Analysis of Organochlorine Pesticides (OCPs) in Gray Whale Scat as an Alternative Method of Toxicant Quantification in Cetaceans

by
Amy Margaret Kutnerian

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Chemistry
(Honors Scholar)

Presented April 20, 2018
Commencement June 2018

AN ABSTRACT OF THE THESIS OF

Amy Margaret Kutnerian for the degree of Honors Baccalaureate of Science in Chemistry presented on April 20, 2018. Title: Analysis of Organochlorine Pesticides (OCPs) in Gray Whale Scat as an Alternative Method of Toxicant Quantification in Cetaceans.

Abstract approved:_____

Staci Simonich

A method was developed to quantify organochlorine pesticides in whale scat. This method would ideally replace analysis of pesticides in blood and tissue samples, which are difficult to acquire. Scat samples were collected from one stranded gray whale and three live whales. The samples were extracted with pressurized liquid extraction, cleaned up by solid phase extraction, and analyzed by gas chromatography/mass spectrometry. Compounds detected in whale scat included hexachlorobenzene, β-hexachlorocyclo-hexane, chlordanes, dichlorodiphenyltrichloroethane and its derivates (DDTs), and dieldrin. Lipid content of one sample was determined and compared to published literature values obtained through tissue sample analyses of the same population. The relationship between pesticide concentrations in scat and tissue samples is not known and must be established before scat analysis can replace tissue analysis as an approach to toxicant quantification in whales. The method developed in this study will allow rapid analysis of a large number of scat samples, thereby providing a means by which this relationship cap be identified.

Key Words: organochlorine pesticides, scat, gray whales, persistent organic pollutants

Corresponding e-mail address: hallsofstone2941@gmail.com

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APPROVED:

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Staci Simonich, Mentor, representing Chemistry

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Leigh Torres, Committee Member, representing Marine Mammal Institute

_____

Kim Anderson, Committee Member, representing Environmental and Molecular Toxicology

_____

Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Amy Margaret Kutnerian, Author

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INTRODUCTION

Pesticides comprise a group of chemicals that can be divided into four main categories: rodenticides, fungicides, insecticides, and herbicides[1]. Pesticides were widely manufactured and used during the 20th century, particularly after World War II[2]. Their most notorious compounds, such as dichlorodiphenyltrichloroethane (DDT), chlordane, dioxins, and furans, belong to a class of compounds called "persistent organic pollutants", or "POPs". They have a wide range of uses[2], such as insecticides in agricultural practices[1–3], protection against disease-carrying vectors (DDT and its role as a malaria preventative, for example), and in anti-fouling paints and other industrial applications[4]. POPs were so widely used that an estimated 1.3 million tons were produced between the 1930s and the 1970s[5].

The stimulus that triggered widespread concern about the use of POPs was Rachel Carson's *Silent Spring*, which detailed the long-term effects of pollutant exposure, particularly DDT[2]. Studies began to draw correlations between pesticide exposure and adverse health effects. POPs are now known to cause a variety of acute and chronic health problems in both humans and animals[6]. Long-term health effects in humans caused by POP exposure include the following: respiratory illness[1], birth defects[1], Parkinson's disease[1], kidney damage[1], lowered IQ[1], brain damage in children[1], blood disorders[1], reproductive toxicity[1,2], endocrine disruption[1,2], abnormal fetal development[1,2], neurological damage[1,2], cancer[1,7], and abnormal immune system function[1,2]. The most common adverse health effects in animals are cancer[7,8], reproductive toxicity[7–11], endocrine disruption[9,10], abnormal fetal development[7,9,10], hormone disruption[10,11], neurological concerns[10], and immunosupression[8–12]. POPs also pass from mother to

offspring via the placenta and during lactation. POP uptake, metabolization, and toxic health effects have been studied extensively in marine, Artic, and high trophic-level mammals. These studies include Arctic land species such as polar bears, foxes, seals, sea lions, and walruses[10], seabirds such as eagles, ospreys, ducks, puffins, loons, herons[10], gulls, penguins, and murres[13]. Other studies focus on a wide range of marine predators such as whales, porpoises, dolphins, and sharks[3,12,14,15].

