Environmental Science & Technology

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Fin Whale as a Sink of Legacy and Emerging Contaminants: First Integrated Chemical Exposomics and Gene Expression Analysis in Cetaceans

Published as part of Environmental Science & Technology special issue "Ocean Health".

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Cite This: *Environ. Sci. Technol.* 2025, 59, 11477−11492

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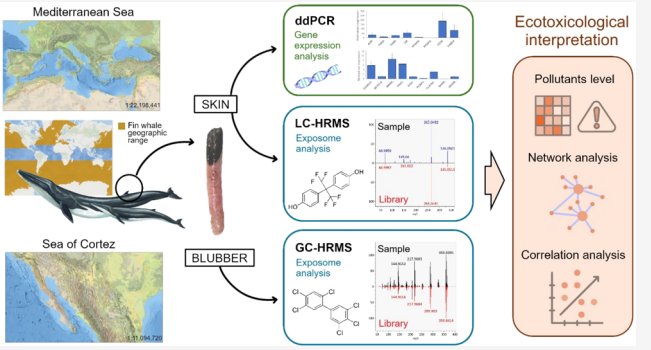
ABSTRACT: Cetaceans face numerous anthropogenic chemical stressors in global oceans, yet there is a lack of studies that simultaneously assess their cumulative exposure to both legacy and emerging contaminants and their combined effects. To evaluate the susceptibility of fin whale (*Balaenoptera physalus*) to chemical pollution, this study employed for the first time a multidiagnostic molecular approach that integrates chemical exposomics and gene expression analysis in live-sampled skin and blubber biopsies from two distinct populations: the endangered Mediterranean subpopulation (Italy) and the vulnerable population from the Sea of Cortez (Mexico). Both marine regions are biodiversity hotspots characterized by different anthropogenic impacts, making them ideal for the assessment of heterogeneous contaminants exposure and their effects. Results revealed distinct exposure profiles in the two populations, with Mediterranean fin whales exhibiting higher concentrations of legacy pollutants such as polychlorinated biphenyls (PCBs), as well as plasticizers, perfluoroalkyl substances (PFAS), while both populations showed traces of pharmaceuticals and lifestyle-related chemicals (e.g., paracetamol, diclofenac, nicotine, UV filters) and other substances not previously reported in whales. Supported by 32 network correlations with gene expression relevant to transcriptional regulation, endocrine disruption, lipid homeostasis, and inflammation, our findings suggest that complex anthropogenic chemical exposures may compromise the health and reproductive viability of the endangered Mediterranean fin whales, affirming their importance as a global sentinel species, which reflects marine ecosystem integrity within the "One Health" framework.



KEYWORDS: *cetaceans, LC-HRMS, GC-HRMS, pharmaceuticals, PCBs, PBDE, gene expression, biomarkers*

INTRODUCTION

The health of cetaceans, due to their habitat, migratory habit, long life span, and mainly belonging to high trophic level, reflects the general health of our oceans and the complex interplay between marine organisms and the environment. Whales and dolphins, in particular, are known to bioaccumulate environmental chemical contaminants in their fat and storage tissues (blubber).[1−5] Based on all of these premises, these species can be considered a suitable sentinel of ocean health.

After the blue whale (*Balaenoptera musculus*), the fin whale (*Balaenoptera physalus*) is the second largest animal on the planet. It is found in all of the major oceans, from polar to tropical waters, with the highest population density occurring in temperate and cold waters. This species is globally listed as Vulnerable (IUCN Red List of Threatened Species) and faces several anthropogenic stressors, including collision with vessels,[6] noise,[7] pathogens,[8] and a variety of pollution including marine litter and microplastics,[1,9,10] and chemical contaminants[11] that may threaten population stability in the long term.

Although fin whales are considered a sentinel of ocean health,[12] little is known about their exposure to anthropogenic pollutants, including thousands of widespread emerging organic contaminants of concern that are not globally regulated. These chemicals have applications in industry, pharmaceuticals, personal care products, flame retardants, plasticizers, current-use pesticides, and lifestyle-related products. The cumulative

Received: January 31, 2025
Revised: April 18, 2025
Accepted: April 21, 2025
Published: June 2, 2025

effects that these complex mixtures of chemicals may exert on cetaceans are not clear, and studies have so far been limited to the analysis of a small number of targeted contaminants at a time.[2,13−15]

To advance our understanding of chemical pollution in whales, a new versatile and high-throughput approach, such as exposomics, could be effective. The exposome concept was originally proposed as a paradigm for human health research[16,17] and aims to comprehensively characterize environmental exposures that together may impact health through molecular-initiated events.[18] Chemical exposomics in particular focuses on the analysis of small molecules and environmental pollutants, combining untargeted analyses and suspect screening with and multiclass targeted analysis by high-resolution mass spectrometry (HRMS).[19,20] This wide-scope approach offers a novel framework to improve our understanding of the multiple factors influencing animal health and well-being, yet it has been rarely applied to wild animals,[21,22] and never in whales.

Current studies on whale chemical exposures have primarily focused on single classes of pollutants, particularly those initially regulated by the Stockholm Convention, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and chlorinated dioxins or furans, while more recent attention has been paid to microplastics and their additive plasticizers.[1,9] In addition, few studies have established causal links between exposure and health impacts in cetaceans, although variation of gene expression[23−26] and epigenetic modifications[27] are considered a reliable early warning signal of toxicological effect.

Here, skin-blubber biopsies were collected from free-ranging fin whales[28] in the Mediterranean Sea (Ligurian Sea, Italy-France) and the Sea of Cortez (Mexico). In this case study, the Mediterranean Sea was chosen as a threatened biodiversity hotspot[29] where the declining fin whale population (classified as Endangered) currently consists of less than 2500 mature individuals.[30] In contrast, the Sea of Cortez is another biodiversity hotspot with lower human impacts, serving as a major feeding and breeding zone for large marine mammals. Through valuable and strategic nonlethal biopsy sampling, representative of the fin whale live populations,[31] we aim to demonstrate how chemical exposomics and gene expression analysis of the same samples can be integrated to identify biomarkers of chemical stressors. To capture the wide chemical space represented by legacy pollutants, emerging contaminants, as well as endogenous metabolites, the chemical exposomics workflows included both gas chromatography (GC) and liquid chromatography (LC)-HRMS untargeted and multiclass targeted analysis for priority substances. Despite the limited starting material of only a few milligrams of skin-blubber tissue, posing challenges to perform sensitive chemical exposomics and gene expression analyses together, in this work, we showcase a reliable and efficient method to perform multiomics ecotoxicological analysis in cetaceans for the first time. Given the ethical, animal care, and logistic restrictions and implications, this nonlethal eco-exposome approach can help to unravel how complex environmental chemical exposures are affecting the health of the target fin whale populations and others worldwide.

MATERIALS AND METHODS

Sampling and Sex Determination. Whales' biopsies were obtained in accordance with the relevant guidelines and regulations.[32] Mediterranean samples were collected under sampling permit no. 0018799/PNM issued by the Italian Ministry for Environment and Energy Security and the Italian National Institute for Environmental Protection and Research. All samples from the Gulf of California were obtained under the appropriate collecting permits issued by the Mexican Wildlife Agency; permit numbers: D0070(2) 0598, D00700(2) 14093, D00750−1537, and SGPA/DGVS/0576. The permits include the necessary ethical approval for sample collection, analysis, and use for scientific studies.

