.02.2020	-65.222363,-64.304492	Tier II
Ant-2020-7	Adélie penguin (*Pygoscelis adeliae*)	Pooled eggs (n = 6)	15.12.2019	Yalour Island -65.233515,-64.166806	Tier II
Ant-2020-8	Adélie penguin (*Pygoscelis adeliae*)	Pooled eggs (n = 7)	15.12.2019	Yalour Island -65.233268,-64.167437	Tier II
Ant-2020-9	Gentoo penguin (*Pygoscelis papua*)	Pooled eggs (n = 5)	30.11.2019	Galindez Island, Marina and Penguin Point -65.248631,-64.241463	Tier II
Ant-2020-10	Gentoo penguin (*Pygoscelis papua*)	Pooled eggs (n = 5)	30.11.2019	Galindez Island, Marina and Penguin Point -65.248631,-64.241463	Tier II

However, since many eggs from Penguin Point contained embryos, two composite samples with eggs from both sites were prepared (random selection of eggs for the composite samples). For P. adeliae, eggs were assigned to composite samples based on their position in the sequence (even- *vs.* odd-numbered). To ensure proportional representation, an equal fraction (e.g., 95%) of the contents of each egg was used to form the composite samples, rather than pooling equal masses from each egg. For each composite sample, 5 (P. papua) or 6-7 (P. adeliae) eggs were used. The four composite samples were designated Ant-2020-7 and Ant-2020-8 (P. adeliae) and Ant-2020-9 and Ant-2020-10 (P. papua), respectively. After preparation and mixing, the composite samples were freeze-dried [Supplementary Figure 2]. The original water content was 78% for eggs from both species. Water content was determined by weighing before and after freeze-drying of the composite samples.

Pre-treatment of biota samples

All the biota samples except the penguin eggs [Table 1] were delivered to the Laboratory of Analytical Chemistry of the National and Kapodistrian University of Athens. Prior to analysis, the samples underwent lyophilization and homogenization. As a pre-lyophilization step, the wet samples were frozen at -80 °C for no less than 5 h. Freeze-drying was performed at -55 °C and 0.05 mbar using a LyoQuest-55 laboratory lyophilizer (Telstar, Spain), in accordance with the laboratory's internal standardized operating procedure (SOP). Then, the samples were homogenized using a pestle and mortar that had been cleaned with milli-Q water, methanol, and acetone. After homogenization, samples were placed in amber glass vials and stored at -80 °C until further analysis. Part of the lyophilized material was dispatched to Environmental Institute (Slovakia) and the University of Florence (Italy) for analysis of POPs and POP-like contaminants.

Instrumental analysis

Elemental analysis (Cd, Pb, Ni, Hg)

All glassware and polypropylene bottles were pre-cleaned with acidified Milli-Q water before analysis. Subsequently, 0.1 g of freeze-dried sample was weighed into a Teflon container, followed by the addition of 5 mL of 65% HNO_3. The samples underwent digestion using the MARS X-Press microwave (CEM Corporation, USA). The digestion process was carried out according to the following program: the first stage involved 1,600 W power, with a 2-minute ramp time from 25 to 165 °C with no hold time; the second stage involved 1,600 W power, with a 3-minute ramp time from 165 to 175 °C, with a 5-minute hold time. After digestion, samples were brought to a final volume of 20 mL using ultrapure water. The supernatant was diluted 1:20 with Milli-Q water and prepared for analysis using the iCAP QC ICP-MS instrument (Thermo Scientific, USA). The analyte isotopes used for quantitation include ^{111}Cd, ^{208}Pb, ^{60}Ni, and ^{202}Hg, while the internal standards used for quantification purposes include ^{103}Rh for Cd, ^{72}Ge for Ni, and ^{191}Ir for Pb and Hg. Kinetic energy discrimination (KED) was adopted for interference correction.

For penguin egg samples, total mercury (Hg) was measured by Fraunhofer IME using a solid mercury analyzer to ensure interference-free detection. This method eliminates the need for digestion, reducing contamination risks. Mercury quantification is achieved through automatic sample combustion at approximately 1,000 °C in an oxygen current, followed by catalytic conversion of combustion gases. Elemental mercury is trapped as an amalgam and then measured using atomic absorption spectrometry (AAS). The process follows strict quality assurance (QA), with calibration verified using certified reference materials. The limit of detection (LOD) for mercury in penguin eggs was 0.05 ng/g wet weight (ww), while the limit of quantification (LOQ) was 0.145 ng/g ww [Supplementary Table 2].

POPs and POP-like contaminants

The samples were analyzed for: (1) Novel organophosphorus flame retardants (PFRs) and Dechlorane Plus; (2) Dioxins and dioxin-like compounds (DLCs); (3) PBDEs; (4) Polychlorinated biphenyls (PCBs); (5) Novel BFRs; and (6) HCB. Due to quantity and budget limitations, Tier I samples were analyzed for DLCs, PFRs, and Dechlorane Plus. Tier II samples were analyzed for BFRs and HCB. All samples were analyzed for PCBs and PBDEs.

Novel organophosphorus flame retardants (PFRs) and Dechlorane Plus

The biota samples underwent QuEChERS extraction to measure novel organophosphorus flame retardants and dechlorane plus. The extraction started with spiking 50 ng/g of labeled internal standards to 0.5 g of lyophilized sample in a 20 mL centrifuge tube. The mixture was equilibrated at ambient temperature for 15 min, followed by the addition of 10 mL ACN and 1 min of vortexing. The mixture was then transferred to a separate centrifuge tube preloaded with 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride.

The mixture underwent 1-minute vortexing, followed by centrifugation at 3,250 rpm for 5 min. Samples were cleaned up following the QuEChERS extraction, where the aliquot of the upper layer was transferred into a centrifuge tube with 50 mg of Z-Sep Plus sorbent and 150 mg of anhydrous magnesium sulfate. The samples were vortexed for 1 min and centrifuged at 3,250 rpm for 5 min. Finally, 10 µL of labeled injection standard was added to the cleaned extract for injection into gas chromatography-tandem mass spectrometry (GC-MS/MS) with electron impact ionization.

The Agilent 7890 GC (Germany) was operated in splitless injection mode using a Restek split liner with glass frit (4 mm × 6.3 mm × 78.5 mm). The splitless purge valve was activated 1 min after a 2 μL injection. Hydrogen was used as the carrier gas at a flow rate of 1.6 mL/min in the Restek Rxi-5Sil MS column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness). The analysis was performed using a 5975 triple quadrupole mass spectrometer system (Agilent, Germany) with electrospray ionization.

