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Ultrasonic extraction-based analysis of persistent organic pollutants in blubber from false killer whales

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Abstract

In view of the toxic effects of persistent organic pollutants (POPs), fast and effective assessment of their concentrations in marine mammals is important for understanding individual and population level health impacts. This study developed an ultrasonic-based method that is less time-consuming, uses minimal solvent, and thus more sustainable than the gold-standard Soxhlet method for accurate analysis of organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs) and benzene hexachlorides (BHC) in false killer whale blubber. This method was developed by comparing concentrations of POPs obtained using the traditional Soxhlet and novel ultrasonic extraction methods using blubber from false killer whales ($n = 4$) that were stranded in the Hawaiian Islands. The average total POPs extracted from the four killer whales and adjusted for lipid weight (lw) were $11,379.57 \pm 3,303.03$ ng/g lw for Soxhlet extraction and $14,310.39 \pm 4,469.00$ ng/g lw for the ultrasonic method, indicating a greater extraction efficiency of the ultrasonic method. The results further revealed that false killer whale FKW2013018 (Σ OCs $15,447.38 \pm 812.17$ ng/g lw and Σ PCBs $5,205.32 \pm 253.46$ ng/g lw) and false killer whale FKW2016016 (Σ OCs $18,164.90 \pm 1,433.15$ ng/g lw and Σ PCBs $4,913.32 \pm 447.29$ ng/g lw) accumulated organochlorines and PCBs at very high and potentially toxic levels. The low ratio of 4,4'-DDT/4,4'-DDE (0.026 ± 0.004) using both extraction methods indicated that the stranded false killer whales experienced long-term exposure to 4,4'-DDT, leading to the bioaccumulation of the stable 4,4'-DDE metabolite. The levels of OCs, PCBs and BHCs in this study were below toxic

thresholds levels. However, in view of the susceptibility of cetaceans with reduced lipid content to the toxic effects of POPs, cetaceans with low lipid content (as a result of starvation, fasting or extended migration events) may be at higher risk to the negative health impacts of organic pollutants.

Keywords: Ultrasonic extraction, Soxhlet extraction, POPs, bioaccumulation, false killer whale.

1. Introduction

The industrial boom that followed World War II and the need to control disease outbreaks in many countries led to the production of persistent organic pollutants (POPs) such as organochlorines (OCs) and polychlorinated biphenyls (PCBs) and their release and accumulation in the environment. These compounds proved beneficial for industrial use, pest control and food production¹. The widespread distribution of OCs and PCBs eventually became a global concern because of their toxicity to humans, wildlife and aquatic organisms, resulting in their ban or restriction in the latter part of the 20th Century². Persistent organic pollutants are capable of long-range environmental transport³, bioaccumulation in living tissues⁴ and resistance to degradation⁵. As a result, POPs continue to be observed in various aquatic and terrestrial systems as they biomagnify along the food chain. The distribution of POPs in the environment occurs through agricultural runoff⁶, wind circulation and deposition⁷, and ocean transport⁸.

Organochlorine pesticides and PCBs are absorbed following inhalation, oral or dermal exposure and are readily distributed in the lymph and blood to all body tissues and ultimately accumulate in the lipid content of the tissue, regardless of exposure route⁹. These contaminants have been found in the blubber of cetaceans, adipose tissue of multiple other species, maternal milk, blood, and umbilical cord tissue of pregnant women, with strong potential for maternal-to-fetus transfers¹⁰⁻¹². In humans, exposure to OCs, and PCBs and their metabolites has been reportedly associated with reduced birth weight¹³, preterm and small-for-gestational age birth¹⁴, neurodevelopmental delay¹⁵, and immunotoxicity¹⁶. In cetaceans that are apex predators in the food web, OCs, PCBs, and benzene hexachlorides are a recognized threat. There is a need to better understand the threat that these POPs pose to individual and population level cetacean health, marine ecosystem health as well as insight into mechanisms of human exposure. Since OC pesticides and PCB industrial compounds are lipophilic in nature, the determination of lipid content (or lipid weight, lw) in animal tissues enables appropriate assessment of contaminant body

burden in marine mammals. In whales and other cetaceans, blubber is a specialized form of adipose tissue containing between 8.9% and 77.4% lipids¹⁷. There is a need to develop effective methods of extracting them from fatty matrices and concentrating these compounds for mass spectrometry-based exposure studies to more accurately quantitate the degree of exposure and assess potential risk to the health of organisms.

Over the years, Soxhlet extraction has proven to be the gold standard method of extracting organic pollutants from various matrices¹⁸. However, this method is time consuming, energy demanding, solvent intensive, and contributes to a high carbon footprint in laboratories¹⁹. Furthermore, samples are normally extracted at the boiling point of a solvent for a long period of time, thus, risking the possibility of target compounds being thermally degraded²⁰. To address these problems, efforts are being made to develop more sustainable methods of extraction such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurized-assisted extraction (PLE)²⁰. While MAE is cheap and time efficient, it can overheat samples. Moreover, the effectiveness of MAE is dependent on the physicochemical properties of target substances as microwave energy is absorbed only by polar particles²¹. This makes MAE solvent-selective since the choice to extract the solvent must be based on its ability to absorb microwave energy. SFE requires less time than Soxhlet extraction but has the drawback of being complex and expensive²². Although PLE is time efficient, it requires high temperatures with potential for thermal degradation of analytes²³. Reducing laboratory footprints, saving energy, and minimizing organic solvent usage are tangible benefits of identifying an alternative extraction procedure to Soxhlet that yields comparable results.

Ultrasound-assisted extraction, which is based on the cavitation of samples carried out by sound waves, offers the advantages of being simple and easy to operate, while reducing extraction time, temperature, and solvent use²². Consequently, ultrasonic extraction is gaining increasing attention globally, with a wide range of applications²⁴⁻²⁷. This study was aimed at developing an ultrasound-assisted method of extraction for OCs and PCBs in whale blubber and directly comparing the effectiveness of this method with traditional Soxhlet extraction. Although two forms of ultrasonic extraction exist, ultrasonic bath and ultrasonic probe, we focused on the ultrasonic bath that allows for simultaneous extraction of multiple samples, thereby significantly reducing extraction time and energy usage. To demonstrate its effectiveness, results from the

analysis of OCs and PCBs were directly compared using ultrasonic and Soxhlet extractions of the blubber of stranded false killer whales in the Hawaiian Islands.

2. Materials and methods

2.1 Sample collection

Samples were part of a previously reported, population-level study on persistent pollutant levels in Hawaiian false killer whales²⁸. Blubber was collected at necropsy from false killer whales stranded on the island of Hawaii between 2013 and 2016. Life history information, stranding year and location, animal identity and cluster (when known) of the stranded false killer whales is listed in **Table 1**. Carcass condition followed the Geraci and Lounsbury²⁹ 1-5 scale of fresh dead to mummified where animals ranged from: freshly deceased but sighted alive to moderately decomposed (1-3) in three cases, with one individual in advanced decomposition (4). Blubber samples that weighed approximately 10g were frozen at -20°C in Whirl-Pak® specimen bags until 2023. Then, external blubber was removed using a titanium scalpel blade to ensure that no blubber used for these investigations came into contact with the specimen bags. A core internal sub-sample of the blubber weighing 50mg from each individual false killer whale was then used for this current study.

Table 1. False killer whales stranded in the Hawaiian Islands between 2013 and 2016 where blubber samples were collected at necropsy and used for the development of the ultrasonic extraction method presented herein.

Date of Stranding ^a	Location of Stranding	ID and Cluster Identity	Condition Code ^b	Sex	Body Length (cm)	Reproductive Status
10-04-2013	Ka Lae, Hawai'i	HIPc162(3)	1	M	445	Mature
11-07-2015	Ka Lae, Hawai'i	HIPc198(3)	2-3	F	401	Mature
09-28-2016	Ka Lae, Hawai'i	HIPc164(3)	1	M	502	Mature
11-21-2016 ^a	Ka'alu'alu Bay, Hawai'i	Unknown	4	F	397	Mature?

^aStranding dates represent the date of initial observation but this individual was found in a remote area in a moderate state of decomposition and complete examination of the reproductive tract was not possible.