Biopsies of fin whale (*B. physalus*) were collected from the NW Mediterranean Sea (SPAMI Pelagos Sanctuary, Italy; $n = 17$ specimens) between 2018 and 2019, and from the Sea of Cortez (Gulf of California, Mexico; $n = 9$ specimens) between 2018 and 2019 (Table S1). Biopsies were collected in the dorsolateral area below the dorsal fin using a 68 kg draw weight recurve crossbow (Barnett Panzer V). We obtained about 1 g of tissue samples using sterilized stainless steel biopsy tips (30 mm × 8 mm) attached to 18″ bolts. The samples were immediately stored in liquid nitrogen after biopsy collection and stored at −80 °C until laboratory analysis. However, for some specimens, the skin or blubber sample was not enough to perform the complete set of analysis, and details on the number of analyzed tissue samples are reported in Table S1. Sex was determined through PCR amplification of ZFY/ZFX gene following the protocol described by Berube and Palsbøll, 1996.[33]

Chemical Exposomics Analysis. Exposomics analyses were performed at the National Facility for Exposomics, SciLifeLab (Sweden). Skin and blubber tissues were processed in a positive pressure HEPA filter clean laboratory and handled under a laminated fume hood. Dissected blubber and skin samples underwent dedicated polar and nonpolar solvent extractions which were then analyzed by GC- and LC-HRMS Orbitrap,[16,34,35] respectively. This approach leveraged the distinct properties of each tissue, with the blubber (hypodermis) having strong partitioning for lipophilic contaminants and the skin (epidermis) serving as a matrix for more polar compounds. The lipophilic fraction of skin was not analyzed, as any lipophilic compounds were likely already captured at higher concentrations in the blubber. Raw GC and LC data were preprocessed with the open-source software MS-DIAL, and chemical annotations (Level 2 confidence)[36] were assigned by spectral matching on NIST20 and MassBankEU libraries, respectively. Detailed sections relevant to sample extraction, analytical methods, data quality control, and data analysis are reported in the Supplementary methods.

Gene Expression Analysis. The analysis was performed at the University of Siena on the Mediterranean fin whales for 16 out of 17 skin samples available as one sample did not yield enough total RNA to proceed (Table S1). The gene expression analysis was restricted to the Mediterranean population, since not enough skin tissue was available from Mexican samples. A set of 17 genes was selected as molecular markers of exposure to emerging and legacy contaminants (Supplementary Data 2). Total RNA was isolated from 20 to 40 mg of skin biopsies and retrotranscribed in cDNA following published methods.[26] Primers (Table S3) were designed using online tools Primer-BLAST[37] and Primer3.[38] Direct quantification of transcript levels was performed using droplet digital PCR (ddPCR).[25] Gene expression results were obtained by normalizing to the reference gene tyrosine 3-monooxygenase (*YWHAZ*).[39]

Data Analysis and Statistics. Multivariate analyses of exposome data were performed with SIMCA v.17. Heatmaps were generated from normalized peak area for each compound using the R package pheatmap (version 1.0.12). Correlations

https://doi.org/10.1021/acs.est.5c00844
Environ. Sci. Technol. 2025, 59, 11477−11492

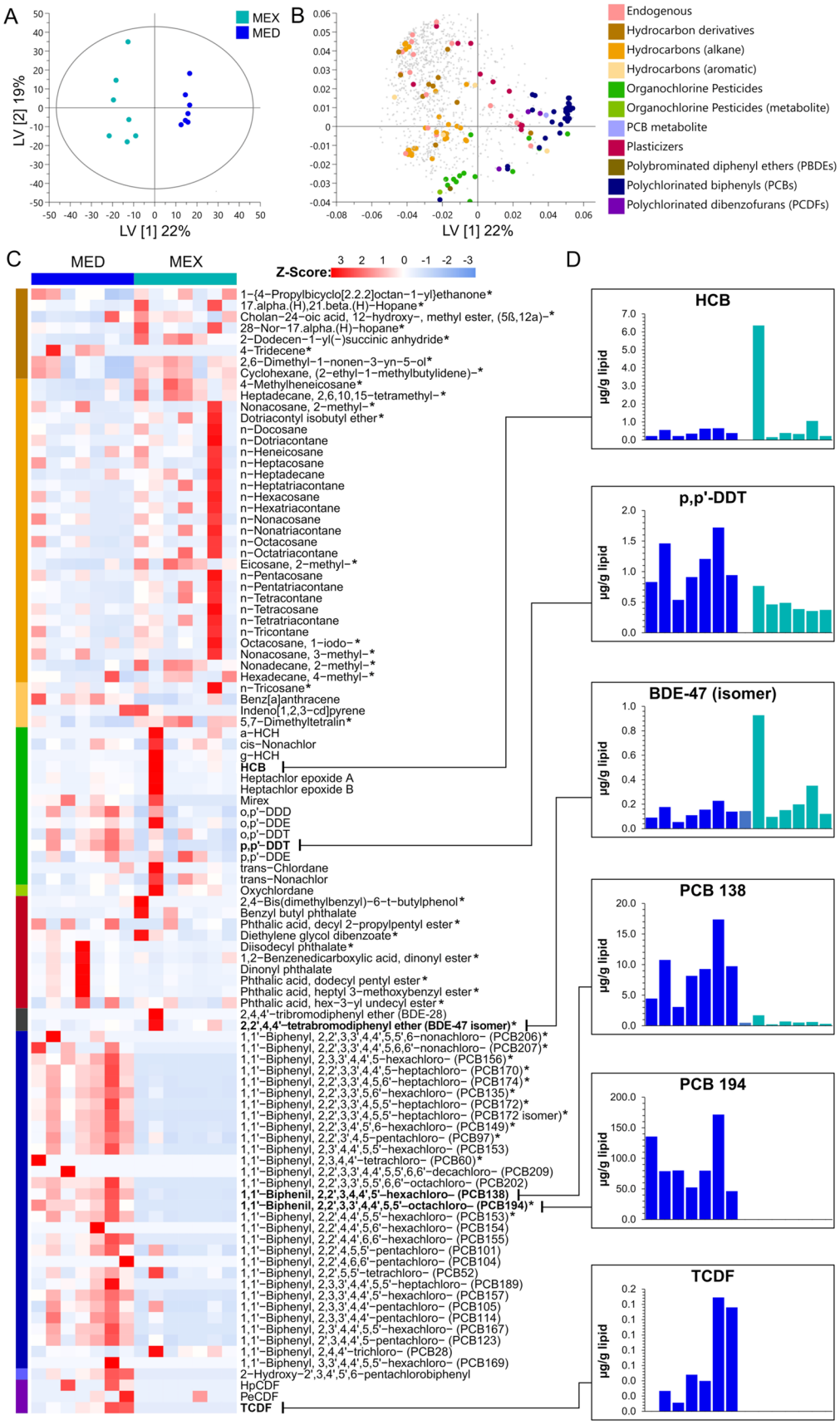


Figure 1. Chemical exposome profiling of fin whale blubber by GC-HRMS for two fin whales' populations. Multivariate discriminant analysis results are shown in (A) scores and (B) loadings plots of an OPLS-DA model (1 + 1 + 0); (R^2Xcum = 40.6%, R^2Ycum = 95.7%, Q^2cum = 63.4%, CV-ANOVA p -value = 0.04) explaining the variation of targeted and untargeted chemical exposome profiles of blubber extracts analyzed by GC-HRMS from fin whale (*B. physalus*) populations of the Mediterranean Sea (n = 7; MED) and the Sea of Cortez (n = 7; MEX). In total, the model accounted for x = 1176

https://doi.org/10.1021/acs.est.5c00844
Environ. Sci. Technol. 2025, 59, 11477−11492

Figure 1. continued

molecular features, including endogenous biomolecules and legacy environmental contaminants which were quantified (Level 1) or semiquantified (Level 2) by GC-HRMS. (C) Heatmaps of the normalized values are shown for a selection of 101 confirmed (Level 1) or annotated (Level 2) molecules arranged according to the different chemical classes. Asterisks indicate annotation confidence Level 2, no asterisks Level 1. (D) Zoom-in barcharts showing concentrations (μg/g lipid) quantified in blubber of the two whale populations for some of the most relevant legacy contaminants: organochlorine pesticides (HCB, *p,p′*-DDE), tetrabromodiphenyl ether (BDE-47 isomer), polychlorinated biphenyls (PCB-138, PCB-194), and polychlorinated dibenzofurans (TCDF).

between chemical compounds and gene expression levels were investigated by computing Spearman's correlation coefficients (P) through a partial correlation analysis including whales' sex as a factor using the R package psych (version 2.4.3). Pairwise correlation *p*-values were adjusted using the Benjamini-Hochberg procedure, and correlations (FDR < 0.05) were visualized through a network diagram using the R package igraph (version 2.0.3.9033). Network nodes were clustered according to the Louvain method for community detection and plotted using the Fruchterman-Reingold force-directed layout algorithm with ρ as the weight parameter.

RESULTS AND DISCUSSION

Mapping Chemical Exposomes in Skin and Blubber for Two Fin Whale Populations. We profiled fin whale exposure to legacy and emerging contaminants via trace-level chemical exposomics on tissue biopsies of free-ranging populations from the Mediterranean Sea (Italy-France) and the Sea of Cortez (Mexico). This approach enabled targeted analyses of known priority contaminants across multiple chemical classes alongside untargeted screening for biomolecules and unexpected chemical exposures.