DLCs and PBDEs

For the analysis of dioxins, DLCs, and PBDEs, two distinct sample preparation methods were employed. The portion of biota samples intended for the analysis of dioxin and DLCs underwent accelerated solvent extraction (ASE) followed by fractionation into PCDD/F, planar PCBs, and non-planar PCBs. The portion of biota samples intended for the determination of PBDEs underwent modified solid-phase extraction (SPE), where each lyophilized biota sample was first spiked with a mixture of 13C-labeled compounds. Subsequently, the samples, mixed with an equivalent amount of a water:1-propanol (85:15, v/v) solution, were applied to conditioned and end-capped SPE columns with 2 g C18 (Alltech, USA).

The analytes were eluted using an n-hexane:dichloromethane mixture (1:1, v/v), and the resulting eluate was subsequently concentrated. Following this, the extract underwent cleanup on a multi-layer florisil-silica/sulphuric acid column and was eluted with a mixture of n-hexane:dichloromethane (9:1, v/v). The eluate was gently evaporated to dryness under a stream of nitrogen. Just prior to GC injection, a ^{13}C-labelled recovery standard solution was added to ensure accuracy in the analysis process.

HRGC-HRMS DFS (Thermo Finnigan, Germany) was used for the determination of DLCs, while MAT 95 XP HRMS (Thermo Finnigan, Germany) was used for the determination of PBDEs. HRMS instrumentations were operated in splitless injection mode, coupled to an HP 6890 gas chromatograph (Hewlett-Packard, USA). Helium was used as carrier gas at a flow rate of 0.8 mL/min in the DB-5 MS column of 60 m (0.25 mm i.d. × 0.25 μm film thickness). Quantification was performed with reference to the proportion of the two most abundant ions of natural (^{12}C) compounds and ^{13}C-labeled ones monitored. Calibration was performed with five standard solutions containing the measured ^{12}C- and ^{13}C-labeled compounds.

Novel BFRs and HCB

The extraction and analysis of HCB and NBFRs were performed using validated methodologies to ensure accurate quantification. Soxhlet extraction was conducted on lyophilized biological samples using a 3:1 (v/v) dichloromethane-hexane mixture for a minimum of 12 h, with the addition of ^{13}C-labeled internal standards as surrogates to evaluate extraction efficiency. The resulting extracts were concentrated to 10 mL using a rotary evaporator, and a 1 mL aliquot was reserved for lipid content determination. Lipid content was assessed gravimetrically by solvent evaporation at 70 °C, with repeated heating and weighing cycles until a stable mass was achieved.

A multi-step cleanup procedure was employed to eliminate the lipidic fraction and potential interferences. The extracts were purified using a multi-layer silica gel column, packed sequentially with silica gel, acidic silica gel, additional silica gel, and a sodium sulfate layer, conditioned with 100 mL of hexane, and eluted with 200 mL of hexane.

Purified samples were analyzed by GC-MS using an Agilent 6890N gas chromatograph coupled to an Agilent 5973 single quadrupole mass spectrometer operated in negative chemical ionization (NCI) mode. HCB was analyzed using an Agilent J&W DB-5ms (Cat #122-5532) (30 m × 0.25 mm i.d., 0.25 μm film

thickness) column, with an oven temperature program starting at 100 °C (30 s hold) and ramping at 8 °C/min to 310 °C. NBFRs were analyzed using an Agilent J&W DB-5ms (Cat #122-5511) (15 m × 0.25 mm i.d., 0.10 μm film thickness) column, with an initial oven temperature of 90 °C (1 min hold), followed by a ramp of 20 °C/min to 220 °C. The injection was performed in splitless mode (1 μL injection volume) with helium as the carrier gas, and detection was carried out in selected ion monitoring (SIM) mode for improved sensitivity.

Quality control (QC) measures included the analysis of laboratory blanks to verify the absence of contamination and repeated testing of certified reference material (lyophilized fish tissue, Wellington Laboratories) to validate the accuracy of the method.

CECs

Wide-scope target and suspect screening of CECs

CECs with a wide range of physicochemical properties were simultaneously extracted from biota matrices using validated, generic sample preparation protocols. ASE followed by SPE was applied prior to analysis by LC-HRMS and GC-HRMS. For data treatment, wide-scope target and suspect screening methodologies were adopted. All samples underwent two generic sample preparation methods to accommodate the diverse properties of the extracted compounds. Compounds with higher polarity, lower volatility, and thermal instability were extracted using a protocol optimized for LC-compatible analytes. Conversely, a separate sample preparation protocol was used to extract more volatile and thermally stable compounds suitable for GC analysis.

LC-amenable contaminants

ASE followed by mixed-mode SPE was applied for the extraction of CECs from biota matrices [Supplementary Section 3]. Briefly, 1 g of the sample was homogenized with 4 g of sodium sulfate dispersant (Na_2SO_4). Following the addition of isotopically labeled internal standards, samples were extracted using Dionex™ ASE™ 350 (Thermo Scientific, USA) with MeOH:ACN (2:1, v/v). The resulting extract was pre-concentrated via rotary evaporation followed by volume adjustment to 15 mL with Milli-Q water. A defatting step was performed with hexane. Milli-Q water was added to adjust the final volume to 50 mL. Subsequently, samples underwent cleanup using in-house mixed-mode SPE cartridges comprising Oasis HLB (200 mg) and a mixture of Strata-X-AW, Strata-X-CW, and Isolute ENV+ (300 mg total mixture). The resulting extract was evaporated to dryness using a nitrogen stream and reconstituted with methanol/water (1:1 v/v). The reconstituted sample was homogenized via vortex stirring, and a 4-fold sample enrichment was achieved during the preparation process.

Final extracts were filtered through Regenerated Cellulose filters and underwent LC-HRMS analysis. The HPLC system included a Dionex UltiMate 3000 RSLC HPG-3400 pump (Thermo Scientific, USA) coupled with the Acclaim TM RSLC 120 C18 column of 100 × 2.1 mm with 2.2 μm diameter (Thermo Scientific, USA). The quadrupole time of flight mass spectrometer (QTOF-MS) used was Maxis Impact (Bruker Daltonics, USA). Details of the LC-HRMS system are included in the Supplementary Section 4[22].

GC-amenable contaminants

The extraction of GC-amenable CECs involved ASE and SPE [Supplementary Section 5]. Briefly, 1 g of the sample was homogenized with 4 g of Na_2SO_4 dispersant, followed by the addition of isotopically labeled internal standards. The CECs were extracted using Dionex™ ASE™ 350 (Thermo Scientific, USA) with hexane:dichloromethane (2:1, v/v) at 100 °C. Following the addition of 50 μL of isooctane as a keeper, the resulting extract was pre-concentrated via rotary evaporation at 30 °C to achieve a final volume of 10 mL.