^bGeraci and Lounsbury²⁹

2.2 Sample preparation, extraction and cleanup

The processes involved in sample preparation, extraction and cleanup are described schematically in Figure 1. Briefly, replicate blubber samples (50 mg each; $n = 4$) were weighed combined with anhydrous sodium sulfate (Na_2SO_4) and homogenized using mortar and pestle. The mixture was transferred into a 10-mL vial and 20 μL of 200 $\mu\text{g}/\text{mL}$ 4,4'-DDT-d8 (catalog #34021, Sigma-Aldrich Co., Bellefonte, PA, USA) was added as a surrogate standard. Thereafter, 2 mL of *n*-hexane:dichloromethane (4:1) mixture was added to the vial followed by ultrasonic extraction²⁴ at 40 kHz in an ultrasonic bath for 30 min in order to extract the analytes (Branson model 5800). A previous study of the extraction efficiency of various solvents and solvent ratios led to the adoption of *n*-hexane:dichloromethane at the ratio of 4:1 (v:v)³⁰. After centrifugation of the extract, the supernatant was transferred into a new vial. The extraction process was repeated a second time using another 2 mL of hexane:dichloromethane (4:1) mixture. After two extraction processes, the extracts were combined in a 10-mL vial. To determine the percentage of total lipids, an empty 2-mL vial was weighed after which 10% of the extract was transferred into the vial and dried under nitrogen. The net weight corresponding to extractable organic content was determined as a proxy of lipid content. The remaining 90% extract was evaporated to a minimal volume of 100 μL followed by extract cleanup. The cleanup process was performed using Supelclean™ EZ-POP NP (Supelco Cat. No. 54341-U, Sigma-Aldrich Co., Bellefonte, PA, USA) following the manufacturer's protocol. In brief, the SPE cartridge was conditioned using 10 mL acetone under gravity, followed by drying under vacuum (-10 to -15" Hg). The extract was added to the top of the column and allowed to fully penetrate the SPE frit. The analytes were eluted using 15 mL (2 x 7.5 mL) acetonitrile at a drop rate of approximately 1 drop/second. The eluate was evaporated under nitrogen to a final volume of 500 μL and transferred into a 2-mL vial. The final step was to add 20 μL of 10 $\mu\text{g}/\text{mL}$ 4,4'-DDD-d8 (HPC Standards Inc., Atlanta, GA, USA, catalog #684690) as a deuterated analytical internal standard before gas chromatography-triple quadrupole mass spectrometry (GC-QqQ) analysis.

To assess the effectiveness of the ultrasonic method for efficient recovery of target analytes, a comparative analysis was performed using the traditional Soxhlet extraction method. Therefore, equivalent blubber samples were additionally extracted in replicates ($n = 4$) using the Soxhlet method as detailed in USEPA Method 3540C¹⁸. In brief, homogenized samples were extracted using dichloromethane (DCM) following an 80-cycle Soxhlet extraction process. As in ultrasonic

extraction, 10% of the extract was used to determine the total lipid content of blubber samples gravimetrically. This was done by evaporating the 10% extract and measuring the net mass of lipid remaining (M_{net}). The percentage lipid in the initial sample is obtained by multiplying M_{net} by 10 (since only 10% of the extract was used) to obtain M_{lipid} , and applying the following formula:

$$\text{Percentage Lipid} = \frac{M_{lipid}}{M_{sample}} \times 100\%$$

The remaining 90% of the extract was evaporated to a minimal volume of 50 μ L followed by extract cleanup and final evaporation of the eluate to a final volume as described above. As with ultrasonic extraction, deuterated internal standard (4,4'-DDD-d8) was added to the final volume of extract obtained from Soxhlet extraction before GC-QqQ analysis.

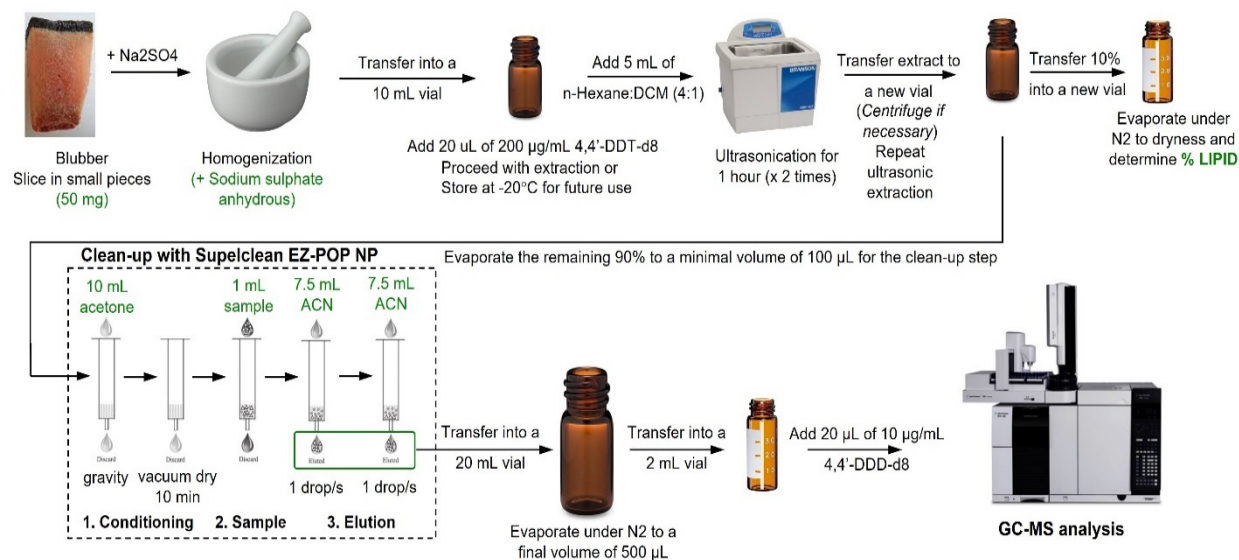


Figure 1. Schematic illustration of the ultrasonic extraction process.

2.3 POP analysis

Pure reference standards of organochlorine pesticide mixture (Mix AB#1, catalog #32291) and PCB congener mixture (Method 8082A, catalog #32416) were obtained from Restek Corporation, Bellefonte, PA, USA. Chemical analysis of organochlorines and polychlorinated biphenyls was performed using an Agilent 7890A gas chromatography system coupled to a 7000B triple quadrupole mass spectrometer. The GC capillary column used was DB-5MS (30 m, 0.25 μ m

film thickness, 0.25 mm inner diameter). Each sample (1 μ L) was transferred to the GC column splitless at an injector temperature of 250 $^{\circ}$ C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The GC temperature program was as follows: 70 $^{\circ}$ C (hold 2 min), 70 $^{\circ}$ C to 180 $^{\circ}$ C at 12 $^{\circ}$ C/min (hold 0 min), 180 $^{\circ}$ C to 195 $^{\circ}$ C at 3 $^{\circ}$ C/min (hold 3 min), 195 $^{\circ}$ C to 220 $^{\circ}$ C at 3 $^{\circ}$ C/min (hold 0 min), 220 $^{\circ}$ C to 280 $^{\circ}$ C at 15 $^{\circ}$ C/min (hold for 8 min). The total run time was 39.5 min. Electron ionization mass spectra were recorded in MRM mode (mass range 30-550 m/z) according to the relevant m/z values for precursor and product ions (Table 2). Peak areas were integrated using Agilent Mass Hunter B.06.00 software (Agilent Technologies, Santa Clara, CA, USA). Samples were randomly injected in the GC-MS, and each batch contained quality controls (pooled blubber extract sample) and blanks (containing only the extraction solvent).