During and after the 1970s, several POPs were banned from use or manufacture around the world via multiple treaties, agreements, and conventions[2,15]. The most notable global effort was the 2001 Stockholm Convention, which banned or limited the use of twelve POPs labelled the "dirty dozen"[2]: PCBs, DDT, chlordane, dieldrin, aldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, dioxins, and furans. Several countries had already made individual efforts to ban some of these compounds before the Stockholm Convention. For example, the United States Congress banned PCB production and limited use of the remaining stocks in 1978. Congress also gradually restricted the use of DDT between 1969 and 1972[2], as did most other developed countries[10]. China banned the production of DDT in 1983[4]. Most developed nations have restricted use of the most dangerous POPs, and developing nations are beginning to follow[2]. However, while POP concentrations initially decreased after such measures, they now remain stable across the globe[15].

Pesticides and other POPs are stable in the environment because they break down slowly. DDT degrades to dichlordiphenyldichloroethane (DDD), and both DDT and DDD can degrade to dichlorodiphenyldichloroethylene (DDE)[3,8], which does not undergo further degradation. Compounds such as HCB and HCH do not degrade at all. POPs have

a wide range of environmental half-lives[16], and some, such as DDT, can last for several decades[1]. The longevity of some POPs allows for long-range transport. Furthermore, POPs are hydrophobic[5], which means they attach to sediments and other organic components in the environment. They are also lipophilic[5], so they tend to get stored in adipose tissue in animals, a process referred to as bioaccumulation[1,10]. All of these factors mean POPs have the lifespan and chemical characteristics to travel anywhere and affect any animal in the world.

One method of POP transport is through the air. POPs can volatilize or sorb (attach) to airborne particles[7,16]. These gases and particles can then be carried via wind currents for thousands of miles, depending on their volatility and environmental half-life[7,16]. From there, POPs can return to the soil or ocean through precipitation or dry deposition much farther away from their sources[2]. Snow is particularly good at removing POPs from the atmosphere, resulting in higher POP concentrations at higher elevations[7]. This transport capability means that POPs have also appeared in locations as remote and as far from their sources as the Arctic and Antarctic circles[2].

Because they are hydrophobic, POPs have low solubility in water and sorb to organic particles instead[2,17]. An estimated 35-50% of pesticide chemicals sprayed on agricultural crops are almost immediately deposited onto the soil below via direct application or rainfall[1]. Sediments in aquatic systems act as sinks for POPs due to their sorptive nature[15,17]. This POP build-up can occur in river beds, lakes, and on ocean floors. Re-suspension of POPs and sediments occurs when these reservoirs are disturbed by natural processes like tidal movement and storms[17], or by anthropogenic activities such as dredging[15,17] and bottom-trawling[15,17]. As a result, the POPs are reintroduced to the

aquatic system and can be carried by river and ocean currents thousands of miles from the source. These processes are particularly impactful during natural events such as storms and floods, when turbulent waters kick up sediments.

POPs can be transported via migratory species such as sea birds and marine mammals[2,18], a process called biotic transport. One example of biotic transport can be found in whales, which move nutrients and chemicals both vertically and horizontally in the ocean. Whales consume the POPs contained in their prey, and then travel up to thousands of miles on their migratory journey before excreting these compounds through defecation[18]. Whales also ingest POPs and nutrients from the ocean floor and release them at the ocean's surface[18]. Finally, when whales die, their carcasses fall to the bottom of the ocean, where they are consumed by bottom-feeding biota[18]. This is one of the reasons POPs can be found in "pristine" environments such as the Mariana and Kermadec Trenches[5].