The samples were then cleaned up by SPE using Strata® FL-PR Florisil (170 µm, 80 Å, 5 g / 20 mL, Giga Tubes) cartridges (Phenomenex, USA). Another 50 µL of isooctane was added to each sample, followed by pre-concentration by rotary evaporation at 30 °C to achieve 10 mL. A nitrogen stream at 30 °C was used to dry the extracts to a final volume of 250 µL. Each sample was homogenized using vortex stirring for 1 min. A 4-fold sample enrichment was achieved during preparation.

Final extracts were filtered through Regenerated Cellulose filters and underwent GC-HRMS analysis. The system consisted of a Varian 450 GC (Bruker Daltonics, USA), a CP-8400 AutoSampler (Agilent, Germany), and a Maxis Impact QTOF-MS (Bruker Daltonics, USA). GC was operated in splitless injection mode with an injection volume of 1 µL. A Restek Rxi-5Sil MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was employed, with helium as the carrier gas at a constant flow rate of 1.5 mL/min. Details of the GC-HRMS system are included in the Supplementary Section 6.

Data treatment

Wide-scope target screening

Target screening was realized utilizing *in-house* databases consisting of 2,236 contaminants. LC target list is available as S21 UATHTARGETS on the NORMAN Suspect List Exchange[23]. Data analysis was performed using DataAnalysis 5.1 and TASQ Client 2.1 software by Bruker Daltonics (Germany). Clear detection criteria were applied, including screening parameters such as mass accuracy within ±2 mDa, retention time deviation within ±0.2 min, and isotopic pattern fitting below 100 mSigma for confirmation of positive findings. The HRMS data processing workflow was described in detail elsewhere[22]. The presence of characteristic adduct and fragment ions further supported the identification of the detected analytes. Screening detection limit (SDL) was established for contaminants screened by the wide-scope method, denoting the lowest concentration level at which a compound was consistently detected in all spiked samples, at the expected retention time and within specific mass error of the precursor ion. SDL determination in the in-house method involved satisfaction of thresholds for retention time and mass accuracy of the precursor ion, yielding a generic reporting value after method validation. Compound-specific validation was performed for quantification, with compound-specific LOD and LOQ values calculated post-treatment and analysis of samples spiked with detected compounds and structure-related isotope labeled compounds. Contaminants detected in traces below LOQ (concentration levels between LOD and LOQ) were reported as below quantification limit (< LOQ). For statistical treatment, Directive 2009/90/EC suggested the substitution of < LOQ with LOQ/2[24].

Suspect screening

Suspect screening for environmentally relevant pollutants was performed using the NORMAN SusDat database (https://www.norman-network.com/nds/susdat/, retrieved 1 October 2021) on all raw chromatograms imported into the NORMAN Digital Sample Freezing Platform (DSFP)[25]. DSFP is a tool that was developed to unveil suspect presence and identify unknown compounds in environmental samples. Calibration was carried out using calibrant masses to recalibrate the entire chromatogram via the HPC fitting algorithm implemented in DataAnalysis 5.1 (Bruker Daltonics, Germany), ensuring mass accuracy better than 2 mDa across the m/z range of 50-1,000 throughout the chromatographic run. Data were subsequently exported in mzML format using CompassXport version 3.0.9.2 (Bruker Daltonics, Germany). Chromatograms acquired in bbCID mode were separated into low and high collision energy layers. Subsequently, all mzML files and associated metadata - including instrumental parameters, sample and matrix-specific information, and retention time data for RTI calibrants - were uploaded to the DSFP. DSFP integrates a SOP for processing mzML files and their metadata, enabling the automated generation of Data Collection Templates (DCTs). This data reduction process condenses complex LC-HRMS information into structured DCTs for streamlined analysis and sharing. Detected suspected compounds were semi-quantified

using the structural similarity method. 2D-based chemical similarity was employed to identify the most similar reference standard compound. This involved calculating two-dimensional linear fragment descriptors based on the original definitions of atom pairs and atom sequences, using the Tanimoto coefficient as the similarity metric. Due to the extensive calibration curve database (> 1,000 compounds in positive and negative ionizations), the semi-quantification uncertainty for all detected compounds was maintained below one order of magnitude. All LC-HRMS and GC-HRMS data from this study were uploaded to the NORMAN DSFP. All HRMS data are available at https://doi.org/10.60930/zkqd-pn07.

QA/QC

Extensive QA/QC procedures were applied throughout both sample preparation and instrumental analysis workflows. Prior to extraction, a mixture of internal standards was added to each sample to monitor the recovery of target compounds. In addition, samples spiked with a known mix of CECs were included in each analytical batch to validate method performance. Procedural (reagent) blanks were carefully prepared and analyzed to assess potential contamination introduced during sample preparation and analysis. To ascertain retention time stability during instrumental analysis, a mixture of known compounds (RTI calibrant substances) was employed[26]. Furthermore, a QC sample was injected every 10 injections to validate instrument performance and ensure the sensitivity of the instrument. For QC purposes and quantification of the metal analysis, the certified reference material ERM-CE278k (trace elements in muscle tissue) was also subjected to analysis. These QA/QC procedures collectively ensured the reliability, accuracy, and precision of the analytical results.

RESULTS AND DISCUSSION

Metals analysis (Cd, Pb, Ni, and Hg)

The results of metals analysis in the fourteen tested samples, along with their LODs, LOQs, and environmental quality standards (EQSs; listed on the WFD[19]) for fish, are provided in Table 2.

Cadmium was detected at a few thousands ng/g levels in the sea stars sample (Ant-2019-1), a concentration that was 100 to 1,000 times higher than that found in fish (Ant-2020-4) and Tier II samples. Significantly higher concentrations of lead were detected in macrophytes (Ant-2019-3; 1,239 ng/g) and fish (Ant-2019-4; 1,564 ng/g). The macrophytes were also highly contaminated with nickel at 1,854 ng/g, which was 2-3 orders of magnitude higher than that detected in other samples. The penguin egg sample Ant-2020-3 showed the highest level of mercury at 248 ng/g, whereas the concentration of mercury detected in the fish sample (Ant-2019-4) slightly exceeded the respective EQS value of WFD. More fish samples from Antarctica are needed to reveal the level of mercury contamination and the extent of exceedance of EQS in the region. Overall, metal concentrations in the 2020 samples were lower than those measured in 2019. The levels of these metals in fish biota samples from the Mediterranean Region (generally at hundreds to thousands ng/g; up to 5,660 ng/g[27]) are higher than that in Antarctica. Nonetheless, the different species and matrix of analysis shall be considered.

These metal contamination patterns may reflect both natural geochemical variability and localized anthropogenic influence[28]. The relatively high mercury concentration in penguin eggs is consistent with maternal transfer of methylmercury during egg formation, as previously observed in Antarctic birds[29]. Although most detected concentrations are below European EQS, the occasional exceedance - especially for mercury - warrants further investigation due to the neurotoxicity and biomagnification potential of this element in cold ecosystems with slow contaminant turnover.