GC-HRMS Analysis of Blubber Extracts Shows Higher Levels of Polychlorinated Biphenyls in Mediterranean Fin Whales. A total of 1196 molecular features were detected across all blubber samples (Figure 1A), with 123 chemical identities consisting of 58 target compounds confirmed with reference standards (Level 1) and 62 putatively annotated on spectral libraries (Level 2).[40] Many of these GC-amenable compounds were legacy halogenated contaminants (Supplementary Data 1), of which 29 were quantified using reference standards and 12 semiquantified with a close structural analogue (Table S4). Among the quantified analytes were various congeners of the industrial polychlorinated biphenyls (PCBs; n = 24 homologues), organochlorine pesticides and their metabolites (n = 14), e.g., heptachlors, nonachlors, hexachlorocyclohexanes, oxychlordane, and dichlorodiphenyltrichloroethanes (DDTs), two polychlorinated dibenzofurans (PCDFs), and a putative tetrabromodiphenyl ether flame retardant ($C_{12}H_6Br_4O$, positional isomer of BDE-47). Multivariate discriminant analysis (OPLS-DA; Figure 1A) significantly explained 22% (LV1) of the variation in these 1196 chemical signals between the Mediterranean and Mexican populations. Scores and loadings plots (Figure 1A), and exposome heatmaps of GC-HRMS xenobiotic profiles (Figure 1B) revealed distinct clustering by population.

Among the major chemical drivers differentiating the two populations were higher levels of PCBs in the Mediterranean samples (Figure 1). PCBs were the most ubiquitous contaminants, with detection frequency (DF) between 14 and 100% depending on the congener. Overall, PCBs were more frequently detected in the Mediterranean Sea samples, usually at 10−100-fold higher concentrations compared to those from the Sea of Cortez. A total of 17 congeners showed elevated levels in the Mediterranean Sea samples (Welch *t* test, p-values from 0.04 to <0.001) (Table S4 and Supplementary Data 1).

In our previous studies of fin whales and cetaceans from the Mediterranean (2012−2015), we reported blubber concentrations of 18−30 PCB congeners (up to 7 chlorine atoms), with single congeners ranging from 0.1 μg/g lipid to 15 μg/g lipid in the case of PCB-153.[1,41] Here, 30 distinct PCBs were detected, including 17 confirmed PCBs (Level 1), ranging from trichloro- to decachloro, and one putative PCB metabolite (2-hydroxy-2′,3,4′,5′,6-pentachlorobiphenyl, Level 2) (Table S4 and Supplementary Data 1). Among Level 1 congeners, the highest concentrations were found for hexachloro congeners 2,2′,3,4,4′,5′-PCB (PCB-138) and 2,2′,4,4′,5,5′-PCB (PCB-153), up to 17.4 and 10.9 μg/g lipid, respectively (DF 93−100%, *p*-value = 0.003−0.004) (Table S4), consistent with our previous reports.[41] Among Level 2 congeners, an octachloro isomer ($C_{12}H_2Cl_8$; RI = 2718) tentatively identified as 2,2′,3,3′,4,4′,5,5′-PCB (PCB-194; RI diff = 57) and semiquantified with the isomer PCB-202, was detected only in Mediterranean samples (Welch *t* test, *p*-value = 0.002) with the highest concentration of 171.7 μg/g lipid (median 80 ± 45.4, DF 50%). Numerous highly chlorinated congeners were also putatively identified, including 9 hexachloro-, 5 heptachloro-, 3 octachloro-, 2 nonacholoro-, and the fully chlorinated decachloro- 2,2′,3,3′,4,4′,5,5′,6,6′-PCB (PCB-209) (Table S4 and Supplementary Data 1).

Other chlorinated legacy pollutants, e.g., DDT and DDE isomers (*p,p′*-, and *o,p′*-) and the associated metabolite *o,p′*-DDD, were also frequently detected (DF 93−100%) and quantified (Level 1). Levels of DDTs and metabolites were generally twice as high in Mediterranean whales, with a significant difference for the levels of *o,p′*-DDD (p = 0.048) and *p,p′*-DDT (p = 0.004) detected up to 1.7−2.0 μg/g of lipid. It is well known that *o,p′*-isomer exhibits stronger estrogenic effects compared to *p,p′*-DDT.[42,43] The related DDE (*p,p′*- and *o,p′*-) isomers were present at similar levels in both populations, with the highest levels of *p,p′*-DDE (DF 100%) up to 64 μg/g of lipid (Table S4).

The chlorinated furan 2,3,7,8-tetrachlorodibenzofuran (TCDF; Level 1) was detected and quantified only in 6 samples from the Mediterranean Sea (DF 43%) up to 0.15 μg/g lipid (median 0.04 ± 0.06 μg/g). Hexachlorobenzene (HCB, Level 1) was found at similar median concentrations in all samples (Med: 0.33 μg/g lipid; Mex: 0.39 μg/g lipid) with maximum concentrations of 6.4 μg/g in one sample from the Sea of Cortez (Table S4).

A putative tetrabromophenyl ether (isomer of BDE-47; Level 2; DF 100%) was found at median concentrations of 0.12 μg/g lipid (Med) and 0.15 μg/g lipid (Mex), similar to what we previously reported in the Mediterranean Sea (ca. 0.3 μg/g),[41] but was also found at higher levels up, to 0.71 μg/g, in the Sea of Cortez.

Phthalate plasticizers were detected in blubber, including dinonyl phthalate and benzyl butyl phthalate (both Level 1; not

https://doi.org/10.1021/acs.est.5c00844
Environ. Sci. Technol. 2025, 59, 11477−11492