Table 2. MRM monitoring m/z values and analytical conditions

Compound	Precursor ion	Product ion	RT (min)	Left Delta RT	Right Delta RT	CE (V)
α -BHC	218.8	>> 180.8	13.437	0.30	0.30	10
PCB 5	221.9	>> 151.9	13.494	0.30	0.30	30
β -BHC	218.8	>> 180.8	14.128	0.30	0.30	10
γ -BHC	218.8	>> 180.8	14.368	0.30	0.30	10
PCB 18	255.8	>> 185.8	14.689	0.30	0.30	30
δ -BHC	218.8	>> 180.8	15.237	0.30	0.30	10
PCB 31	255.8	>> 185.8	16.258	0.50	0.50	30
Heptachlor	271.7	>> 236.7	16.752	0.30	0.30	20
PCB 52	291.7	>> 219.8	17.613	0.20	0.20	30
Aldrin	262.7	>> 192.8	18.251	0.30	0.30	30
PCB 44	291.7	>> 219.8	18.481	0.30	0.30	30
HpCl epoxide	352.7	>> 262.7	20.264	0.20	0.20	20
PCB 66	291.7	>> 219.8	20.765	0.20	0.20	30
γ -Chlordane	372.7	>> 336.7	21.566	0.20	0.20	10
PCB 101	325.7	>> 255.7	22.082	0.20	0.20	30
α -Chlordane	372.8	>> 336.7	22.273	0.30	0.30	10
PCB 87	325.7	>> 255.7	23.548	0.20	0.20	30
4,4'-DDE	317.7	>> 245.8	23.757	0.30	0.30	30
PCB 110	325.7	>> 255.7	24.036	0.30	0.30	30
PCB 151	359.6	>> 289.6	24.677	0.30	0.30	30
4,4'-DDD	234.8	>> 164.9	26.222	0.20	0.20	30
PCB 153	359.6	>> 289.6	26.907	0.20	0.20	30
PCB 141	359.6	>> 289.6	27.601	0.20	0.20	30
PCB 138	359.6	>> 289.6	28.289	0.20	0.20	30
4,4'-DDT	234.8	>> 164.9	28.307	0.20	0.20	25
PCB 187	393.6	>> 323.7	28.896	0.20	0.20	30

PCB 183	393.6	>>	323.7	29.09	0.30	0.30	30
Endrin ketone	316.7	>>	280.7	29.671	0.30	0.30	10
Methoxychlor	226.9	>>	211.8	30.315	0.20	0.20	20
PCB 180	393.6	>>	323.7	30.492	0.20	0.20	30
PCB 170	393.6	>>	323.7	31.133	0.30	0.30	30

BHC: Benzene hexachloride; PCB: Polychlorinated biphenyl; DDE: Dichlorodiphenyldichloroethylene; DDD: Dichlorodiphenyldichloroethane; DDT: Dichlorodiphenyltrichloroethane.

2.4 Data analysis

Targeted data analysis was performed using Agilent Mass Hunter Qualitative Analysis (version B.06.00) and Agilent Mass Hunter Quantitative (QQQ) Analysis (version B.06.00) software. Compounds were identified, confirmed, and integrated using accurate mass, retention time and MS/MS information. All detected compounds were quantified using standard calibration curves and their responses were corrected using the internal standard response. Individual compounds were adjusted for lw by first calculating the total mass of compound (in ng), followed by determining the total mass of lipids. The total mass of lipid was calculated by multiplying the original sample weight by percentage lipid. Finally, the lipid-normalized concentration (in ng/g lw) was obtained by dividing the total mass of compound by the total lipid weight. The mean of the duplicate or triplicate values were used in the statistical analyses comparing the Soxhlet vs the Ultrasonic methods of blubber extraction using Microsoft Excel and R³¹. Mean concentrations ($\pm$ SE) of POPs were tabulated or shown in boxplots.

2.5 Extraction efficiency of ultrasonic method

To assess the extraction efficiency of the ultrasonic bath method, the recovery of isotopic internal standard (IS) that are chemically analogous to target analytes were determined using the following equation:

$$\text{Percentage recovery} = \frac{C_r}{C_s} \times 100\%$$

Whereas C_r is the recovered concentration of the internal standard after extraction, C_s is the spiked concentration of the internal standard before extraction. The value of C_r was determined from the linear calibration curve equation. On the other hand, the value of C_s was determined by calculating for C_2 in the dilution equation $C_1V_1 = C_2V_2$ (where $C_2 = C_s$). C_1 is the original

concentration of the IS; V_1 the volume of the IS added to the sample before extraction; V_2 the final volume of the extract before GC-MS analysis.

3. Results and Discussion

3.1 Lipid content of blubber and extraction efficiency of the ultrasonic method

The lipid content of blubber samples in this study ranged from 9.0% to 10.4%. This is lower than that obtained in other studies involving biopsy sampling of blue whales in different environments such as 24-63% in Svalbard Archipelago, Norway³² and 33-64% in blue whales from the Gulf of St. Lawrence³³. In a study of POPs in a blue whale that was stranded on the coast of Taiwan, percentage lipids was 22.6%³⁴. The low lipid content in this study indicates that the whales used in this study may have experienced limited food intake or starvation. Previous studies showed that during extended migration and starvation, the lipid content of blubber decreases^{35,36}. The lipid in the blubber of marine organisms undergoing chronic starvation are utilized for energy needs³⁴. Studies have shown that cetaceans with reduced blubber content are more susceptible to the toxic effects of lipophilic POPs due to the redistribution of these pollutants throughout the body^{37,38}. Lower lipid content may also be a result of long-term storage at -20 °C^{39,40}.

Comparison of the conventional Soxhlet vs Ultrasonic methods of blubber extraction from false killer whales and subsequent POP analysis provided novel results for a more efficient and effective and, perhaps, standardized approach for future studies involving POP exposure in cetaceans. Determination of extraction efficiency using the ultrasonic approach with various isotopically labeled surrogate standards showed that the mean ($\pm$ SE) percentage recovery ranged from $82.3 \pm 1.1\%$ to $119.1 \pm 2.4\%$ for organochlorine surrogate (4,4'-DDT-d8). In a previous study using the ultrasonic method, researchers compared the extraction efficiencies of different solvents and solvent mixtures such as *n*-hexane:diethyl ether (9:1, v:v), dichloromethane:petroleum ether (1:4, v:v) and *n*-hexane:petroleum ether (1:1, v:v)⁴¹. Their results showed that the mixture of *n*-hexane:petroleum ether (1:1, v:v) gave the highest recovery, ranging from 78 ± 2 to $100 \pm 6\%$ ⁴¹. On the other hand, they found that recoveries increased when sonication time increased from 30 to 45 min. An additional increase to 60 min did not produce any advantage. They concluded that three consecutive extractions of 15 min each (total of 45 min) using a 25 mL mixture of *n*-hexane:petroleum ether (1:1, v:v), resulting in mean ($\pm$ SE) percentage recoveries of between 67 ± 5 and $120 \pm 7\%$ for OC pesticides, was enough for the ultrasonic extraction of POPs⁴¹. Thus,

the ultrasonic extraction approach used herein resulted in higher extraction efficiency (82 to 119%), which may be attributed to the use of *n*-hexane:dichloromethane at the ratio of 4:1 (v:v), which is ideal for lipophilic compounds³⁰. It should be noted, however, that with different mass-labelled standards, the extraction efficiencies may vary among different lipophilic POPs based on such factors as the degree of PCB chlorination and the specific position of the chlorine atoms (congener structure)⁴²⁻⁴⁴. Future studies will employ the ultrasonic method for the extraction of standard reference materials (e.g., SRM NIST 1945 whale blubber or other fatty matrices) and compare percentage recoveries of OCs, PCBs, BHCs, and PAHs with those of Soxhlet method.

3.2 GC-QQQ analysis of POPs

The mass spectrometer data revealed the detection of all compounds in the pure standard mixture (Figure 2), enabling the quantification of POPs using calibration curves.