Table 2. Results of metals analysis

Sample	Cd	Pb	Ni	Hg
Ant-2019-1	1,621	261	261	26
Ant-2019-2	360	621	305	10
Ant-2019-3	113	1,239	1,854	6.7
Ant-2019-4	35.0	1,564	37.5	24.7
Ant-2020-1	140	21.9	267	45.8
Ant-2020-2	51.6	52.1	285	25.2
Ant-2020-3	2.9	96.1	288	248
Ant-2020-4	7.3	64.0	278	19.4
Ant-2020-5	11.9	56.9	280	16.2
Ant-2020-6	17.9	61.6	287	23.7
Ant-2020-7	0.8	30.7	283	141
Ant-2020-8	2.1	34.7	277	262
Ant-2020-9	3.4	23.7	271	62.6
Ant-2020-10	1.5	17.4	287	62.1
LOD	0.10	1.30	0.97	0.16, 0.005 (for Ant-2020-7 until 10)
LOQ	0.30	3.90	2.91	0.48, 0.0145 (for Ant-2020-7 until 10)
EQS (for fish)	-	-	-	20

Values are expressed in ng/g wet weight. LOD: Limit of detection; EQS: environmental quality standards.

Novel organophosphorus flame retardants (PFRs), novel BFR and Dechlorane Plus

The determination of 13 organophosphorus flame retardants (PFRs) was performed in the Tier I samples, including Triisobutyl phosphate (TIBP), Tributyl phosphate (TBP), Tris(2-chloroethyl) phosphate (TCEP), Tris(2-chloroisopropyl) phosphate (TCPP), Tris(3-chloropropyl) phosphate, Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), Triphenyl phosphate (TPHP), Tris(2-butoxyethyl) phosphate (TBEOP), 2-Ethylhexyl diphenyl phosphate (EHDP), Tris(2-ethylhexyl) phosphate (TEHP), Tri-o-cresyl phosphate (TOCP), Tris(methylphenyl) phosphate (TPCP-isomers), and 1,2-Bis(2,4,6-tribromophenoxy) ethane (BTBPE). Moreover, Tier I samples were also tested for two isomers of Dechlorane Plus (syn-Dechlorane Plus and anti-Dechlorane Plus). The detected levels, LOD, and LOQ of the compounds are included in Table 3 and Supplementary Table 4. Supplementary Table 4 shows all the analytical results for non-polar contaminants. No isomers of Dechlorane Plus were detected, while 5 PFRs (TIBP, TCE, TCPP, TPHP and EHDP) were detected, even below the LOQ levels, in the Tier I samples. The order of the number of PFRs detected in the samples was fish (4) > sea urchin (3) > sea stars (2) > macrophytes (0). Moreover, the determined levels of PFRs are two to three orders of magnitude lower than that in the fish biota collected in the Swedish lakes and coastal areas[30]. Nonetheless, all PFRs were detected at levels below the predicted no-effect concentration (PNEC) for biota: 5.15 ng/g ww for TCEP; 5.08 ng/g ww for EHDP; 953 ng/g ww for TCPP; 984 ng/g ww for TPHP. Like PFRs, BFRs were detected sporadically in the samples at low concentration levels (0.06-0.35 ng/g ww), not exceeding the PNECs.

The sporadic detection and low concentrations of novel PFRs and BFRs in the samples likely reflect the limited environmental dispersion of these compounds to Antarctica, owing to their more recent introduction as replacements for legacy flame retardants (e.g., PBDEs), their lower global usage volumes, and their physicochemical properties that may limit long-range atmospheric transport[16,31].

PCBs, PBDEs and dioxins, DLCs

PBDEs were distinguished from NBFRs and grouped with other legacy contaminants due to their historical widespread use. The determination of PCBs and PBDEs was conducted in all samples, while DLC analysis

Table 3. Results of PFRs and Dechlorane Plus analysis

Compound	LOD	LOQ	Sea stars (Ant-2019-1)	Sea urchin (Ant-2019-2)	Macrophytes (Ant-2019-3)	Fish (Ant-2019-4)
Triisobutyl phosphate (TIBP)	0.02	0.05	< LOQ	< LOD	< LOD	< LOD
Tributyl phosphate (TBP)	0.04	0.08	< LOD	< LOD	< LOD	< LOD
Tris(2-chloroethyl) phosphate (TCEP)	0.02	0.05	< LOQ	0.20	< LOD	0.35
Tris(2-chloroisopropyl) phosphate (TCPP)	0.03	0.06	0.1	0.06	< LOD	0.13
Tris(3-chloropropyl) phosphate	0.04	0.07	< LOD	< LOD	< LOD	< LOD
Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)	0.05	0.09	< LOD	< LOD	< LOD	< LOD
Triphenyl phosphate (TPHP)	0.03	0.06	< LOD	< LOD	< LOD	0.12
Tris(2-butoxyethyl) phosphate (TBEOP)	0.10	0.25	< LOD	< LOD	< LOD	< LOD
2-Ethylhexyl diphenyl phosphate (EHDP)	0.03	0.06	< LOD	< LOD	< LOD	0.17
Tris(2-ethylhexyl) phosphate (TEHP)	0.03	0.06	< LOD	< LOD	< LOD	< LOD
Tri-o-cresyl phosphate (TOCP)	0.10	0.25	< LOD	< LOD	< LOD	< LOD
Tris(methylphenyl) phosphate (TPCP-isomers)	0.04	0.08	< LOD	< LOD	< LOD	< LOD
1,2-Bis(2,4,6-tribromophenoxy) ethane (BTBPE)	0.10	0.25	< LOD	< LOD	< LOD	< LOD
Syn-dechlorane plus	0.01	0.03	< LOD	< LOD	< LOD	< LOD
Anti-dechlorane plus	0.01	0.03	< LOD	< LOD	< LOD	< LOD

Values are expressed in ng/g wet weight. PFRs: Novel organophosphorus flame retardants; LOD: limit of detection; LOQ: limit of quantification.

was performed only in Tier I samples. The determined concentrations, along with the LOD and LOQ of the 56 analyzed compounds, are provided in Supplementary Table 4. PCBs exhibited the highest concentration levels compared to PBDEs and DLCs, with cumulative concentrations reaching 3,986 ng/g ww in Sea stars (Ant-2019-1), followed by 2,829 ng/g ww in Sea urchin (Ant-2019-2) and 158 ng/g ww in Fish (Ant-2019-4). The most significant contributors were PCB 118 and PCB 105, accounting for 64.5% and 22.7% of the total PCB concentration, respectively. In all other samples, cumulative PCB concentrations remained below 47 ng/g ww. Sea stars (Ant-2019-1) and Sea urchins (Ant-2019-2) also exhibited the highest PBDE loads, with cumulative concentrations of 14.5 and 11.6 ng/g ww, respectively. PBDE concentration levels ranged between 0.47-5.31 ng/g ww in penguin eggs, 0.35-0.53 ng/g ww in Penguin Muscles (ANT-2020-3 and ANT-2020-4), 0.40-6.83 ng/g ww in Crabeater Seal Muscles (ANT-2020-5 and ANT-2020-6), and 0.32-0.52 ng/g ww in Seal placenta samples (ANT-2020-1 and ANT-2020-2).