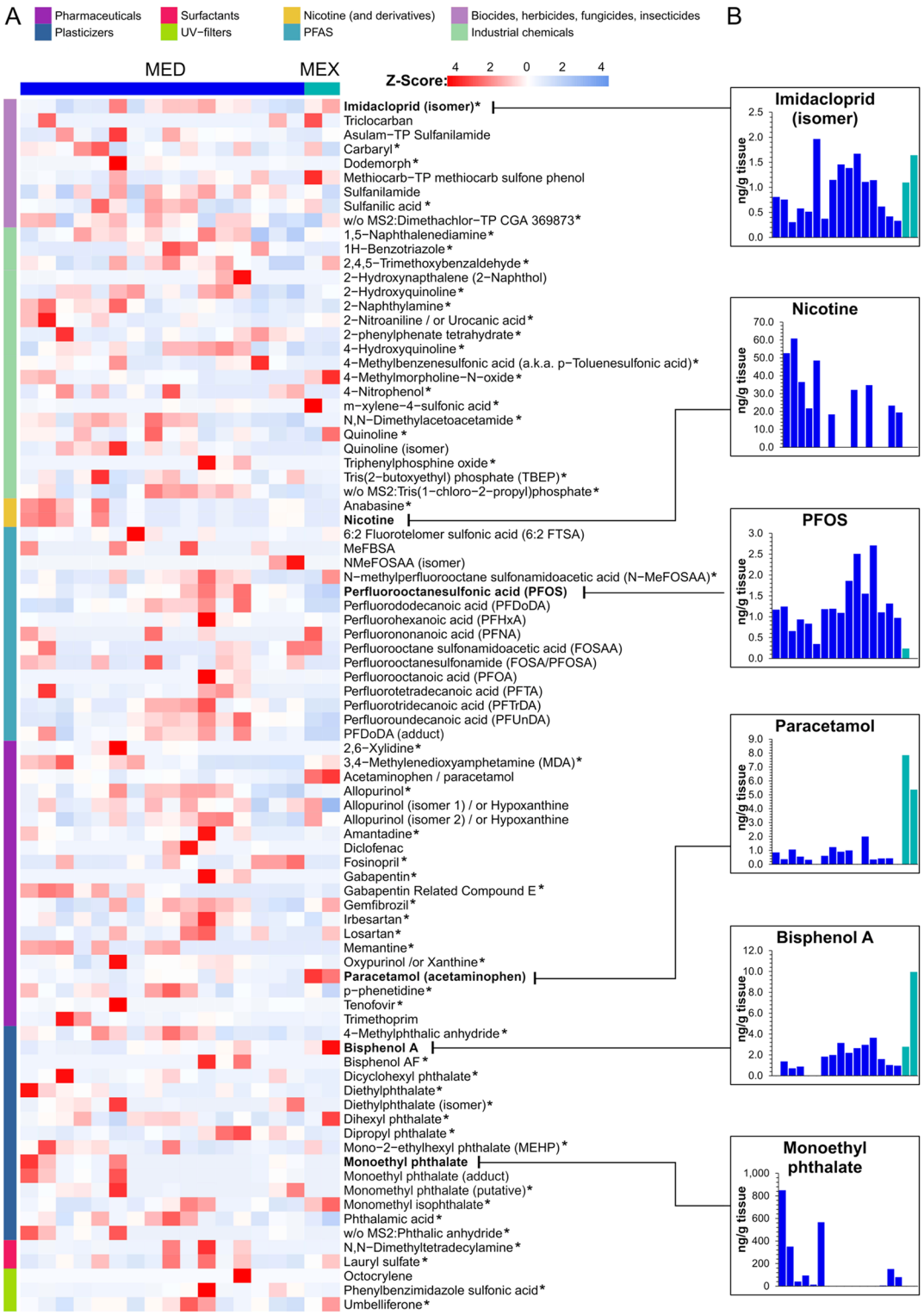


Figure 2. Chemical exposome heatmaps and concentrations determined by untargeted LC-HRMS of fin whale skin. (A) Heatmap of the normalized values are shown for a selection of 81 xenobiotic molecules assembled according to the different molecular classes identified as emerging contaminants in the skin of fin whale (*B. physalus*) specimens of the two study areas (Med $n = 16$; Mex $n = 2$). Asterisks indicate identification confidence level 2, no asterisk level 1. (B) Zoom-in barcharts showing concentrations (ng/g tissue) quantified in skin samples from the two whale populations for some of the most relevant emerging contaminants: imidacloprid (insecticide), nicotine, perfluorooctanesulfonic acid (PFOS), paracetamol (pharmaceutical), bisphenol A, and monoethyl phthalate (plastic additives).

quantified due to high background), along putative identifications (Level 2) for diethylene glycol dibenzoate, diisodecyl phthalate, and 2,4-bis(dimethylbenzyl)-6-t-butylphenol (Figure 1 and Supplementary Data 1).

Several alkane hydrocarbons—which were previously observed to distribute to lipids in marine organisms[44]—were found higher in blubber of whales from the Sea of Cortez (Figure 1). Previous studies described different natural or anthropogenic

https://doi.org/10.1021/acs.est.5c00844
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sources, including seepage of crude oil, combustion of fossil fuel and biomass, production from sea vegetation or deep-water hydrocarbon seeps, as possible exposure sources of alkanes for whales inhabiting the Sea of Cortez (Guaymas Basin).[45,46]

No significant differences were found in blubber contaminant burdens of males ($n = 9$) and females ($n = 5$) when considering both populations together (two-tailed Welch's t test). However, within the Mediterranean population, significant sex differences were observed for several lipophilic legacy contaminants, including PCBs (PCB-52, PCB-97, PCB-138, PCB-153, PCB-155, PCB-157), chlorinated pesticides (HCB, *cis*-Nonachlor, *trans*-Nonachlor, *p,p′*-DDT, *o,p′*-DDT, *o,p′*-DDD) and tetrabromophenyl ether, all of which were more abundant in males ($p < 0.05$, two-tailed Welch's t test, Supplementary Data 1). This result may be explained by the transfer of contaminants from females to the offspring during gestation and lactation.[47] These differences were not observed within the Mexican population, where only two females were sampled, both showing lipophilic pollutant concentrations comparable to males (Table S4).

LC-HRMS Analysis of Skin Reveals Widespread Exposure to Emerging Contaminants. Parallel untargeted and targeted LC-HRMS analyses enabled the quantification and discovery of a range of polar and semipolar chemicals, many of which have rarely been investigated in cetaceans. Over 45,000 molecular features were detected across ESI+ and ESI− modes, yielding 387 chemical identities, including 353 structural annotations based on spectral library matching (Level 2) for both endogenous compounds and xenobiotics (102 in ESI+ and 251 in ESI−) (Supplementary Data 1). Additionally, 23 target analytes (Level 1) were quantified with reference standards, and 6 putative compounds (Level 2) were semiquantified using close structural analogues (Table S4).

In total, 21 pharmaceutical substances (Levels 1 and 2) were detected in skin samples from both regions (Figure 2, Table S4, and Supplementary Data 1). Notably, we report emerging contaminants, including the antipyretic drug acetaminophen/paracetamol (Level 1, DF 89%) detected at the highest concentrations in samples from the Sea of Cortez (max. 7.84 ng/g); the nonsteroidal anti-inflammatory drug diclofenac (Level 1, DF 28%, max 0.18 ng/g); and sulfanilamide (Level 1, DF 22%, max 14.9 ng/g), potentially resulting from direct exposure to the antibacterial drug or as transformation product of the carbamate herbicide asulam (not detected).[48]

Other putative annotations (Level 2) included the pharmaceuticals fosinopril, gemfibrozil, gabapentin, trimethoprim, irbesartan, losartan, amantadine, memantine, allopurinol, and its metabolite oxypurinol (Figure 2 and Supplementary Data 1). Many of these substances were previously monitored and detected in a global study on pharmaceuticals in rivers,[49] indicating that they enter, persist in coastal seas, and accumulate in the marine food web following their manufacture, use, and disposal in populated areas. However, studies on pharmaceuticals in cetaceans are scarce, with one recent investigation reporting trace levels (<1 ng/g tissue) in stranded dolphins from the Bay of Biscay (Atlantic Ocean, Spain).[50]

Previous studies reported high concentrations of pharmaceuticals in Mediterranean coastal waters, including analgesics like ibuprofen, and lipid regulators such as gemfibrozil,[51] which lowers blood lipids by activating proliferator-activated receptors (PPARs).[52] Paracetamol was previously reported among other pharmaceuticals measured in offshore surface waters of the Western Mediterranean, including the Pelagos Sanctuary;[53] however, no studies have documented its presence in pelagic marine organisms, including fin whale preys (e.g., krill). The xanthine allopurinol, a commonly prescribed antigout medication in Europe,[54] and its metabolite oxypurinol (both Level 2), are known to persist in wastewater treatment plant effluents[55] and were detected for the first time in fin whale skin samples in this study (Supplementary Data 1).

The tobacco alkaloids nicotine (Level 1) and anabasine (Level 2) were detected in the Mediterranean whale skin samples at concentrations up to 60.8 ng/g (DF 56−67%; Figure 2 and Table S4). Current knowledge of the environmental concentrations of these substances in marine environments is limited, as most studies have focused on freshwater systems and laboratory contexts to evaluate their effects on aquatic organisms, particularly in relation to the discharge of cigarette butts into the environment.[56] Recently, in a laboratory setting, nicotine leached from smoked cigarette butts has been shown to bioaccumulate at high concentrations in fish (rainbow trout) indicating the possibility of accumulation in marine food webs.[57]

A total of 20 perfluoroalkyl and polyfluoroalkyl substances (PFAS) were detected in most of the analyzed specimens (28−100% DF), including 13 confirmed (Level 1) and quantified (Figure 2 and Table S4). The most ubiquitous PFAS (DF 83−100%) were perfluorooctanesulfonate (PFOS), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoic acid (PFDoDA), and perfluorotridecanoic acid (PFTrDA), with median concentrations from 0.1 to 4 ng/g in skin. Concentrations of PFOS, PFUnDA, PFDoDA, PFTrDA, as well as *N*-methylperfluorobutanesulfonamide (MeFBSA) and perfluorooctanesulfonamide (FOSA/PFOSA) were higher in the Mediterranean Sea (p-value <0.001−0.05; Table S4), consistent with PFAS levels previously reported in cetaceans, e.g., in the skin of killer whales (*Orcinus orca*) from Greenland[58] and the Northeastern Pacific Ocean[59] or in the liver of stranded striped and bottlenose dolphins from the Mediterranean Sea.[60,61] Here, the highest concentrations were observed for PFOA in samples from the Mediterranean, up to 45.8 ng/g, although with a lower detection frequency (DF 33%). Traces of the short-chain PFSA, perfluorohexanoate (PFHxA), were detected in Mediterranean skin samples (DF 44%) with levels that could be quantifiable in one sample (0.27 ng/g). This finding is surprising given PFHxA's relatively low bioaccumulation potential, but its presence may be linked to the co-occurrence of the precursor compound 6:2 fluorotelomersulfonate (6:2 FTSA), which is more accumulative and has been detected in marine invertebrates.[62] Recent reports have indicated that 6:2 FTSA may be immunotoxic in mouse models.[63]