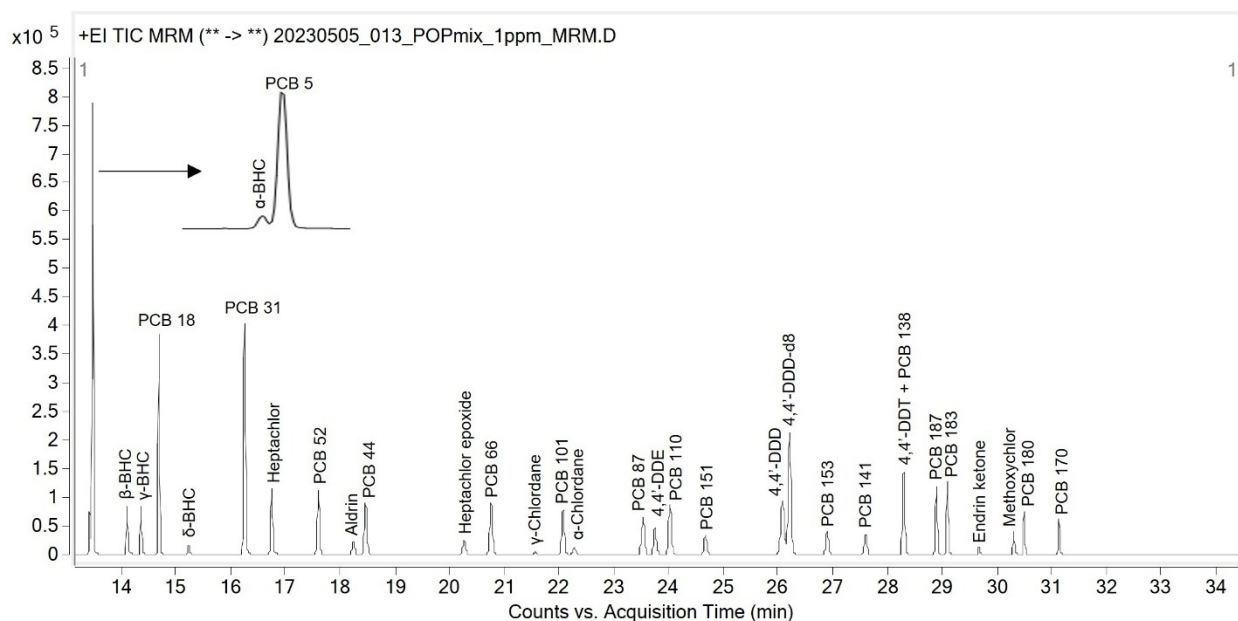


Figure 2. GC-QQQ chromatogram of target compounds in the pure analytical standard mix.

3.3 Comparison of POP concentrations obtained from Soxhlet and ultrasonic extractions

Concentrations of OC pesticides and PCBs obtained from Soxhlet (SE) and ultrasonic extraction (UE) methods are provided in Table 3. While the results from both methods appeared comparable, it seems the ultrasonic method with its high degree of extraction efficiency resulted in greater concentrations of many respective POPs (Table 3) compared to the Soxhlet extraction

method. The summation of the concentrations of POP compounds in the three POP groups, namely, OCs, PCBs and benzene hexachloride (BHC), revealed that, apart from Σ PCBs in sample FKW2016020, the ultrasonic method developed in this study gave higher total concentrations of POPs than 80 cycles of Soxhlet extraction (Figure 3). Total concentrations of organochlorine pesticides (Σ OCs) obtained using the ultrasonic method was significantly greater than that from Soxhlet extraction for samples FKW2013018 ($p > 0.05$) and FKW2016016 ($p > 0.01$) (Figure 3). Similarly, the ultrasonic method gave significantly higher extraction of Σ PCBs for FKW2016016 ($p < 0.05$). While Soxhlet extraction has been the gold standard method of extracting organic pollutants from various matrices over the years, this method is time consuming, energy demanding and solvent intensive, with a feed-to-solvent ratio of 1:10 to 1:25 g/mL⁴⁵. This results in a high carbon footprint in laboratories around the world. There is not a single ideal number of cycles for a Soxhlet extraction, as the optimal number varies depending on the sample's nature, the solvent's polarity and solubility, and the desired compound's thermal stability. However, most Soxhlet extraction processes require as much as 80 cycles until the solvent becomes clear and colorless, indicating the complete exhaustion of soluble constituents from the sample. This process can take several hours, with common ranges from 6-8 hours for highly soluble compounds to 12-24 hours or more for complex or less soluble materials⁴⁶. In addition, because of the higher temperature, this method risks the degradation of thermolabile compounds⁴⁷. Ultrasonic-assisted extraction technique extracts compounds by relying on ultrasound's mechanical action, which enhances the surface contact between solvent molecules and sample matrix^{47,48}. Thus, ultrasound modifies and disrupts tissue materials' physical and chemical characteristics, expedites the release of analytes, and further reinforces the solvent system's mass movement into tissues^{49,50}. In this study, the ultrasonic method was found to quicken the extraction process (1 hour), lower solvent and energy consumption, and increase the recovery of POPs, making it more sustainable than Soxhlet extraction. Additional benefits of ultrasonic techniques is its ability to efficiently preserve the biological integrity of analytes – something crucial for functional activities of phytochemicals and other bioactive compounds⁵¹.

Beyond demonstrating that the ultrasonic extraction approach was more efficient and effective than the conventional Soxhlet method, several specific advantages of this novel method are noteworthy. First, ultrasonic extraction significantly reduced processing time, with complete

extraction achieved within 30–60 minutes, compared to the multi-hour to multi-day cycle required for Soxhlet extraction. This rapid turnaround has practical implications for high-throughput analyses, such as population-level monitoring or rapid-response investigations of marine mammal strandings. Additionally, the ultrasonic method required markedly less solvent (<5 mL per extraction compared to up to 300 mL for Soxhlet), lowering chemical costs, hazardous waste production, and overall environmental impact. The lower operating temperatures also reduced the risk of thermal degradation of thermolabile compounds, which may help preserve the integrity of a broader spectrum of target analytes. The method's ability to process multiple samples in parallel in a single ultrasonic bath further increases analytical throughput and laboratory efficiency.

Methodologically, the ultrasonic approach proved highly effective even with small sample masses (50 mg), enabling robust POP quantification from limited tissue availability such as biopsy cores or archived specimens. This is particularly relevant for studies involving live, free-ranging cetaceans, or for reanalysis of historical samples where tissue reserves are minimal. The method also performed well on lipid-poor blubber, an important consideration when analyzing nutritionally stressed or fasting animals, where the distribution and mobilization of POPs can differ from those in lipid-rich tissues. This adaptability enhances the method's applicability to a wide range of sample types and conditions encountered in marine mammal toxicology.

Despite these benefits, several limitations and considerations should be acknowledged. Optimization of solvent ratios, extraction times, and cycle numbers may be required for different tissue matrices, particularly for blubber with very high or very low lipid content, or for species with differing blubber architecture. While the recoveries observed here were consistently high for most POP classes, some analytes—especially highly chlorinated PCBs—may exhibit matrix-specific extraction variability. In addition, although ultrasonic baths are less costly and more widely available than specialized extraction systems such as supercritical fluid extractors, they may not be present in all laboratories conducting marine mammal contaminant analyses. Care should also be taken to minimize solvent volatilization during extraction and to ensure inter-laboratory calibration when adopting this method for long-term monitoring programs to maintain comparability with legacy Soxhlet-derived datasets.

Table 3. Mean concentrations ($\pm$ SE) of POPs in blubber samples taken from four different stranded false killer whales (FKW) obtained using Soxhlet extraction (SE) and ultrasonic extraction (UE) methods.