The comparatively high concentrations of PCBs in the samples are the result of both environmental persistence and biological accumulation dynamics. PCBs are among the most persistent legacy pollutants in polar environments, with well-documented long-range atmospheric transport and deposition in remote regions such as Antarctica[13]. Their presence in snow, seawater, and sediments has been reported in multiple studies, indicating sustained environmental reservoirs[11,13,32]. Moreover, PCBs are highly lipophilic and bioaccumulative, leading to their progressive magnification through food webs, particularly in long-lived species and higher trophic levels[10,33]. The molecular stability and resistance of PCBs to metabolic degradation further contribute to their elevated concentrations in biota. This dual influence of environmental persistence and trophic transfer likely explains the predominance of PCBs relative to other investigated compounds in our study.

Concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs), as defined in Directive 2013/39/EU, were used to calculate total dioxin and DLC levels expressed in ng/g toxic equivalents (TEQs). TEQs express the toxicity-weighted concentration of mixtures of DLCs, providing a better insight into the potential impact of the mixture. The TEQs for fish, sea stars, macrophytes, and sea urchins were 0.05, 0.9, 0.14, and 0.84 ng/kg,

respectively. The numbers were 7 to 130 times below the EQS of 6.5 ng/kg included in the 2013/39/EC Directive[34]. The results are generally lower than the TEQ found in fish samples in the Baltic Sea, where 40% of the investigated samples exceeded the accepted level[35].

The sum of the concentration of investigated PBDEs (PBDE 28/33, 47, 99, 100, 153, 154, and 183) ranged from 0.32 up to 14.51 ng/kg. The measured PBDE concentrations surpassed the EQS value of 8.5 ng/kg[36] in sea star and sea urchin samples. More samples of these species in Antarctica are required to establish the status of EQS exceedance in the region. Exceedances of EQS of PBDEs were also observed in rivers across Europe, including in Italy[37], Belgium[38], and Germany[39], at levels up to over 100 ng/kg.

Wide-scope target screening of CECs

Wide-scope target screening for 2,236 CECs was conducted for all 14 biota samples. In total, 33 CECs were detected in the samples. The target analysis results, LODs, LOQs, and PNECs of the detected compounds in Tiers I and II samples are provided in Tables 4 and 5, respectively. PNECs were retrieved from the NORMAN Ecotoxicology Database[40]. Detected compounds were assigned to four main chemical groups based on the main use, including pharmaceuticals and personal care products (PPCPs), stimulants, plant protection products (PPPs), and industrial chemicals (ICs), along with their respective TPs. Among the 33 detected CECs, 42% were PPCPs, 30% were ICs, 18% were PPPs, and 9% were stimulants and TPs. Methylparaben was the most frequently detected contaminant, appearing in 13 out of the 14 samples. It should be highlighted that the oxfendazole (risk quotient 2.76) and methylparaben (risk quotient 9.69) in the fish sample (Ant-2019-4) exceeded the respective PNEC value in fish, which indicates that these two compounds may be of potential environmental concern in the local environment. Exceedance of PNEC is due to the combination of environmental exposure and their intrinsic toxicity.

All detected compounds exhibited low median concentration levels (up to 21.1 ng/g ww), with the exception of the IC lauryl diethanolamide, which had a median concentration of 240 ng/g ww, attributable to its high levels (exceeding 1,000 ng/g ww) in the two Gentoo penguin egg samples. The least contaminated sample, in terms of the number of detected substances and the cumulative concentration of organic pollutants, was Ant-2020-1 (Weddell Seal placenta), with only galaxolidone and methylparaben detected at 9.18 ng/g ww and < LOQ, respectively. In contrast, the Ant-2019-1 (Sea star) sample had up to 12 organic pollutants. The cumulative concentrations in all tested matrices ranged from 12.9 to 271 ng/g ww, with the exception of Gentoo Penguin eggs (2,000 and 2,469 ng/g ww for Ant-2020-9 and Ant-2020-10, respectively), primarily due to lauryl diethanolamide. In an investigation of the same list of CECs in the fish biota in the Danube River Basin, a wider spectrum of CECs (78 compounds) was determined at higher levels (cumulative concentrations over 590 ng/g for all tested samples)[41]. The observed differences in total cumulative concentration levels and number of detected compounds are attributed to the intense anthropogenic pressure in the Danube region versus the remote and relatively isolated conditions of Antarctica[11,41].

Suspect screening of CECs

The suspect screening of all 65,591 substances on the NORMAN Substance Database[42] (as of 2021) was performed in all 14 biota samples. In total, 332 substances were detected (299 in positive ionization mode, 33 in negative ionization mode, and 9 in both ionization modes), including all identified target substances listed in Tables 4 and 5. After excluding target compounds and naturally occurring substances (such as terpenoids, amino acids, fatty acids, nutrients, nucleic acids, oligosaccharides, and various endogenous metabolites), 55 compounds were tentatively identified (IP score higher than 0.50[43]). More confident identification was shown for the compounds 8-Hydroxychinoline (IP score 0.72), Benzamide (IP score 0.71), Dibenzoylmethane (IP score 0.70), 2-Hydroxy-4-methoxybenzophenone (IP score 0.70), Enzacamene

Table 4. Results of wide-scope target screening in Tier I samples, including LODs and LOQs