Many whale species are near the top of their food chains, which means they can accumulate large concentrations of toxic compounds. Bioaccumulation of POPs begins at the lowest levels of the food chain. Terrestrial animals eat plants that are either sprayed directly with pesticides or absorb them and other POPs through soil and groundwater[16]. In aquatic organisms, POPs are not only available via dietary intake, but also aqueous uptake via passive and active diffusion[4,17]. Aquatic uptake of POPs in low trophic-level organisms frequently occurs in regions where anthropogenic sediment disturbances, such as dredging and bottom-trawling, are common[15]. These organisms then become meals for larger species such as squid and fish[13], which eat mass quantities of smaller organisms, while simultaneously taking up POPs from their aquatic environment[4,5,13]. Aqueous POP

intake from the environment diminishes further up the food chain[1], but dietary intake increases as the mass of food consumed increases. Furthermore, because POPs are lipophilic, they tend to be stored in an organism's blubber and liver instead of being excreted through waste[19]. Thus, animals near the top of the food chain accumulate many of the toxins their prey and prey's prey have stored. This means even small levels of POPs found in low trophic levels (such as crustaceans[5]) can biomagnify to harmful levels in animals at higher trophic levels[2,14]. Biomagnification reaches its maximum in apex marine predators such as cetaceans, because their prey accumulate toxins via both dietary and aquatic uptake. This also applies to terrestrial animals that consume aquatic species, such as bears and seabirds.

Whales have a disproportionate level of influence on lower trophic levels. As mentioned above, whales take in nutrients containing iron and nitrogen at depths far below the surface of the water, then release them in fecal plumes at the ocean's surface[18,20]. These nutrients are taken up by phytoplankton that dwell near the water's surface, where they can absorb sunlight. Phytoplankton are at the bottom of the food chain and are consumed by zooplankton, which are then consumed by fish and other organisms[20]. Phytoplankton also take up carbon dioxide from the atmosphere and pull the carbon down into the depths of the ocean when they sink. When whales breach the surface of the water, they create updrafts that allow phytoplankton to remain near the water's surface for a longer period of time. When whale populations were at their peak, they are estimated to have removed tens of millions ($\sim 10^7$) of tons of carbon from the atmosphere by displacing phytoplankton[20]. As a result, whales not only provide nutrients to sustain surface- and bottom-dwelling aquatic food chains, but they are also indirectly

responsible for removing greenhouse gases from the atmosphere. Anything that decreases whale populations will thus have a significant effect on all species.

Whale species have experienced unexpected and dramatic declines in their populations for decades[21]. Some of these population reductions can be explained by whale hunting[18,20]. Others, such as the decline of the Southern Resident killer whale population off the west coast of the United States, mystified scientists in the 1990s[6,21]. Hypotheses about the reasons for these population reductions ranged from noise pollution to reduced prey availability, triggering a wide range of ongoing studies devoted to determining the cause of declining whale populations. Some scientists theorized toxins in the water were the cause. Of these toxicants, PCBs and organochlorine pesticides (OCPs), such as DDT and chlordanes, have been studied the most intensively.

POPs are typically analyzed in a blubber (fat) biopsy taken from the organism[11,22], although liver[19], blood[10,21], and muscle[10] samples can also be analyzed. Blubber and liver samples have especially high lipid content, and are consequently where most POPs tend to bioaccumulate. For marine mammals, such as whales and porpoises, the best opportunities to collect tissue and blood samples often occur when the animal is entangled in fishing nets[12,19]. Other samples may be taken from animals that were killed by hunting or died of natural causes[11,21]. In cetaceans, blubber samples may be collected using darts, although this is an impractical method due to the size of the organisms[21]. Collection of blubber biopsies is also invasive to the organisms and can result in higher levels of stress hormones, which is problematic for studies attempting to analyze hormones in those animals[6]. Finally, the collection of blubber biopsies is strictly

regulated[6]. As a result, sample collection results in small sample sizes and high levels of variation within species and populations.