			FKW2013018			FKW2015015			FKW2016016			FKW2016020			
POP	RT (m)	LOD	SE (ng/g lw)	UE (ng/g lw)	$\Delta\%$	SE (ng/g lw)	UE (ng/g lw)	$\Delta\%$	SE (ng/g lw)	UE (ng/g lw)	$\Delta\%$	SE (ng/g lw)	UE (ng/g lw)	$\Delta\%$	Mean $\Delta\%$
a-BHC	13.437	0.18	21.93 $\pm$ 1.16	22.96 $\pm$ 0.37	4.7	22.21 $\pm$ 3.3	24.55 $\pm$ 1.58	10.5	18.72 $\pm$ 0.26	22.24 $\pm$ 0.79	18.8	9.45 $\pm$ 0.32	10.41 $\pm$ 0.25	10.2	11.0
b-BHC	14.128	0.18	15.46 $\pm$ 0.79	15.67 $\pm$ 0.41	1.4	15.33 $\pm$ 2.33	17.49 $\pm$ 1.14	14.1	13.36 $\pm$ 0.21	15.82 $\pm$ 0.57	18.4	7.68 $\pm$ 0.33	7.39 $\pm$ 0.22	(3.8)	7.5
g-BHC	14.368	0.18	10.81 $\pm$ 0.27	10.81 $\pm$ 0.29	0.0	10.5 $\pm$ 0.13	11.67 $\pm$ 0.30	11.1	11.12 $\pm$ 0.5	10.25 $\pm$ 0.31	(7.8)	10.43 $\pm$ 0.29	9.96 $\pm$ 0.25	(4.5)	(0.3)
d-BHC	15.237	0.18	5.45 $\pm$ 0.16	5.64 $\pm$ 0.32	3.5	5.61 $\pm$ 0.20	6.37 $\pm$ 0.61	13.5	6.15 $\pm$ 0.36	5.11 $\pm$ 0.25	(16.9)	5.52 $\pm$ 0.33	4.87 $\pm$ 0.09	(11.8)	(2.9)
PCB 31	16.258	0.28	11.63 $\pm$ 0.83	8.16 $\pm$ 1.24	(29.8)	9.95 $\pm$ 1.89	11.26 $\pm$ 1.01	13.2	8.45 $\pm$ 0.19	8.26 $\pm$ 0.48	(2.2)	4.53 $\pm$ 0.25	3.7 $\pm$ 0.18	(18.3)	(9.3)
HpCl	16.752	0.18	9.7 $\pm$ 0.21	9.62 $\pm$ 0.22	(0.8)	9.05 $\pm$ 0.19	9.29 $\pm$ 0.25	2.7	9.45 $\pm$ 0.27	8.98 $\pm$ 0.17	(5.0)	8.85 $\pm$ 0.26	8.56 $\pm$ 0.1	(3.3)	(1.6)
PCB 52	17.613	0.12	68.95 $\pm$ 5.35	59.51 $\pm$ 2.74	(13.7)	56.7 $\pm$ 4.26	57.87 $\pm$ 6.5	2.1	55.64 $\pm$ 1.73	64.46 $\pm$ 5.26	15.9	16.74 $\pm$ 2.32	14.76 $\pm$ 2	(11.8)	(1.9)
Aldrin	18.251	0.02	4.58 $\pm$ 0.11	4.85 $\pm$ 0.26	5.9	4.37 $\pm$ 0.13	4.4 $\pm$ 0.13	0.7	4.51 $\pm$ 0.2	4.37 $\pm$ 0.15	(3.1)	4.55 $\pm$ 0.2	4.59 $\pm$ 0.09	0.9	(1.1)
PCB 44	18.481	0.14	18.34 $\pm$ 1.76	12.43 $\pm$ 0.76	(32.2)	14.07 $\pm$ 1.6	14.43 $\pm$ 0.67	2.6	12.84 $\pm$ 0.46	12.24 $\pm$ 0.28	(4.7)	8.91 $\pm$ 0.4	7.35 $\pm$ 0.2	(17.5)	(13.0)
HpCl-epoxide	20.264	0.18	16.58 $\pm$ 0.71	15.75 $\pm$ 0.93	(5.0)	14.65 $\pm$ 1.26	15.75 $\pm$ 0.76	7.5	15.72 $\pm$ 0.19	16.68 $\pm$ 0.43	6.1	9.86 $\pm$ 0.44	9.11 $\pm$ 0.15	(7.6)	0.3
PCB 66	20.765	0.14	36.3 $\pm$ 3.26	27.39 $\pm$ 1.69	(24.5)	32.64 $\pm$ 7.01	32.67 $\pm$ 3.86	0.1	25.37 $\pm$ 0.7	24.2 $\pm$ 1.44	(4.6)	9.39 $\pm$ 0.91	6.83 $\pm$ 0.28	(27.3)	(14.1)
g-Chlordane	21.566	1.82	19.36 $\pm$ 0.42	18.4 $\pm$ 0.15	(5.0)	17.89 $\pm$ 0.38	18.31 $\pm$ 0.51	2.3	18.07 $\pm$ 0.76	18.01 $\pm$ 1.13	(0.3)	17.48 $\pm$ 0.55	18.19 $\pm$ 0.72	4.1	0.3
PCB 101	22.082	0.41	266.41 $\pm$ 30.12	246.53 $\pm$ 14.49	(7.5)	189.57 $\pm$ 17.29	209.63 $\pm$ 28.12	10.6	185.15 $\pm$ 5.28	205.41 $\pm$ 20.23	10.9	43.75 $\pm$ 6.88	29.78 $\pm$ 1.38	(31.9)	(4.5)
a-Chlordane	22.273	1.82	35.74 $\pm$ 3.01	37.31 $\pm$ 1.92	4.4	31.41 $\pm$ 4.81	40.51 $\pm$ 3.94	29.0	29.84 $\pm$ 0.76	35.2 $\pm$ 2.07	18.0	13.85 $\pm$ 1.14	12.33 $\pm$ 0.36	(11.0)	10.1
PCB 87	23.548	0.20	90.6 $\pm$ 8.33	104.12 $\pm$ 13.25	14.9	104.67 $\pm$ 4.9	114.68 $\pm$ 8.3	9.6	102.58 $\pm$ 4.77	96.9 $\pm$ 5.17	(5.5)	69.83 $\pm$ 6.56	50.72 $\pm$ 3.98	(27.4)	(2.1)
4,4'-DDE	23.757	0.24	12121.82 $\pm$ 1597.42	14989.3 $\pm$ 809.42	23.7	3246.09 $\pm$ 725.26	4973.25 $\pm$ 651.91	53.2	12543.28 $\pm$ 396.72	17760.26 $\pm$ 1416.35	41.6	4286.53 $\pm$ 780.48	3954.11 $\pm$ 298.33	(7.8)	27.7
Dieldrin	23.806	18.20	24.85 $\pm$ 2.33	24.37 $\pm$ 3.38	(1.9)	18.37 $\pm$ 1.45	22.85 $\pm$ 4.56	24.4	27.49 $\pm$ 2.75	23.45 $\pm$ 8.89	(14.7)	20.51 $\pm$ 1.64	15.5 $\pm$ 1.43	(24.4)	(4.2)
PCB 110	24.036	0.14	27.06 $\pm$ 4.1	15.21 $\pm$ 0.77	(43.8)	16.77 $\pm$ 1.89	16.29 $\pm$ 1.04	(2.9)	13.89 $\pm$ 0.85	12.27 $\pm$ 0.53	(11.7)	8.93 $\pm$ 0.53	7.23 $\pm$ 0.43	(19.0)	(19.3)
PCB 151	24.677	0.49	158.19 $\pm$ 20.47	172.82 $\pm$ 8.55	9.2	70.17 $\pm$ 12.98	100.53 $\pm$ 10.46	43.3	144.12 $\pm$ 5.29	174.68 $\pm$ 15.07	21.2	49.42 $\pm$ 8.11	37.81 $\pm$ 2.45	(23.5)	12.6
4,4'-DDD	26.222	0.50	104.26 $\pm$ 12.77	115.43 $\pm$ 6.39	10.7	66.93 $\pm$ 11.43	100.59 $\pm$ 10.57	50.3	90.36 $\pm$ 2.68	109.79 $\pm$ 5.72	21.5	41.41 $\pm$ 5.93	33.78 $\pm$ 1.72	(18.4)	16.0
PCB 153	26.907	6.86	1393 $\pm$ 191.72	1645.98 $\pm$ 63.45	18.2	509.36 $\pm$ 89.73	822.33 $\pm$ 87.32	61.4	1226.77 $\pm$ 61.41	1605.91 $\pm$ 150.81	30.9	373.41 $\pm$ 63.49	302.23 $\pm$ 28.52	(19.1)	22.9
PCB 141	27.601	0.50	239.69 $\pm$ 28.63	274.16 $\pm$ 6.14	14.4	103.67 $\pm$ 17.98	141.65 $\pm$ 14.19	36.6	196.62 $\pm$ 8.87	243.99 $\pm$ 21.31	24.1	63.62 $\pm$ 9.59	52.84 $\pm$ 5.41	(16.9)	14.5
PCB 138	28.289	0.70	778.49 $\pm$ 104.14	895.64 $\pm$ 30.34	15.0	296.74 $\pm$ 54.55	478.54 $\pm$ 52.03	61.3	676.48 $\pm$ 27.75	893.17 $\pm$ 72.2	32.0	216.13 $\pm$ 35.6	196.32 $\pm$ 15.99	(9.2)	24.8
4,4'-DDT	28.307	0.40	233.55 $\pm$ 28.71	236.59 $\pm$ 5.33	1.3	229.48 $\pm$ 44.54	295.31 $\pm$ 41.69	28.7	156.56 $\pm$ 8.55	184.61 $\pm$ 7.47	17.9	42.23 $\pm$ 5.55	32.84 $\pm$ 2.62	(22.2)	6.4
PCB 187	28.896	0.89	473.83 $\pm$ 61.66	540.96 $\pm$ 11.23	14.2	170.69 $\pm$ 25.15	273.94 $\pm$ 24.09	60.5	429 $\pm$ 22.19	556.64 $\pm$ 57.48	29.8	132.62 $\pm$ 21.67	108.53 $\pm$ 9.79	(18.2)	21.6
PCB 183	29.090	1.40	155.11 $\pm$ 19.71	175.69 $\pm$ 3.73	13.3	55.59 $\pm$ 6.8	83.55 $\pm$ 6.57	50.3	141.24 $\pm$ 5.96	173.92 $\pm$ 18.07	23.1	47.61 $\pm$ 6.99	39.35 $\pm$ 3.13	(17.3)	17.3
Methoxychlor	30.315	1.82	6.96 $\pm$ 0.46	4.91 $\pm$ 1.27	(29.5)	6.32 $\pm$ 0.82	3.76 $\pm$ 0.07	(40.5)	5.87 $\pm$ 1.34	3.48 $\pm$ 0.5	(40.7)	5.48 $\pm$ 1.09	3.07 $\pm$ 0.18	(44.0)	(38.7)
PCB 180	30.492	0.14	728.85 $\pm$ 102.73	799.77 $\pm$ 87.9	9.7	190.85 $\pm$ 24.66	285.13 $\pm$ 24.22	49.4	555.18 $\pm$ 30.18	669.86 $\pm$ 68.32	20.7	158.94 $\pm$ 26.81	114.46 $\pm$ 9.75	(28.0)	13.0
PCB 170	31.133	0.77	216.94 $\pm$ 28.43	226.88 $\pm$ 39.24	4.6	60.64 $\pm$ 7.91	70.47 $\pm$ 6.35	16.2	178.99 $\pm$ 10.62	171.32 $\pm$ 15.63	(4.3)	53.07 $\pm$ 7.94	39.54 $\pm$ 3.44	(25.5)	(2.2)
ΣPOPs			17,294.44	20,716.86		5,580.29	8,257.07		16,902.82	23,131.48		5,740.73	5,136.16		