Other additive plasticizers were detected by LC-HRMS. Monoethyl phthalate (Level 1) was frequently detected (DF 89%) and found at significantly higher levels in the Mediterranean samples (p-value = 0.05), with the highest concentration of 848.7 ng/g. Diethyl phthalate (Level 2, semiquantified) showed similar trends (DF 67%, p-value = 0.04) but with overall lower concentrations, up to 12.8 ng/g (Level 2, semiquantified; Table S4). Dicyclohexyl phthalate and mono-2-ethylhexyl phthalate (Level 2) were detected at similar levels in every sample (Table S4 and Supplementary Data 1). These data are consistent with our previous reports of phthalate plasticizers in Mediterranean and Mexican fin whales blubber, with single compounds ranging between 5 and 3000 ng/g tissue.[1,64]

Most skin samples (DF 83%) contained detectable concentrations of bisphenol A (BPA, Level 1) up to 9.9 ng/g. Its

https://doi.org/10.1021/acs.est.5c00844
Environ. Sci. Technol. 2025, 59, 11477−11492

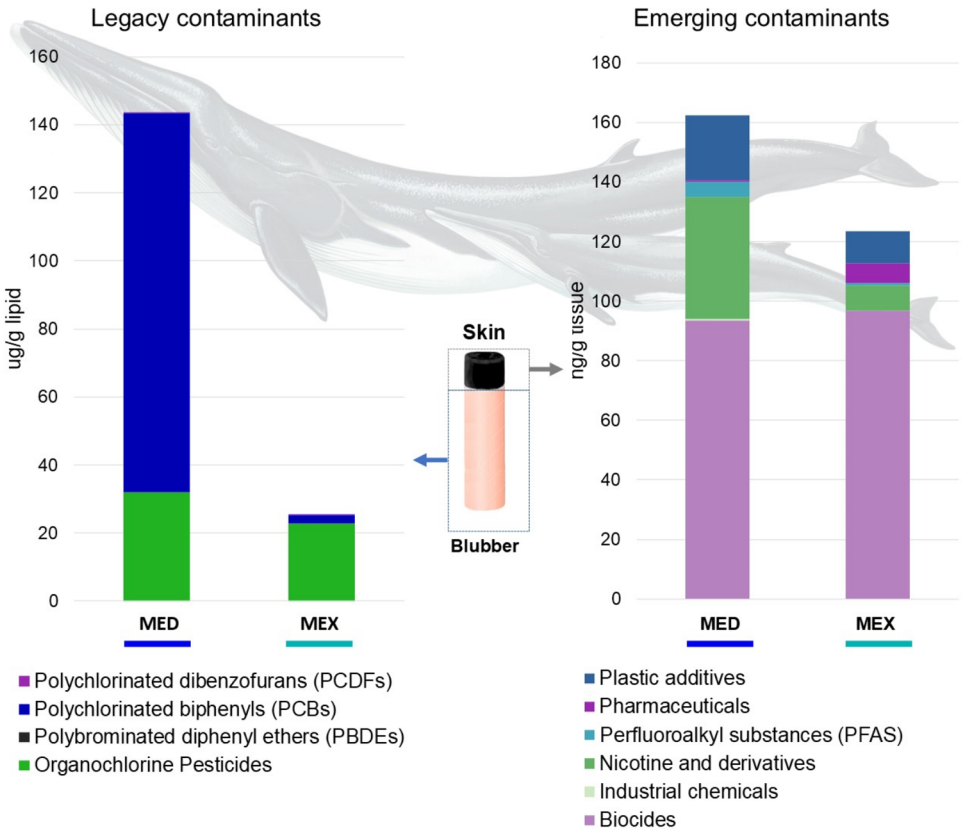

Figure 3. Fin whale as a sink of emerging and legacy contaminants. Cumulative levels of legacy (μg/g lipid) and emerging (ng/g tissue) contaminants in fin whale biopsies from the two study areas, quantified (level 1) by GC-HRMS (in the blubber) and LC-HRMS (in the skin) calculated using the median value of each different contaminant.

putative fluorinated analogue, bisphenol AF (BPAF, Level 2), which can be classified as PFAS, was also detected, although at lower frequency (DF 17%).

In cetaceans, including fin whales, hepatic concentrations of free-BPA detected in liver without enzymatic hydrolysis pretreatment have been reported to range from 0.13 to 498 ng/g,[65] and at similar concentrations (0.8−19.2 ng/g) was also reported in several marine fish species (n = 10 species).[66] A comparative analysis with enzymatic hydrolysis (β-glucuronidase/sulfatase) in marine fish and invertebrates (n = 13 species) showed that free-BPA and BPAF were detected without hydrolysis and represented an average of 20% of total BPA and 50% of total BPAF, with levels up to 4 ng/g and 0.89 ng/g, respectively (n = 13 species).[67] BPAF is commonly employed as a BPA substitute and is widely applied in manufacturing plastic products, such as food contact polymers and electronic materials.[68] The increasing production and use of BPAF have led to its rising detection in aquatic environments,[69] including reports in whales, porpoises, and dolphins.[65] Concerns about BPAF stem from slow degradation rate and significant bioaccumulation, raising potential endocrine toxicity issues to aquatic organisms.[69]

Both phthalates and BPA are ubiquitous contaminants frequently detected in humans, wildlife, and the environment.[70] Phthalates and their metabolites interact with various hormone regulatory systems,[71] while BPA binds to estrogen, androgen, thyroid, and peroxisome proliferator-activated receptors.[72] Both classes of contaminants act as endocrine disruptors, with effects that can be agonistic, antagonistic, or a combination of both, even at very low concentrations.[70,73]

Other industrial contaminants detected in fin whale skin included 2-hydroxynapthalene (a.k.a. 2-naphthol, Level 1), along with putative identifications (Level 2) for tris(2-butoxyethyl) phosphate, triphenylphosphine oxide, quinoline isomers, and the corrosion inhibitor 1*H*-benzotriazole (detected in both ESI+ and ESI−), as well as pesticides and fungicides like carbaryl and dodemorph (Supplementary Data 1). Among these industrial substances, the UV-filter phenylbenzimidazole sulfonic acid (also known as ensulizole, Level 2; two isomers) was detected in all skin samples (Supplementary Data 1). Ensulizole ($C_{13}H_{10}N_2O_3S$), a selective UV−B filter widely used in sunscreens, has been previously reported in Mediterranean loggerhead sea turtles (*Caretta caretta*) dysregulating neuro-endocrine-immune homeostasis and activating pro-inflammatory responses in juvenile organisms.[74,75] Several UV filters, including octocrylene, possess lipophilic properties and have been previously detected in blubber of stranded dolphins,[76,77] but to our knowledge, there are no prior reports of ensulizole in cetacean species.

Fin Whale as a Sink of Emerging and Legacy Contaminants and Sentinel of Ocean Health. Chemical exposomics data from these two whale populations indicate that fin whales act as biological sinks for numerous legacy and emerging contaminants (Table S4). The long lifespans of these animals, up to 90 years, allow for significant bioaccumulation of lipophilic contaminants in their fatty blubber tissues,[78,79] raising concerns about long-term toxicological effects. While these whales primarily inhabit the pelagic environment, typically several miles offshore, our findings reveal for the first time the presence of industrial, agricultural, and municipal pollutants in