In order to circumvent the difficulties associated with blubber analyses, some studies have turned to whale scat (i.e. poop) instead. Scientists have been collecting whale poop for almost two decades[21]. Studies that utilize poop tend to focus on hormone analysis, which can provide information about the impacts of factors such as noise pollution and low prey availability on the whales' stress levels. A novel approach proposed by Lundin et al.[6] was used to analyze POPs in scat. The focus of their study was to quantify toxins such as PCBs and DDTs found in scat collected from the Southern Resident killer whale (SRKW) population in Puget Sound. Their study was optimized for hormone analysis, but may not have been optimal for POP analysis[6,9]. As a result, POP analysis was only partially successful. Small sample size and the additional steps required for hormone analysis may have resulted in false negatives for POPs that would have otherwise been detected. As a result, p,p-DDE was the only pesticide detected in whale scat[6].

The purpose of this research project is to develop a new analytical method for the analysis of POPs in whale scat. This method analyzes a wider range of compounds in a larger sample size than those in previous studies. The focus of this study is on organochlorine pesticides, which were, or are, used in insecticides. These compounds include many of the "dirty dozen", such as chlordane, endosulfan, DDT and its derivatives, hexachlorobenzene (HCB), and chlorpyrifos. These compounds present similar characteristics and health effects as other POPs.

METHOD & METHOD DEVELOPMENT

All method development and optimization was performed with one wet scat sample, which was collected from a stranded male sub-adult (1-2 years old) gray whale in August of 2016 by researchers at the Hatfield Marine Science Center. The sample's moisture content was determined by gravimetric analysis before and after moisture removal, which was performed in a drying oven at 100° C for at least 48 hours. The wet sample was divided into smaller subsamples of approximately 2.5 g wet weight (ww). Lab blanks comprised of diatomaceous earth were run concurrently with each set of subsamples.

Sample Extraction

The 2.5 g subsamples were combined with an average of 8.7 g of diatomaceous earth per 1g wet sample to remove moisture. Each sample was packed in an accelerated solvent extraction (ASE) cell and spiked with deuterated surrogates at 500 pg/µL. Surrogates were added to the sample to normalize loss of analyte that occurs during sample processing. The sample was extracted using pressurized liquid extraction (PLE) on an ASE model 350 with dichloromethane (DCM) and ethyl acetate (EA) twice with the following parameters[23]: cell temperature 100° C, 1500 psi, static time 5 minutes, solvent flush 100% cell volume, 2 static cycles, and an N_2 purge time of 240 seconds. The fractions were collected separately, then mixed with Na_2SO_4 to remove water from the extract. PLE extracts were concentrated to 10 mL, and the lipid content was gravimetrically determined by calculation of the dry weight of a 1% v/v aliquot of the extracts.

Sample Cleanup

The extracts were concentrated under N_2 with a Turbovap II evaporation system at room temperature. The solvents were evaporated and exchanged with hexane, and were then concentrated to 7 mL each. Matrix interferences were removed from each extract on 20 g silica solid phase extraction (SPE) columns (MegaBond Elut, Agilent, Inc.). Columns were conditioned by eluting 50 mL each of hexane, DCM, and ethyl acetate. PLE fractions were loaded onto SPE columns, and then eluted in the following order: 50 mL hexane, 100 mL DCM, and 50 mL EA. The hexane and DCM elutions comprised one fraction, and the EA elution comprised another fraction. Hexane:DCM fractions were solvent exchanged to EA, then all fractions were concentrated to 270 µL each and spiked with labeled internal standards (500 pg/µL) to quantify surrogate loss. The final sample volume was 300 µL. A flowchart for the extraction and clean-up of each sample or subsample is shown in Figure 1.

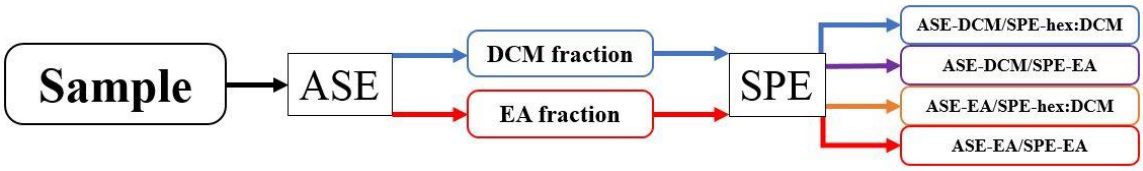

Figure 1: Flowchart for sample processing.