SE: Soxhlet extraction; UE: Ultrasonic extraction; lw: lipid weight; FKW2013018, FKW2015015, FKW2016016 and FKW2016020 are samples from four different stranded whales.

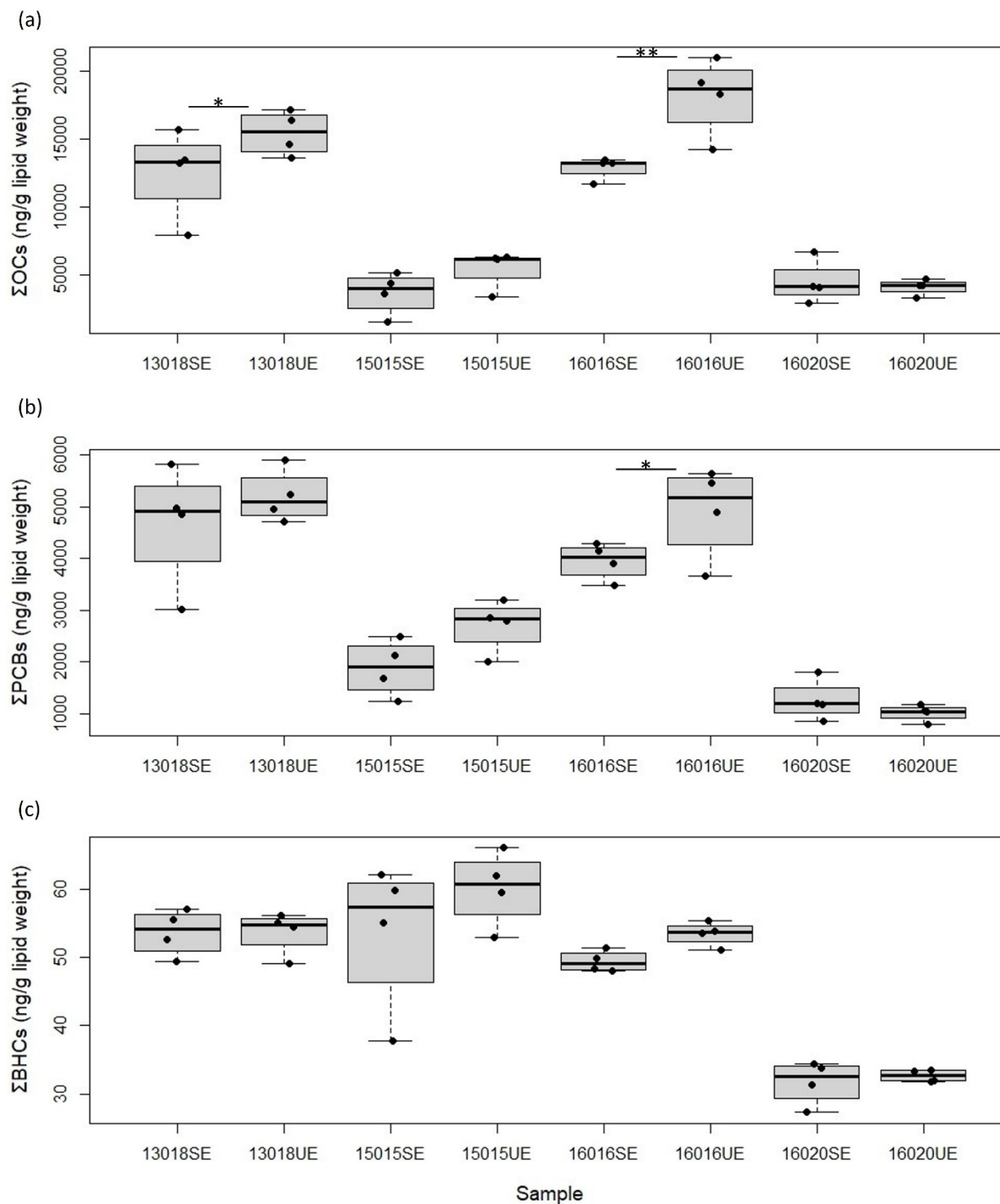


Figure 3. Total concentrations of (a) organochlorines, (b) polychlorinated biphenyls and (c) benzene hexachloride in the different whale blubber samples extracted using Soxhlet (SE) and ultrasonic extractions (UE). The suffix “SE” in sample nomenclature refers to Soxhlet extraction, while “UE” refers to ultrasonic extraction. (* $P < 0.05$; ** $P < 0.01$).

3.4 Bioaccumulation and toxicological implications

Concentrations of POP compounds based on the ultrasonic method showed that the highest total concentrations of organochlorines (Σ OCs 18164.90 ± 1433.15 ng/g lw) and PCBs (Σ PCBs 5205.32 ± 253.46 ng/g lw) were found in blubber samples FKW2016016 and FKW2013018 respectively (Figure 3, Table 4). These concentrations were between two- to four-fold the concentrations found in samples FKW2015018 and FKW2016020. On the other hand, samples FKW2013018, FKW2015015 and FKW2016016 had similar concentrations of Σ BHC. The lowest value of Σ BHC was recorded for sample FKW2016020 (Table 4).

Table 4. Comparison of mean concentrations of POPs ($\pm$ standard error, ng/g lw) in blubber samples obtained in this study (Hawaii) and those obtained in previous studies around the world.