https://doi.org/10.1021/acs.est.5c00844
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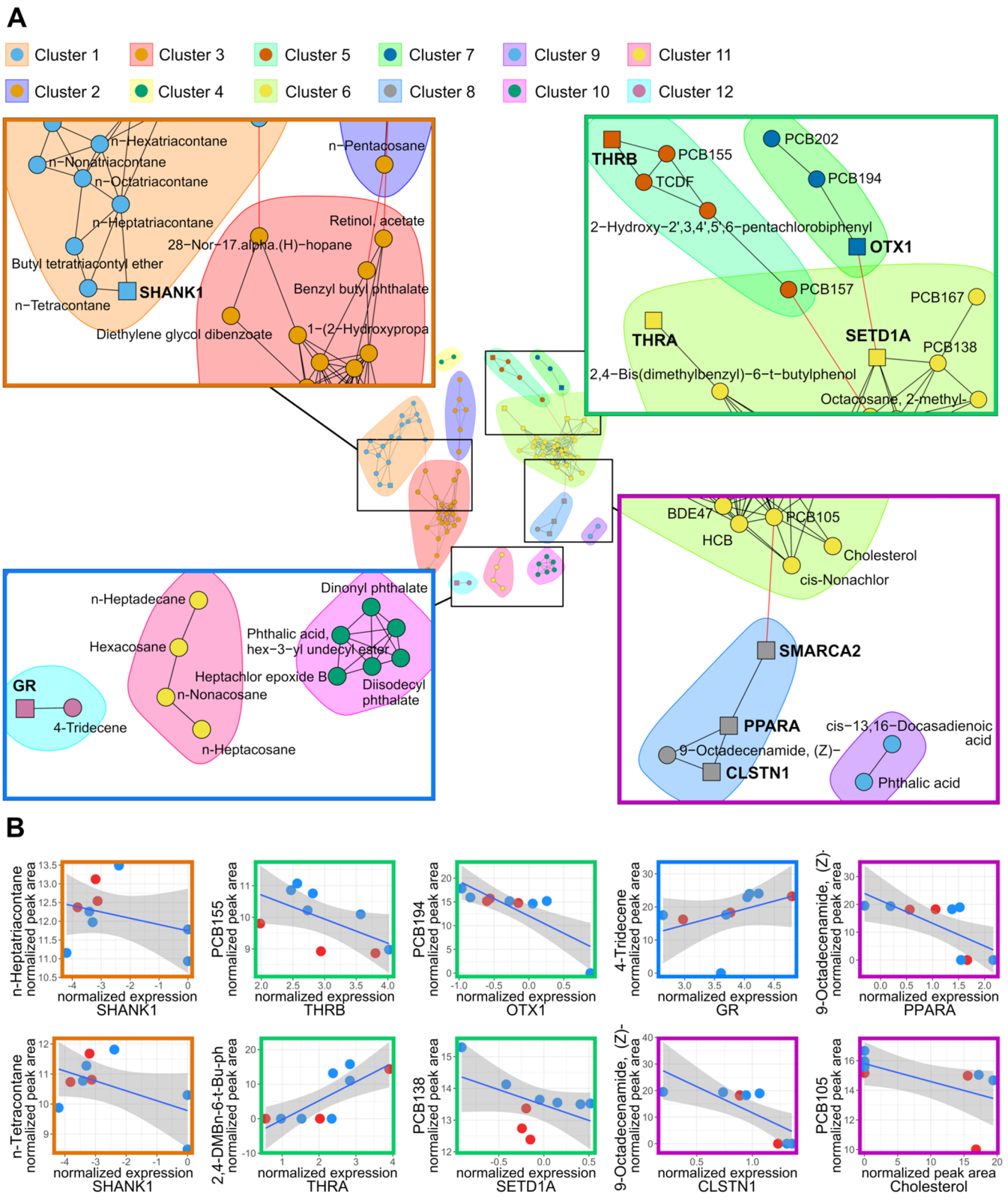

Figure 4. Network map of the whale exposome (GC-HRMS) signatures and gene expression responses. (A) Network of significant correlations based on Spearman's *P* coefficient between gene expression and exposomic data (GC-HRMS) in blubber samples of Mediterranean fin whales. Genes are represented as square nodes; compounds are represented by circles. Red lines represent correlation between nodes belonging to different clusters. Features (nodes) were connected if their correlation was statistically significant (FDR < 0.05, Benjamini-Hochberg) and only connected features with at least one link were displayed. Within this network, 12 clusters were identified using an unsupervised clustering analysis and were annotated with different colors in the network. (B) The most ecotoxicologically relevant correlations are reported in scatter plots of log-transformed data (blue dots = males, pink dots = female) below the network, colored boxes matching the zoomed sections of the network (orange, green, blue, purple).

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Environ. Sci. Technol. 2025, 59, 11477−11492

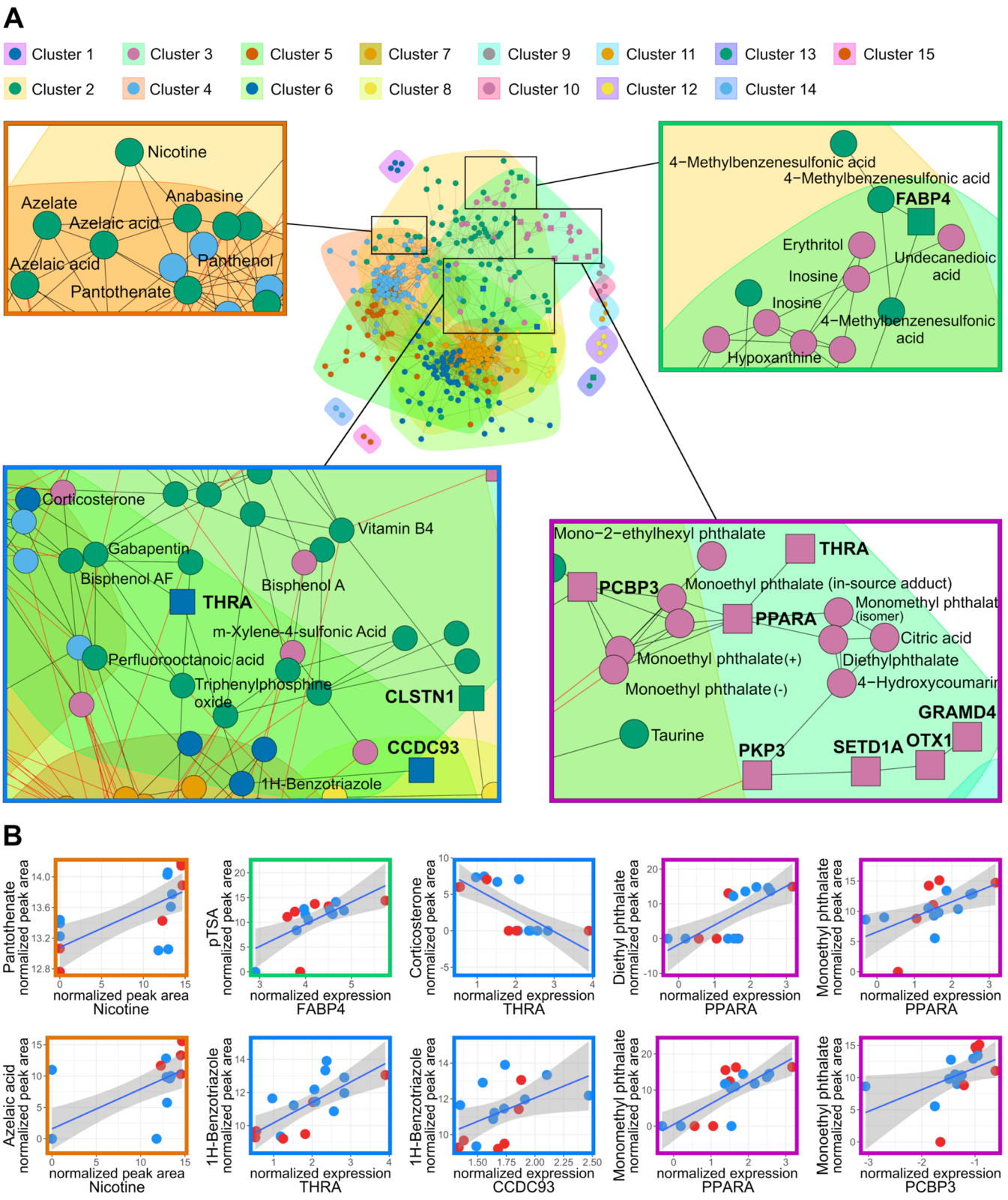

Figure 5. Network map of the whale exposome (LC-HRMS) signatures and gene expression responses. (A) Network of significant correlations based on Spearman's *P* coefficient between gene expression and exposomic data (LC-HRMS) in skin samples of Mediterranean fin whales. Genes are represented as square nodes; compounds are represented by circles. Red lines represent correlation between nodes belonging to different clusters. Features (nodes) were connected if their correlation was statistically significant (FDR < 0.05, Benjamini-Hochberg), and only features with at least one link were displayed. Within this network, 15 clusters were identified using an unsupervised clustering analysis and were annotated with different colors in the network. (B) The most ecotoxicologically relevant correlations are reported in scatter plots of log-transformed data (Blue dots = males, pink dots = female) below the network, colored boxes matching the zoomed sections of the network (orange, green, blue, purple).

https://doi.org/10.1021/acs.est.5c00844
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these pelagic organisms. This highlights an urgent need to investigate the potential health impacts on fin whales, as they can serve as sentinel species for marine ecosystem health.