Instrumental Analysis

All individual fractions were analyzed on an Agilent 6890 gas chromatograph (GC) using a 30 m x 0.25 mm x 0.25 µm DB-5 column (J&W Scientific) with an Agilent 5973N mass spectrometry detector (MSD) in both electron impact (EI) ionization and negative chemical ionization (NCI) modes. Samples were analyzed for 24 compounds in NCI and 26 compounds in EI (target compounds, deuterated surrogates, SIM windows,

and characteristic ions are listed in Tables A and B in the Appendices). ^{13}C-PCB-180 was used as an internal standard to quantify NCI surrogates, and deuterated acenapthene and fluoranthene were used to quantify EI surrogates.

Method Optimization

Sample size. Scat samples were tested at varying masses to identify a sample mass sufficient to contain the compounds of interest at detectable concentrations. Samples of 2.5g, 5g, and 10.1g (wet weight) scat, which correspond to approximately 150, 300, and 600 mg dry weight, respectively, were analyzed by GC/MS.

Fraction analysis: the above sample size analysis was performed while analyzing the fractions of each sample (shown in Figure 1) separately. To reduce the amount of glassware used and time spent analyzing each fraction, samples of 150 mg dry weight (dw) and 300 mg dw were analyzed after all the SPE fractions were combined. A 600 mg dw sample was not analyzed because the sample size test indicated the separate fractions contained too many interferences from the matrix (see Results and Discussion).

Target recovery analysis. To determine the validity of the extraction and clean-up methods, triplicate runs of 150 mg dw samples were packed in ASE cells and spiked with target compounds for a theoretical final concentration of 500 pg/µL per compound. The samples were run through the extraction and clean-up steps detailed above, then spiked with the same deuterated surrogates used to quantify them in sample analysis to a final concentration of 500 pg/µL. The surrogates were used to quantify the actual concentrations of the target compounds in the final solution, which were then used to determine the percent loss from the expected 500 pg/µL.

Sample Collection

Gray whale scat samples were collected by researchers at the Hatfield Marine Science Center from fecal plumes on the surface of the Pacific Ocean near the Oregon Coast between August and October of 2016, lyophilized, and frozen. Three samples, each between 150-350 mg dry weight, were transported to Corvallis, OR, and frozen until analysis.

Final Analytical Method

A finalized method, based on the results of method development (see Results and Discussion), was employed for analysis of the gray whale scat samples. Samples between 150-300 mg dw were packed into individual ASE cells with approximately 20 g of diatomaceous earth to fill the cell, as moisture content had already been removed by lyophilization. Samples were spiked with deuterated surrogates to 500 pg/µL, extracted using pressurized solvent extraction as described in the Sample Extraction section, and cleaned up using solid phase extraction as described in the Sample Cleanup section. All SPE fractions were spiked with internal standards to 500 pg/µL, then analyzed separately by EI and NCI GC/MS. Lipid analyses were attempted gravimetrically with these samples in the same manner as the moisture measurement of the wet sample, but they were unsuccessful. This is likely because the lipid content fell below the detection limit of the scale. Pesticide concentrations in dry samples were analyzed and results reported as ppb dry weight only, without normalization for lipid content. ASE cells packed with diatomaceous earth served as lab blanks, which were extracted and analyzed along with samples.

RESULTS AND DISCUSSION

Method Development

Subsamples are indicated by their masses (e.g. “300 mg dw”) in Figure 2, which shows the number of analytes detected in each subsample. In GC/MS analysis of the 600 mg dw scat subsample, large peaks that did not correspond with the target retention times or qualifying ions increased the signal so much that target peaks were hidden. These peaks were attributed to matrix interferences, which drowned out the target signals such that quantification of most compounds was impossible. Consequently, fewer compounds were identified in the 600 mg dw subsample than in the 300 mg dw subsample. White precipitate was observed in sample vials containing the 600 mg dw subsample extracts stored in the refrigerator between analyses, which could indicate the presence of lipids in the subsample. This suggests the clean-up step was not sufficient to remove matrix interferences in large sample sizes.