Sampling Location	Sampling Year	Σ OCs (ng/g)	Σ PCBs (ng/g)	Σ BHC (ng/g)	Reference/Sample ID
Hawaii Island	2013	15447.38 ± 812.17	5205.32 ± 253.46	53.69 ± 1.57	This study (FKW2013018)
Hawaii Island	2015	5484.08 ± 710.29	2713.03 ± 251.53	60.09 ± 2.74	This study (FKW2015018)
Hawaii Island	2016	18164.90 ± 1433.15	4913.32 ± 447.29	53.43 ± 0.89	This study (FKW2016016)
Hawaii Island	2016	4089.07 ± 301.95	1011.52 ± 81.37	32.65 ± 0.44	This study (FKW2016020)
Gulf of St. Lawrence, Canada	1991-1992	4901 ± 2521 (2551-8454)	3591 ± 1932 (1577-6071)	176 ± 155 (4-378)	Gauthier, Metcalfe and Sears ³³ , Gauthier, Metcalfe and Sears ⁵²
Gulf of St. Lawrence, Canada	1992-1997	3418 ± 2144	2018 ± 912	225.8 ± 322.6	Metcalfe, Koenig, Metcalfe, Paterson and Sears ⁵³
Pacific Islands	1997-2011	9650 (44.4-99100)	6240 (40.8-50200)	48.1 (< 5.42-566)	Bachman, Keller, West and Jensen ¹²
Denmark	2005	85400	96100	87.2	Pedro, Boba, Dietz, Sonne, Rosing-Asvid, Hansen, Provatas and McKinney ⁴
Faroe Islands	2008	4250 (3670-4820)	5160 (2750-7570)	32.2 (21.0-43.0)	Pedro, Boba, Dietz, Sonne, Rosing-Asvid, Hansen, Provatas and McKinney ⁴
Gulf of California, Mexico	2010	3935	4910	N/A	Fossi, Panti, Marsili, Maltese, Coppola, Jimenez, Muñoz-Arnanz, Finoia, Rojas-Bracho and Urban ⁵⁴
Isla de Chiloe, Southern Chile	2011-2013	49.6 (11.0-196)	136 (8.66-470)	20.4 (7.84-77.5)	Muñoz-Arnanz, Chirife, Galletti

					Vernazzani, Cabrera, Sironi, Millán, Attard and Jiménez ⁵⁵
Southeast Greenland	2012-2014	30100 (16100-50200)	48600 (19600-65500)	68.2 (31.0-103)	Pedro, Boba, Dietz, Sonne, Rosing-Asvid, Hansen, Provatas and McKinney ⁴
Svalbard Archipelago, Norway	2014-2018	163-669	106-334	41-82	Tartu, Fisk, Götsch, Kovacs, Lydersen and Routti ³²
Taitung, Taiwan	2020	1089 ± 4.7	1057 ± 49.8	70.6 ± 2.5	Cheng, Wang, Chou, Chang, Wang, Yang and Ko ³⁴

327

328 A comprehensive comparison was made between the levels of POP toxicants obtained in
329 this study and those from previous POP studies of cetacean populations around the world ^{4, 12, 32-}
330 ^{34, 52-55}. By inference, results revealed that the two mature male whales (FKW2013018 and
331 FKW2016016) accumulated organochlorines and PCBs at very high and potentially toxic levels
332 in comparison to the mature female whales (FKW2015015 and FKW2016020) (Table 4). Male
333 whales generally accumulate higher concentrations of POPs than females. This is primarily due to
334 the fact that female whales transfer a portion of their POP burden to their offspring through
335 gestation and lactation, effectively reducing their overall POP load ^{28, 56}. ΣOCs and ΣPCBs in these
336 two animals were far above those found in Gulf of St. Lawrence (Canada), Isla de Chiloe (Chile),
337 Svalbard Archipelago (Norway), and Taitung (Taiwan) but less than those in Denmark and
338 Southeast Greenland. Owing to their lipophilic chemistry, thereby limiting bioavailability,
339 organochlorine pesticides and PCBs are among the most persistent environmental pollutants in
340 aquatic environments around the world ^{1, 57-60}.

341 The predominant congener of OC pesticides in all species was 4,4'-DDE, with a percentage
342 (100 x 4,4'-DDE / ΣOCs) of 95.0 ± 0.6% (Table 3). This was followed by 4,4'-DDT (2.4 ± 0.4%)
343 and 4,4'-DDD (1.1 ± 0.1%). The average ratio of 4,4'-DDT to 4,4'-DDE was 0.026 ± 0.004 (range:
344 0.008-0.08), indicating long-term exposure to DDT. When deposited in water, DDT will adsorb
345 strongly to particulate matter. DDT bioaccumulates in aquatic organisms and biomagnifies up the
346 food chain, with very high concentrations of DDT having been observed in apex predators such as
347 marine mammals from the tropics to the Arctic ^{4, 61}. In living tissues and the environment, 4,4'-
348 DDT metabolizes or degrades to form the stable metabolite 4,4'-DDE through

dehydrohalogenation resulting in loss of HCl and formation of a double bond on the central carbon atoms (Figure 4)^{62, 63}. On the other hand, the less stable metabolite, 4,4'-DDD is formed through reductive dechlorination of 4,4'-DDD (Figure 4)⁶³. Consequently, it has been shown that the stable metabolite, 4,4'-DDE, is found at higher tissue concentrations than DDT and DDD isomers^{9, 33}, especially since DDT was banned in the 1970s in many countries, which limits further input of DDT. Considering the 5.3-year mean half-life of DDT in the U.S.⁹, the extremely low ratio of 4,4'-DDT/4,4'-DDE (0.026 ± 0.004) in this study indicates a long-term bioaccumulation of DDT in the stranded false killer whales studied herein. The US EPA has classified DDT, DDE and DDD as class B2 carcinogens⁹. A number of studies have linked exposures to OCs and other xenobiotics to declines or abnormalities in wildlife species, including fish, birds, and mammals^{10, 64, 65}. Perhaps the best-known effect of DDT is the egg-shell thinning of peregrine falcon^{66, 67}.

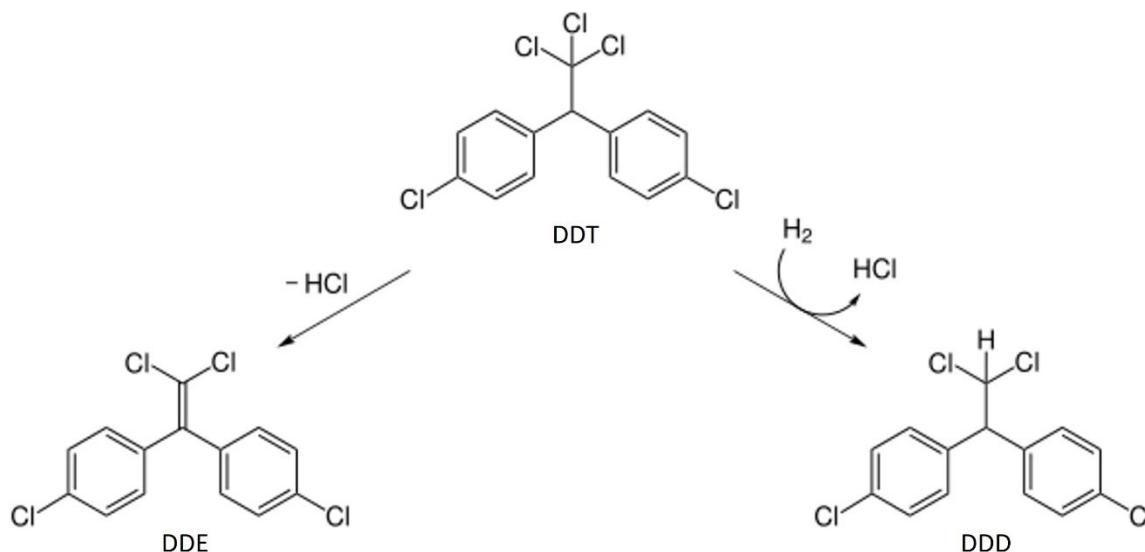


Figure 4. The metabolism of 4,4'-DDT to 4,4'-DDE through dehydrohalogenation and/or to 4,4'-DDD through reductive dechlorination.