We show for the first time that fin whales from two distinct geographic populations exhibit remarkably different exposure fingerprints, reflecting the unique contamination scenarios in their habitats. In the Mediterranean Sea population, levels of legacy contaminants are overall almost twice as high as those in the Sea of Cortez population (Table S4, Figure 3). Consistent with previous studies,[80] total PCB concentrations (Level 1) in the Mediterranean population averaged 111 μg/g lipid weight (Figure 3).

Despite their ban decades ago, levels of organochlorine pesticides, particularly DDTs, remain high in both regions, constituting more than 50% of legacy contaminants in Mediterranean Sea samples and over 80% in Mexican samples. The Mediterranean population appears to have greater exposure to emerging contaminants (Level 1), with plastic additives (1.8 times), PFAS (6.9 times), and nicotine (not detected in Mexican samples) detected at higher levels compared to the Sea of Cortez; however, pharmaceuticals (mainly related to paracetamol concentration) were found at 12 times higher in the Mexican population. Although concentrations of legacy organic pollutants (in blubber) were found up to 1000 times higher than those of emerging contaminants (in skin), the complexity of the contaminant mixture, and in particular the presence of numerous pharmaceuticals, tobacco metabolites, industrial chemicals, and plastic additives (phthalates and BPA), raises significant concerns. These substances may pose potential endocrine and immunotoxicological effects, highlighting the need for further investigation into their combined impact on marine organisms.

Molecular End Points as Warning Signs of Exposure to Legacy and Emerging Chemicals. Network correlation analysis was performed to integrate chemical exposomics data (Supplementary Data 1) with gene expression data (Supplementary Data 2) offering an early warning sign of the biological impact of pollutants in free-ranging fin whales and representing a crucial component of this diagnostic ecotoxicological approach (Figures 4, 5; see full networks in Figures S3, S4). Gene expression analysis focused on Mediterranean fin whales, quantifying 17 molecular end points including nuclear receptors and major targets of endocrine-disrupting chemicals, such as glucocorticoid receptor (*GR*), thyroid hormone receptors α and β (*THRA*, *THRB*), and peroxisome proliferator α and γ (*PPARA*, *PPARG*).[81] Transcription factors that bind to xenobiotics[82] such as the aryl hydrocarbon receptor (*AHR*), and transcripts involved in lipid homeostasis and inflammatory responses[83,84] such as the fatty acid binding protein 4 (*FABP4*), and the cluster of differentiation 36 (*CD36*).

The interplay between epigenetic modifications and environmental exposures has been previously demonstrated in fin whales through methylome sequencing, discovering potential hypo- or hyper-methylated genes in skin biopsies due to organochlorine exposure.[27] The transcripts here analyzed include epigenetic targets, such as SET domain containing 1A histone lysine methyltransferase (*SETD1A*), probable global transcription activator SNF2L2 (*SMARCA2*), orthodenticle homeobox 1 (*OTX1*), plakophilin 3 (*PKP3*), poly(rC) binding protein 3 (*PCBP3*), SH3 and multiple ankyrin repeat domains 1 (SHANK1), calsyntenin 1 (*CLSTN1*), Coiled-Coil Domain Containing 93 (*CCDC93*), and GRAM domain containing 4 (*GRAMD4*).

Notably, correlation network analysis revealed 12 clusters of molecules in the GC-HRMS (blubber) and gene expression data set. Halogenated contaminants (PCBs, PBDEs, and OCPs) cluster alongside cholesterol and the genes *THRA* and *SETD1A* (Cluster 6) (Figure 4A). Higher alkanes and the gene *SHANK1* are grouped in Cluster 1 (Figure 4A), which is linked to the more heterogeneous Cluster 3, composed of endogenous compounds including retinol acetate and Cholest-5-ene, 3-(1-oxobuthoxy)-. Most phthalates detected through GC-HRMS were grouped in Cluster 7 showing no correlation with endogenous molecules or gene expression data (Figure 4A). The analysis also revealed 15 clusters in the LC-HRMS (skin) and gene expression data. Plastic additives, including phthalates and BPA, are in Cluster 3, including also several molecular end points (*PPARA*, *PKP3*, *THRB*, *PCBP3*, *SETD1A*, *OTX1*, *GRAMD4*) (Figure 5A). Most notably, two large and interconnected clusters are present: Cluster 5, comprising endogenous molecules such as essential amino acids, riboflavin, creatinine, cholesterol 3-sulfate, and putative hypolipidemic drug gemfibrozil (Level 2); and Cluster 6, which included pharmaceuticals (paracetamol, losartan, diclofenac), PFAS (PFDA, PFTA, PFTrDA, PFUnDA, PFDoDA), key endogenous molecules (corticosterone, progesterone, retinoic acid), and the genes *THRA* and *CDC93* (Figure 5A).

Subsequent discussion focuses on the significant correlations within the networks; a total of 32 gene-compound correlations are reported, linking both legacy and emerging environmental contaminants to gene expression (Table S5), and full correlation matrices are presented in Supplementary Data 2.

Organochlorine Contaminants (PCBs and TCDF), Thyroid Hormone Receptors, and Epigenetic Markers. Organochlorine contaminants in blubber exhibit a negative correlation with expression of the thyroid hormone receptor *THRB* in the skin samples (Figure 4, Table S5), specifically 2,3,7,8-tetrachlorodibenzofuran (TCDF, ρ = −0.90) and 2,2′,4,4′,6,6′-hexachlorobiphenyl (PCB-155, ρ = −0.91). Both TCDF and PCBs are persistent lipophilic pollutants known to disrupt thyroid function.[85] Previous studies in mammals indicate that PCBs and dioxin-like compounds can impair the hypothalamic−pituitary−thyroid axis,[86−88] leading to an imbalance in the thyroid-stimulating hormone levels.[89] Although data on cetaceans is limited, high PCB levels in bottlenose dolphins (*Tursiops truncatus*) blubber correlate with reduced circulating thyroxine and triiodothyronine hormone levels.[90]

THRA expression positively correlated (ρ = 0.87) with 2,4-bis(dimethylbenzyl)-6-t-butylphenol detected in blubber (Figure 4, Table S5), an alkylphenol used as an antioxidant in plastic formulations[91] and structurally similar to the endocrine disruptor BPA. This compound has been previously detected in marine sediments and lobster tissues, where it was associated with changes in juvenile hormone activity.[92] An imbalance in the thyroid functions and lipid metabolism,[93] is suggested also by endogenous cholesterol in blubber negatively correlating with *o,p′*-DDD and two PCBs (PCB-105, PCB-172) (ρ = −0.89 to 0.92) (Figure 4, Table S5). Additionally, several PCBs congeners (PCB-86, PCB-38, PCB-149, PCB-153) and *o,p′*-DDT showed negative correlations with *SETD1A* expression (ρ = −0.90 to −0.93) (Figure 4, Table S5) which is part of the histone methyltransferase (HMT) complex, while PCB-105 negatively correlated with *SMARCA2* (P = −0.88) (Figure 4, Table S5) involved in chromatin remodeling.[94] Confirming previous findings indicating that methylation of *SETD1A* and *SMARCA2* is linked to organochlorine exposure in fin whales.[27]

https://doi.org/10.1021/acs.est.5c00844
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Alkanes and Epigenetic Markers. Some long-chain alkanes found in fin whale blubber negatively correlate with mRNA level of *SHANK1*, in particular n-tetracontane ($\rho = -0.92$) and n-heptatriacontane ($\rho = -0.88$) (Figure 4, Table S5). Conversely, a shorter-chain alkane, 4-tridecene (Cluster 12, Figure 4), showed a strong positive correlation ($\rho = 0.90$) with the expression of the nuclear glucocorticoid receptor (GR) (Figure 4, Table S5). While linear alkanes are considered less toxic than aromatic hydrocarbons, studies indicate bioaccumulation potential and toxic effects in rats adipose tissue and liver, especially for long-chain alkanes exceeding C-25.[95] The SHANK protein is crucial for neuronal synapses development and functioning, while GR is involved in development, metabolism, and immune responses. These findings may represent early warning signs of exposure, considering also that methylation of the *SHANK1* CpG island has been proposed as a biomarker for chronic lymphocytic leukemia.[96]