Compound	LOD	LOQ	PNEC fish	Chemical group	Sea stars (Ant-2019-1)	Sea urchin (Ant-2019-2)	Macrophytes (Ant-2019-3)	Fish (Ant-2019-4)
Tributylamine	0.16	0.49	221	PPCPs and TPs	< LOD	< LOD	0.912	< LOD
4-Formyl antipyrine*	0.08	0.24	4.24	PPCPs and TPs	1.27	1.50	< LOD	0.51
4-Acetamido Antipyrine	0.25	0.7	28.2	PPCPs and TPs	2.77	4.86	< LOQ	1.09
Benzophenone 3	1.06	3.19	10.5	PPCPs and TPs	13.4	< LOD	85.9	4.69
Bunitrolol	0.344	1.03	5.08	PPCPs and TPs	1.66	< LOD	< LOD	< LOD
Oxfendazole†	0.90	2.70	0.49	PPCPs and TPs	< LOD	< LOQ	< LOQ	< LOQ
Tramadol-N-oxide	0.75	2.26	79.5	PPCPs and TPs	< LOD	< LOD	< LOD	< LOQ
O-Desmethyl-Tramadol	0.29	0.86	37.8	PPCPs and TPs	< LOQ	< LOD	< LOQ	< LOD
Ethylparaben	0.54	1.62	7.15	PPCPs and TPs	< LOQ	< LOQ	7.56	< LOQ
Methylparaben*,†	0.75	2.25	2.56	PPCPs and TPs	5.79	68.9	< LOD	24.8
Salicylic acid	0.50	1.50	2,160	PPCPs and TPs	2.60	< LOD	5.41	7.94
3-hydroxy-Carbofuran	0.86	2.57	5	PPPs and TPs	5.35	2.75	< LOD	< LOQ
DEET (Diethyltoluamide)	0.61	1.84	21.1	PPPs and TPs	< LOD	< LOD	< LOQ	< LOD
Endothal*	1.41	4.66	8.42	PPPs and TPs	< LOD	< LOD	6.43	< LOD
Fenuron*	0.25	0.74	0.64	PPPs and TPs	< LOD	< LOQ	< LOD	< LOD
Isoprocarb	1.95	5.85	9.34	PPPs and TPs	< LOQ	9.09	< LOD	< LOD
3,4,5-Trimethacarb	2.39	7.18	11.6	PPPs and TPs	< LOQ	8.27	< LOD	< LOD
Nicotine	0.89	2.67	4.69	Stimulants and TPs	< LOD	3.89	< LOD	< LOD
Nornicotine*	2.19	6.56	21	Stimulants and TPs	< LOQ	< LOD	15.9	< LOD
Theobromine	1.96	5.88	12.6	Stimulants and TPs	< LOD	< LOD	< LOQ	< LOD

Values are expressed in ng/g wet weight. < LOQ: Below the limit of quantification. *Compounds commonly detected in Tiers I and II samples. †Exceedance of PNEC for fish sample. LODs: Limits of detection; LOQs: limits of quantification; PNEC: predicted no-effect concentration; PPCPs: pharmaceuticals and personal care products; TPs: transformation products; PPPs: plant protection products.

(IP score 0.70), PEMA (2-Phenyl-2-ethylmalonamide) (IP score 0.70), Acetanilide (IP score 0.68), Brefeldin A (IP score 0.68), 4-hydroxy-2(1H)-Quinolinone (IP score 0.67), Telbivudine (IP score 0.65), Salmeterol (IP score 0.65). The identification confidence level for these compounds adheres to the categorization scheme available elsewhere[43].

The chemicals detected were semi-quantified, and the results are presented as a heatmap in Figure 1. Figure 1 reveals a separation between the Tier I samples collected in 2019 (Ant-2019-1 to Ant-2019-4) and the Tier II samples from 2020, with Tier I showing a higher number of detected suspect compounds. Tier I consisted of organisms from lower trophic levels (sea stars, sea urchins, macrophytes, and fish) that are in direct contact with sediment and water, where many contaminants first accumulate[11,32]. In contrast, Tier II included higher-trophic organisms such as Weddell seal placentas, crabeater and gentoo seal muscle tissues, and pooled penguin eggs, which may exhibit different bioaccumulation profiles due to trophic transfer, metabolic transformation, or maternal offloading[33,44]. These differences in species ecology, exposure routes, and sample matrix complexity contribute to the distinct chemical fingerprints[31].

The detected compounds included 26 PPCPs, 26 ICs, and 2 PPPs. The detailed semi-quantitative results are available in Supplementary Table 5 and aggregated in Supplementary Table 6. Most of these compounds were registered as REACH substances by the European Chemicals Agency (ECHA). Some of these substances are produced in very high tonnage, such as caprolactam (1,000,000-10,000,000 tonnes), methyl 4-pentylbenzoate, dipropyl phthalate, and dibenzoylmethane (1,000-10,000 tonnes), as well as 4-heptylbenzoic acid, 4-hydroxybenzophenone, N,N-dimethyldec-9-enamide, and 2-hydroxy-4-methoxybenzophenone (100-1,000 tonnes). The remaining compounds fall within the 1-10 or 10-100 tonnes production range.

Table 5. Results of wide-scope target screening in Tier II samples

Compound	Chemical group	Placenta				Muscles						Pooled eggs					
		Ant-2020-1	Ant-2020-2	LOD	LOQ	Ant-2020-3	Ant-2020-4	Ant-2020-5	Ant-2020-6	LOD	LOQ	Ant-2020-7	Ant-2020-8	Ant-2020-9	Ant-2020-10	LOD	LOQ
Galaxolide	PPCPs and TPs	< LOD	< LOD	3.07	9.20	< LOD	< LOD	< LOD	< LOD	3.07	9.20	< LOQ	< LOQ	< LOQ	< LOD	1.80	5.41
Galaxolidone	PPCPs and TPs	9.18	< LOD	3.03	9.10	< LOD	< LOD	5.46	< LOD	1.52	4.55	14.5	9.24	< LOQ	< LOQ	0.767	2.30
Methylparaben*	PPCPs and TPs	< LOQ	11.6	2.50	7.50	4.46	11.6	5.88	52.9	1.06	3.19	< LOQ	< LOQ	< LOQ	8.19	0.848	2.55
4-Formyl antipyrine*	PPCPs and TPs	< LOD	14.0	1.30	3.89	< LOD	< LOD	3.29	13.1	0.65	1.94	6.20	4.57	4.76	12.0	0.27	0.82
Hydrocortisone	PPCPs and TPs	< LOD	< LOD	1.41	4.22	< LOD	< LOD	18.1	< LOQ	1.41	4.22	< LOD	< LOD	< LOD	< LOD	0.83	2.48
O-Desmethyl-Tramadol	PPCPs and TPs	< LOD	< LOQ	0.75	2.26	< LOD	< LOD	< LOD	< LOQ	3.09	9.26	< LOD	< LOD	< LOD	< LOD	1.82	5.45
Nornicotine*	Stimulants and TPs	< LOD	< LOD	1.92	5.77	< LOD	< LOD	< LOD	6.92	1.92	5.77	< LOD	< LOD	< LOD	< LOD	1.13	3.39
Endothal*	PPPs and TPs	< LOD	< LOQ	0.84	2.51	< LOD	< LOQ	< LOD	< LOD	0.41	1.23	< LOD	< LOD	< LOD	< LOD	0.49	1.48
Fenuron*	PPPs and TPs	< LOD	< LOQ	1.41	4.23	< LOQ	< LOQ	< LOD	< LOD	1.06	3.18	< LOD	< LOD	< LOD	< LOD	0.62	1.87
Benzododecinium (Benzyldimethyldodecylammonium)	ICs	< LOD	< LOD	2.90	8.71	< LOD	< LOD	< LOQ	< LOD	2.90	8.71	33.6	< LOD	< LOQ	13.3	2.90	8.71
Didecyldimethylammonium [DADMAC (C10:C10)]	ICs	< LOD	< LOD	0.38	1.15	< LOD	< LOD	< LOQ	< LOD	0.38	1.15	1.86	< LOQ	< LOD	< LOD	0.38	1.15
Hexadecyltrimethylammonium	ICs	< LOD	< LOD	1.22	3.66	< LOD	< LOD	< LOD	< LOD	1.22	3.66	4.39	< LOD	< LOD	< LOD	1.22	3.66
Lauryl diethanolamide	ICs	< LOD	240	29.0	87.1	< LOQ	< LOD	106	< LOD	29.0	87.1	< LOD	< LOD	1.959	2.426	29.0	87.1
N,N-Dimethyldodecylamine	ICs	< LOD	< LOD	0.56	1.67	14.6	7.44	3.88	< LOD	0.56	1.67	9.73	1.92	5.49	5.69	0.56	1.67
N,N-Dimethyldodecylamine N-oxide	ICs	< LOD	< LOD	1.79	5.38	< LOD	< LOD	<LOD	< LOD	1.79	5.38	< LOD	< LOD	21.1	< LOD	1.79	5.38
N,N-Dimethyltetradecylamine	ICs	< LOD	< LOD	0.15	0.44	4.63	< LOD	1.81	< LOD	0.15	0.44	8.38	0.48	< LOD	< LOD	0.15	0.44
N,N-Dimethyltetradecylamine N-oxide	ICs	< LOD	< LOD	0.50	1.49	1.94	< LOD	<LOD	< LOD	0.50	1.49	3.92	< LOD	< LOD	< LOD	0.50	1.49
N-Methyldodecylamine	ICs	< LOD	< LOD	0.51	1.52	3.46	< LOD	3.24	< LOD	0.51	1.52	41.0	1.75	8.18	2.83	0.51	1.52