The predominant congeners of PCBs in the false killer whales studied herein were hexachlorinated biphenyls ($56.3 \pm 0.5\%$) followed by hepta-chlorinated congeners ($30.6 \pm 0.3\%$). The most abundant PCB isomer is 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153), which accounted for $30.1 \pm 0.5\%$ of the entire PCBs present in the cetacean population. This is in contrast to some previous studies in which the lower chlorinated biphenyls were found to be predominant^{34, 55}. For example, in a study of POPs in blue whales in the Southern hemisphere, Muñoz-Arnanz, Chirife, Galletti Vernazzani, Cabrera, Sironi, Millán, Attard and Jiménez⁵⁵ found that penta-, tetra-, and

tri-chlorinated biphenyls, and tetra- and tri-brominated biphenyls were the most prevalent among PCBs. The result of a recent study of organic contaminants in stranded blue whale on the coast of Taiwan showed that penta- and hexa- congeners were predominant ($41.3 \pm 0.8\%$ and $37.7 \pm 0.4\%$ respectively) ³⁴. In the present study, pentachlorobiphenyls accounted for only $9.8 \pm 0.5\%$ of the PCBs. At $5.5 \pm 0.2\%$, 2,2',4,5,5'-Pentachlorobiphenyl (PCB 101) was the most abundant of the pentachlorobiphenyl congeners. Similar to our results, Gauthier, Metcalfe and Sears ³³ found that PCBs 101 and 153 were among the most abundant PCB congeners present in the blubber of whales from Gulf of St. Lawrence, Canada.

In living organisms, PCBs undergo xenobiotic transformation to make them more polar and excretable via glomerular filtration ⁶⁸. This process is dependent on the number of chlorine atoms present, and begins with the oxidation of one of the benzene rings by cytochrome P450 monooxygenase ⁶⁹. The monooxygenase cytochrome P450 is known for its role in the detoxication and degradation of xenobiotics, and the type of cytochrome P450 determines where oxidation occurs ^{70, 71}. Phenobarbital-induced P450s catalyzes oxidation to the meta-para positions, while 3-methylcholanthrene-induced P450s catalyzes ortho-meta oxidations ⁷⁰. While PCBs that are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are directly toxic, the majority of PCB toxicity arises from its ability to stimulate the production of bioactivating enzyme systems such as the mixed-function oxidases (MFOs), which consequently leads to an increased bioactivation of otherwise nontoxic foreign compounds ⁷². Among MFO inducers are the hexa-chlorinated congener PCB 138 and the hepta-chlorinated congener PCB 170 ⁷². In this study, PCB 138 was the second most abundant PCB congener (after PCB 153), with percentage abundance of $17.3 \pm 0.2\%$. The predominance of the higher chlorinated (hexa- and hepta-) congeners in this study is of great concern in view of their toxicity and environmental persistence.

Total concentrations of benzene hexachloride (Σ BHC) in the four whales in this study were much less than the levels in the two samples from Gulf of St. Lawrence, Canada, but considerably similar to the levels at other locations (Table 4). The two most abundant BHCs were α -BHC and β -BHC at $38.3 \pm 0.7\%$ and $27.4 \pm 0.4\%$ respectively, while δ -BHC had the lowest mean concentrations in the blubber samples. Most BHCs are unintentionally released to the environment as by-products of industrial production. For example, while the use of α -BHC and β -BHC was phased out years ago, these chemicals have been produced as byproducts of lindane. For each ton

of lindane produced, around 6-10 tons of α -BHC and β -BHC are also produced as byproducts⁷³. Therefore, there are large stockpiles leading to site contamination. In human and animal studies, birth defects, cancers, and neurologic and immune system disorders have been linked to PCBs and BHCs^{2, 10, 74}, in addition to being capable of long-range transport^{59, 60, 75}.

The basis for the standings of the false killer whales in the present study was not the intent of this study. Nonetheless, the results herein indicated these stranded whales had relatively low total lipid content and were chronically exposed to relatively high concentrations of various OCs, PCBs, and BHCs. While there is no direct link and until pathology results indicate otherwise, the association between POP exposure and stranding of the false killer whales, aligns with other combined studies of POP exposure and strandings in cetaceans.

4. Conclusions

Many persistent organic pollutants (POPs) are resistant to biodegradation and photolytic degradation and are consequently transported in the environment across international boundaries far from their sources resulting in their deposition in environments where they have never been used. Inherently, they persist in these environments and have the potential for toxic and adverse health effects on the flora and fauna that inhabit terrestrial and aquatic environments. In view of the reported toxic effects of OC pesticides, PCBs and BHCs on aquatic mammals, accurately determining their concentrations in marine organisms is important for unravelling probable causes of strandings and individual and population declines. This will positively enhance approaches targeted at conserving and preserving threatened and endangered species. While Soxhlet extraction has been the gold standard method of extracting organic pollutants from various matrices over the years, this method is time consuming, energy demanding and solvent intensive, resulting in high carbon footprint in laboratories around the world. This study developed an ultrasonic-based process for accurate analysis of POPs in whale blubber samples that is more efficient and effective. The results from the ultrasonic method compared favorably with those of Soxhlet method, with the ultrasonic method extracting more of the contaminants in most cases than the gold-standard Soxhlet method. Analysis of POPs in the stranded false killer whales herein revealed that two of the four whales accumulated OCs and PCBs at high concentrations, despite having relatively low lipid contents. The low ratio of 4,4'-DDT/4,4'-DDE (0.026 ± 0.004) in this study indicates a long-term bioaccumulation of DDT in the stranded cetacean populations and further highlights the

environmental persistence and bioaccumulative nature of OCs. The levels of OCs, PCBs and BHCs in this study were below toxic thresholds. However, in view of the susceptibility of cetaceans with reduced lipid content to the toxic effects of POPs, stranded cetacean populations with low lipid content (as a result of starvation, fasting or extended migration events) may be at higher risk to organic pollutants. In any case, owing to their adverse impacts on terrestrial and aquatic animal life, coupled with their environmental persistence and long-range transport, POPs continue to pose significant threat to cetaceans and other marine species around the globe.

Thus, ultrasonic extraction represents a practical and environmentally sustainable approach for large-scale biomonitoring of POPs in marine mammal tissues, though broader adoption will require attention to tissue variability and calibration across laboratories.”

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

MOE, EB, MMM, DRB, SEH, KLW and CED conceived and designed the research; MOE performed the experiments; MOE, EB and MMM analyzed data; MOE, EB and KLW wrote the manuscript; MMM, SEH, DRB, and CED reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

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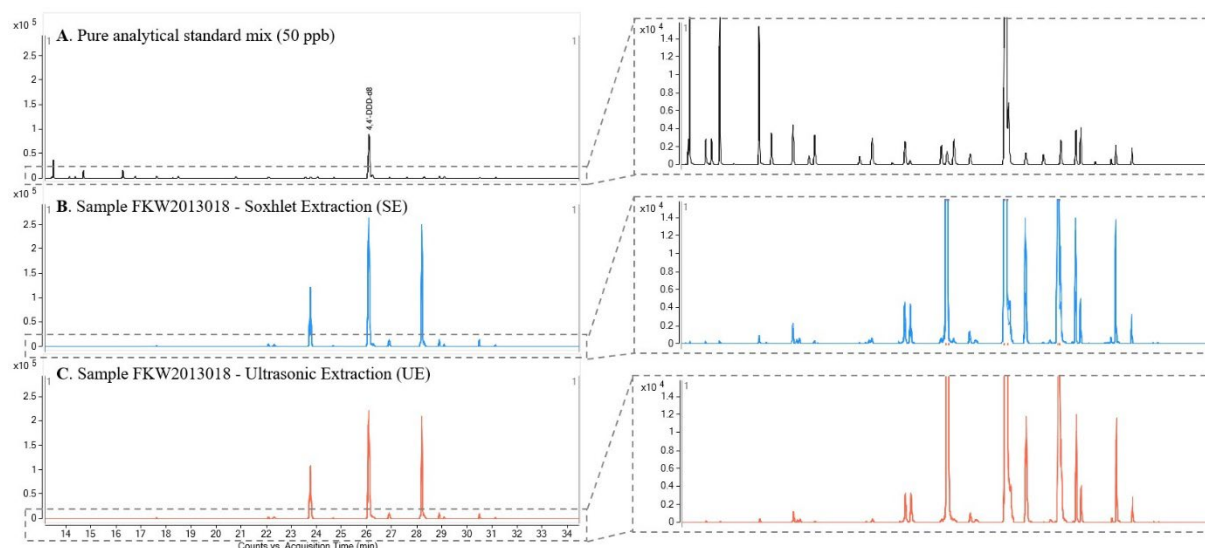
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729 **Supplementary Data**



Supplementary Figure 1. GC-QQQ chromatograms of target compounds in the pure analytical standard mix (A), four replicates of extracted sample FKW2013018 using Soxhlet extraction (B), and four replicates of extracted sample FKW2013018 using Ultrasonic extraction (C).

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