Phthalates and Nuclear Receptors PPARs. Phthalates presence in filter-feeding whales from the Mediterranean Sea and Sea of Cortez has been linked to high concentrations of microplastics in feeding areas,[1] as microplastics can act as vectors for these associated chemicals upon ingestion. In skin samples, various phthalates correlate with *PPARA* expression, e.g., monoethyl phthalate ($\rho = 0.74$), diethyl phthalate ($\rho = 0.71$), and phthalic anhydride ($\rho = 0.81$) (Figure 5, Table S5). Phthalates are known peroxisome proliferators (PP) which interact with PPARs, disrupting normal transcriptional activity and affecting lipid metabolism and inflammation pathways.[97,98] Moreover, phthalates correlated with expression of Poly(RC)-Binding Protein 3 (*PCBP3*) ($\rho = 0.71-0.82$) (Figure 5, Table S5), suggesting this post-transcriptional regulator (e.g., via mRNA silencing or translation activation[99]) may mediate phthalates' biological effects in marine mammals.

Benzotriazole and Thyroid Hormone Receptors. Benzotriazoles are used extensively as industrial anticorrosive agents and UV stabilizers, and are persistent pollutants of emerging concern due to their water solubility and resistance to degradation.[100,101] Notably, skin levels of 1*H*-benzotriazole (ESI+) positively correlated ($\rho = 0.73$) with the expression of the thyroid hormone receptor α (*THRA*) (Figure 5, Table S5). Endocrine-disrupting properties of benzotriazole have been documented in fish.[102,103] Moreover, 8 out of 13 benzotriazole UV stabilizers displayed endocrine activity in humans by interacting with estrogen and androgen receptors.[104] Interactions with thyroid hormone pathways have also been reported, with exposure in zebrafish leading to alterations in *THRA* and *THRB* gene expression.[105] Our findings suggest that benzotriazole contamination in free-ranging fin whales may exhibit potential endocrine-mimicking activity as reported in laboratory studies for other species.

Toluenesulfonic Acid and Fatty Acid Binding Proteins. Skin levels of 4-methylbenzenesulfonic acid (i.e., *p*-toluenesulfonic acid) were positively correlated with the expression of fatty acid binding protein 4 (*FABP4*) ($\rho = 0.79$) (Figure 5, Table S5), a gene implicated in the regulation of glucose and lipid metabolism, as well as inflammatory responses.[106] Toluenesulfonic acid is widely employed as a synthetic precursor of pharmaceuticals,[107] as well as in the plastics and paints industries,[108] and is recognized as a refractory organic pollutant that is challenging to remove through conventional wastewater treatments.[109] Given these characteristics and the lack of information about the potential biological response to this substance, these findings warrant further investigation.

Tobacco Metabolites and Endogenous Fatty Acids. Skin level of nicotine was strongly correlated with another alkaloid found in tobacco, anabasine ($\rho = 0.96$). While nicotine exposure may potentially originate from the dispersion in the marine environment of cigarette butts,[57,110] both nicotine and anabasine have historically been used as insecticides.[111] Nicotine levels also positively correlated with two endogenous compounds, azelaic acid and pantothenic acid (both $\rho = 0.71$) (Figure 5, Table S5), which are known to mediate inflammatory responses. Interestingly, a metabolomic study in human blood linked smoking habits with an increase of endogenous pantothenic acid (vitamin B5),[112] a key precursor of coenzyme A (CoA) potentially affecting multiple metabolic pathways.[113]

Other Endogenous Compounds. Several endogenous biomolecules were detected by both GC-HRMS ($n = 15$ Level 2) and LC-HRMS ($n = 24$, 5 at Level 1, and 235 at Level 2), the latter including progesterone (Level 1; average 2.4 ng/g), L-thyroxine (Level 1; average 9.1 ng/g), corticosterone (Level 2), and other putative steroid hormones (Figure S2, Table S4). Corticosterone inversely correlated with *THRA* expression in the skin ($\rho = -0.76$) and positively correlated with several endogenous biomolecules such as prostaglandin E2, progestins, (medroxyprogesterone, norethindrone), and steroid hormones (17a-ethynylestradiol, meso-hexestrol, 17-α-methyltestosterone, exemestane) (Supplementary Data 2). These imbalances of the interconnected thyroid and corticosterone endocrine signaling pathway may reflect an early signal of stress.[114] Blubber levels of 9-octadecenamide, also known as oleamide, an endogenous compound biosynthesized from oleic acid, negatively correlated with the expression of *PPARA* ($\rho = -0.88$) and *CLSTN1* ($\rho = -0.78$) (Figure 4, Table S5). Oleamide's bioactivity involves activation of PPARs in *in vitro* studies,[115,116] and affects neuronal and vascular tissues binding to endocannabinoid receptors,[117] potentially involving *CLSTN1*, a calcium-binding protein of the postsynaptic membrane.[118] It is important to mention that further research should be conducted on the putatively annotated hormones, to distinguish endogenous from synthetic hormones, which may enter the marine environment through urban and industrial discharges[119] potentially mimic natural hormones and acting as endocrine disruptors.[120]

In conclusion, this study employs a multidisciplinary approach to pioneer the application of chemical exposomics and gene expression analysis in cetaceans. Our findings provide insights into the toxicological effects of cumulative xenobiotics exposure on these species for the first time, highlighting the pervasive presence of legacy and emerging chemicals, including pharmaceuticals (e.g., paracetamol, trimethoprim, diclofenac), industrial substances (e.g., BPA, PFAS, benzotriazole), and lifestyle consumer molecules (e.g., nicotine), previously undetected in pelagic cetaceans living far from their point sources.

These results underscore the urgent need to comprehend the distribution and potential effects of unregulated substances on the global marine biodiversity. Furthermore, this research sheds light on the intricate relationship between whales and their chemical environments, unveiling how cumulative exposure to contaminants threatens the health of the Mediterranean fin whale population, classified as endangered.[30]

The exposome profile and associated molecular biomarkers indicate exposure to endocrine-disrupting substances that could interact with important cellular receptors, potentially compromising reproductive health in these declining fin whale

https://doi.org/10.1021/acs.est.5c00844
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populations.[6] This pioneering study transcending the traditional single-chemical focus paradigm advances cetacean ecotoxicology by integrating human exposome research to the environmental monitoring of endangered species, bridging ecosystem, and biodiversity within a "One Health" framework.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.5c00844.

Extended GC-HRMS and LC-HRMS methods and results, samples metadata and outliers, isotope-labeled internal standards, primer sequences, endogenous compound heatmaps, cumulative pollutants level, and full list of gene-exposome correlations and networks (PDF)

Complete exposomics data set and MS-DIAL preprocessing parameters (Supplementary Data 1) (XLSX)

Gene expression data and full correlation matrices (Supplementary Data 2) (XLSX)

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Author Contributions

M.C.F. and C.P. conceptualized the study. G.L., S.P., J.W.M., M.C.F., and C.P. developed the methods. M.C.F., C.P., M.B., M.R., and J.U. collected and contributed the samples and sample information. G.L., M.B., S.P., and I.A. carried out laboratory work. G.L. and S.P. performed the data analysis and visualization. M.C.F., C.P., and J.W.M. provided guidance and advised the project. M.C.F. and C.P. funded the study. M.C.F., C.P., G.L., S.P., and J.W.M. wrote the original draft of the manuscript. All authors contributed review and editing to the manuscript and gave final approval for publication.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the Interreg MED project: Plastic Busters MPAs, cofunded by the European Regional Development Fund (grant agreement no. 4MED17_3.2_M123_027). We thank the CIMA Foundation (A. Moulins, P. Tepsich, A. Borroni, and A. Sechi) for assistance with whale sampling in the Mediterranean Sea and L. Viloria for the sampling activities in the Sea of Cortez. J.W.M. and S.P. acknowledge Swedish national funding to the National Facility for Exposomics (SciLifeLab). Exposomics analyses at SciLifeLab were supported by grant 4MED17_3.2_M123_027 to M.C.F.

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