Values are expressed in ng/g wet weight. < LOQ: Below the limit of quantification. *Compounds commonly detected in Tiers I and II samples. LOD: Limit of detection; LOQ: limit of quantification; PPCPs: pharmaceuticals and personal care products; TPs: transformation products; PPPs: plant protection products; ICs: industrial chemicals.

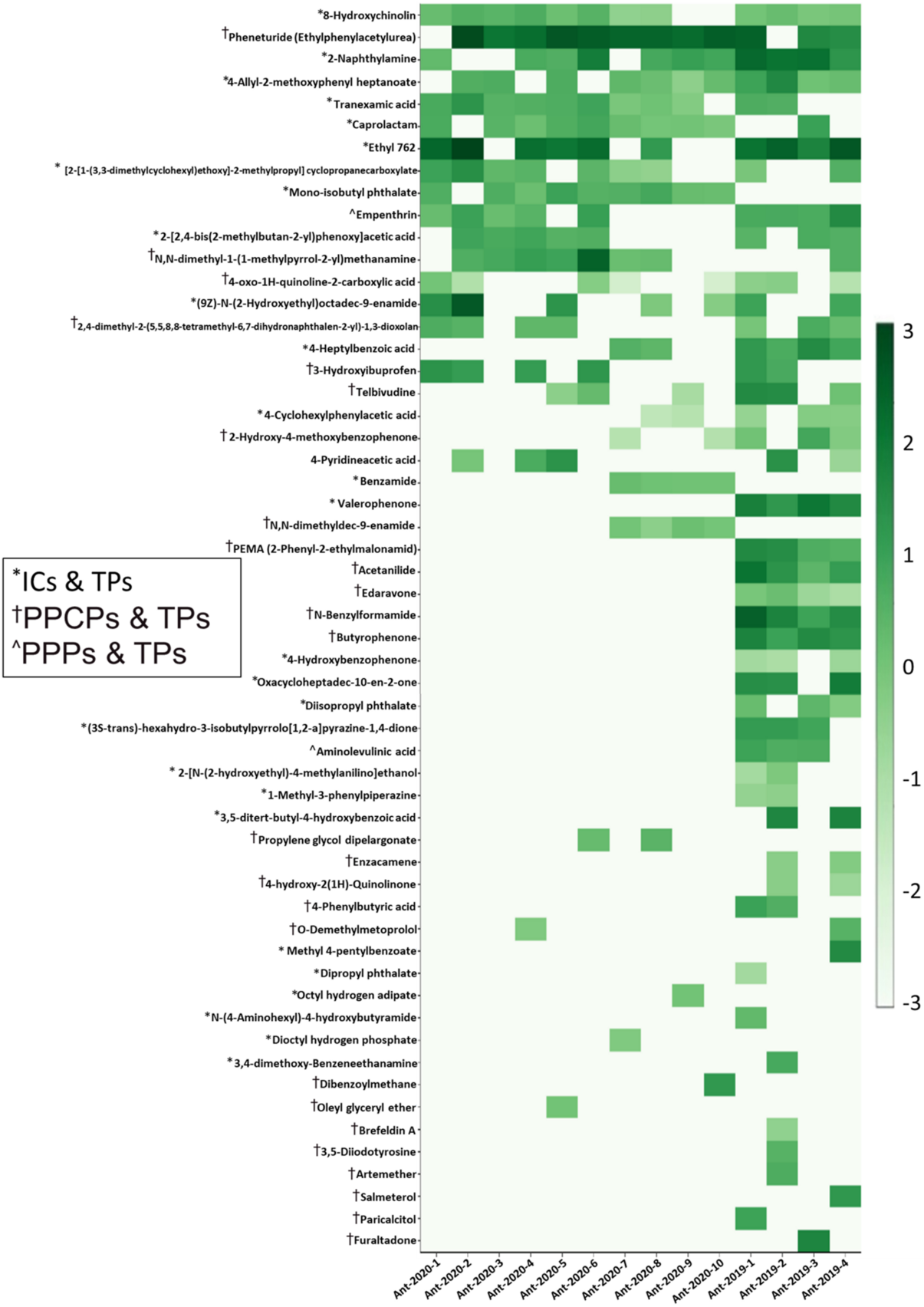
*8-Hydroxychinolin
†Pheneturide (Ethylphenylacetylurea)
*2-Naphthylamine
*4-Allyl-2-methoxyphenyl heptanoate
*Tranexamic acid
*Caprolactam
*Ethyl 762
* [2-[1-(3,3-dimethylcyclohexyl)ethoxy]-2-methylpropyl] cyclopropanecarboxylate
*Mono-isobutyl phthalate
^Empenthrin
*2-[2,4-bis(2-methylbutan-2-yl)phenoxy]acetic acid
†N,N-dimethyl-1-(1-methylpyrrol-2-yl)methanamine
†4-oxo-1H-quinoline-2-carboxylic acid
*(9Z)-N-(2-Hydroxyethyl)octadec-9-enamide
†2,4-dimethyl-2-(5,5,8,8-tetramethyl-6,7-dihydronaphthalen-2-yl)-1,3-dioxolan
*4-Heptylbenzoic acid
†3-Hydroxyibuprofen
†Telbivudine
*4-Cyclohexylphenylacetic acid
† 2-Hydroxy-4-methoxybenzophenone
4-Pyridineacetic acid
*Benzamide
* Valerophenone
†N,N-dimethyldec-9-enamide
†PEMA (2-Phenyl-2-ethylmalonamid)
†Acetanilide
†Edaravone
†N-Benzylformamide
†Butyrophenone
*4-Hydroxybenzophenone
*Oxacycloheptadec-10-en-2-one
*Diisopropyl phthalate
*(3S-trans)-hexahydro-3-isobutylpyrrolo[1,2-a]pyrazine-1,4-dione
^Aminolevulinic acid
* 2-[N-(2-hydroxyethyl)-4-methylanilino]ethanol
*1-Methyl-3-phenylpiperazine
*3,5-ditert-butyl-4-hydroxybenzoic acid
†Propylene glycol dipelargonate
†Enzacamene
†4-hydroxy-2(1H)-Quinolinone
†4-Phenylbutyric acid
†O-Demethylmetoprolol
* Methyl 4-pentylbenzoate
*Dipropyl phthalate
*Octyl hydrogen adipate
*N-(4-Aminohexyl)-4-hydroxybutyramide
*Dioctyl hydrogen phosphate
*3,4-dimethoxy-Benzeneethanamine
†Dibenzoylmethane
†Oleyl glyceryl ether
†Brefeldin A
†3,5-Diiodotyrosine
†Artemether
†Salmeterol
†Paricalcitol
†Furaltadone
Ant-2020-1
Ant-2020-2
Ant-2020-3
Ant-2020-4
Ant-2020-5
Ant-2020-6
Ant-2020-7
Ant-2020-8
Ant-2020-9
Ant-2020-10
Ant-2019-1
Ant-2019-2
Ant-2019-3
Ant-2019-4
3
2
1
0
-1
-2
-3
*ICs & TPs
†PPCPs & TPs
^PPPs & TPs

Figure 1. A heatmap showing the results of suspect screening of the 14 biota samples. The green color scale represents the logarithm of the estimated concentrations, expressed in ng/g wet weight.

Examples of detected pharmaceuticals include the analgesic drug acetanilide, the antiviral drugs telbivudine and brefeldin A, the blood pressure regulator metabolite O-Demethylmetoprolol, the metabolite of the pharmaceutical primidone (2-phenyl-2-phethylmalonamide), the bronchodilator agent salmeterol, the antineoplastic agent edaravone, and seven other pharmaceuticals. Comparison of chemical occurrence data with PNEC resulted in exceedance for 21 compounds [Supplementary Table 6], with ethyl 762, empenthrin and pheneturide (ethylphenylacetylurea) showing the highest frequency of PNEC exceedance (8, 9 and 12 times respectively). However, this is just a rough prioritization given the uncertainties in PNEC prediction and concentration prediction.

It is important to acknowledge that the presented study focused on screening a high number of substances in a small number of samples (10 individual samples and 4 pooled samples of 23 penguin eggs), which were collected in an opportunistic manner, limiting our ability to assess temporal trends and assess variability within species. As a result, the findings should be interpreted as exploratory, providing preliminary insight into the chemical burden of local wildlife. Nonetheless, they highlight the presence of diverse legacy and emerging contaminants and underscore the need for further investigation of legacy and emerging contaminants in Antarctica to better characterize chemical pollution in the polar regions.

CONCLUSIONS

A holistic investigation of legacy and emerging chemical pollutants was performed on 14 biota samples collected from Antarctica. The four studied Water Framework Directive heavy metals (lead, cadmium, nickel and mercury) were detected in all studied samples. Although most detected concentrations were below EQS, the occasional exceedance, especially for mercury, necessitates further investigation due to its known neurotoxicity and bioaccumulative behavior. In the Tier I samples, 4 among the 13 studied PFRs (TCEP, TCPP, TPHP and EHDP) were detected sporadically below their respective PNECs, while no isomers of Dechlorane Plus were detected. These low detection frequencies likely reflect limited long-range environmental transport and lower global usage volumes of these newer flame retardants compared to legacy compounds. Dioxins, DLCs, and PBDEs showed high detection frequencies among the analyzed samples. No exceedance of EQS of DLCs was observed. High concentration levels were measured for PCBs, especially PCB105 and PCB118. Total PBDE concentrations in sea stars and sea urchins exceeded the EQS of 8.5 ng/kg. The widespread detection and elevated levels of PCBs and PBDEs in the samples reinforce their persistence and bioaccumulation potential in cold ecosystems. A wide-scope target screening (2,236 compounds) and suspect screening (65,591 compounds) of all 14 biota samples were conducted using LC-HRMS techniques. A total of 33 contaminants spanning multiple chemical classes were identified through wide-scope target screening. Of these detected contaminants, 42% were PPCPs, and another 30% were ICs and TPs. Methylparaben was the most frequently detected compound, occurring in 93% of the samples. It exceeded the respective PNEC value in the fish sample together with oxfendazole. The detection of these compounds highlights the potential risk posed by pharmaceuticals and additives even in remote regions, calling for further ecotoxicological assessment. Through suspect screening efforts, 55 additional compounds were identified, with PPCPs and ICs each accounting for 26 of these compounds. The majority of these compounds are registered as REACH substances by the ECHA, with some being produced in very high tonnage, exceeding 1,000,000 tonnes. Their detection in Antarctic biota emphasizes the global reach of anthropogenic chemical pollution and underscores the value of suspect screening for identifying lesser-known but potentially hazardous substances. Overall, contamination levels in the Antarctic biota samples were lower than those reported in comparable European studies, such as those conducted in the Danube

Riber Basin, in both the number of detected contaminants and their concentrations. It is recommended to further investigate the occurrence of legacy and emerging contaminants in Antarctica to better characterize the status of chemical pollution in the polar regions.

DECLARATIONS

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Availability of data and materials

All acquired LC-HRMS and GC-HRMS chromatograms from this study were uploaded to the NORMAN DSFP. All HRMS data are available at https://doi.org/10.60930/zkqd-pn07.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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