The number of compounds detected in each sample size category is shown in Figure 2. The ideal sample size for scat analysis was determined to be 300 mg dry weight. Because some compounds were detected in the 150 mg dw subsample, and because the mass of scat collected from gray whales is

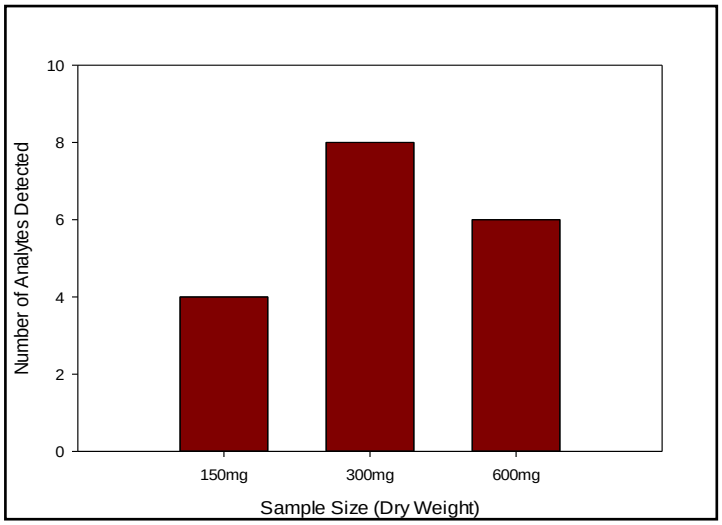

Figure 2: Number of compounds detected in varying sample sizes.

usually small, subsequent sample analyses were done with a range of sample sizes between 150-300 mg dry weight.

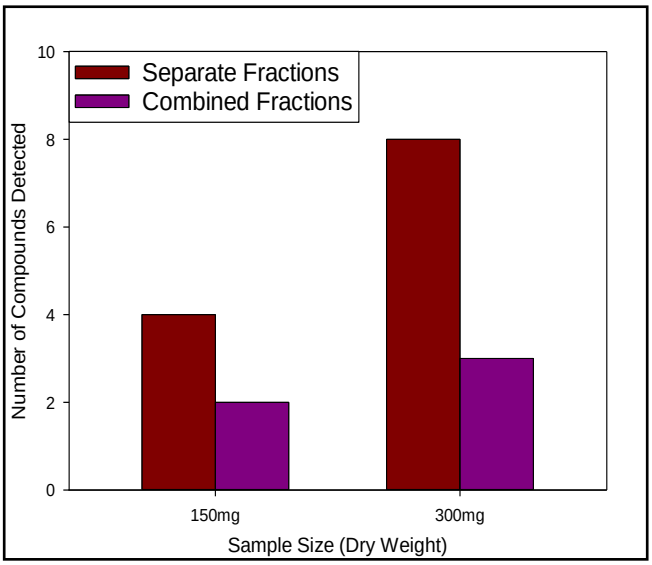

Figure 3: Number of compounds detected in analysis of separate and combined fractions.

Figure 3 compares the results of sample analyses for the combined SPE fractions and the sum of the separate SPE fractions. Fewer compounds were detected in the combined SPE fractions than in separate ones. This can be attributed to the same matrix interferences that masked analyte signals in the 600mg subsample. Subsequent SPE sample fractions were analyzed separately to minimize the impact of matrix interferences.

Figure 4 shows the recoveries of target compounds from the scat samples. Analytes eluted almost exclusively in the PLE-DCM + SPE-hex:DCM fraction, while two compounds, trans-nonachlor and cis-nonachlor, had very low recoveries (<1%) in the

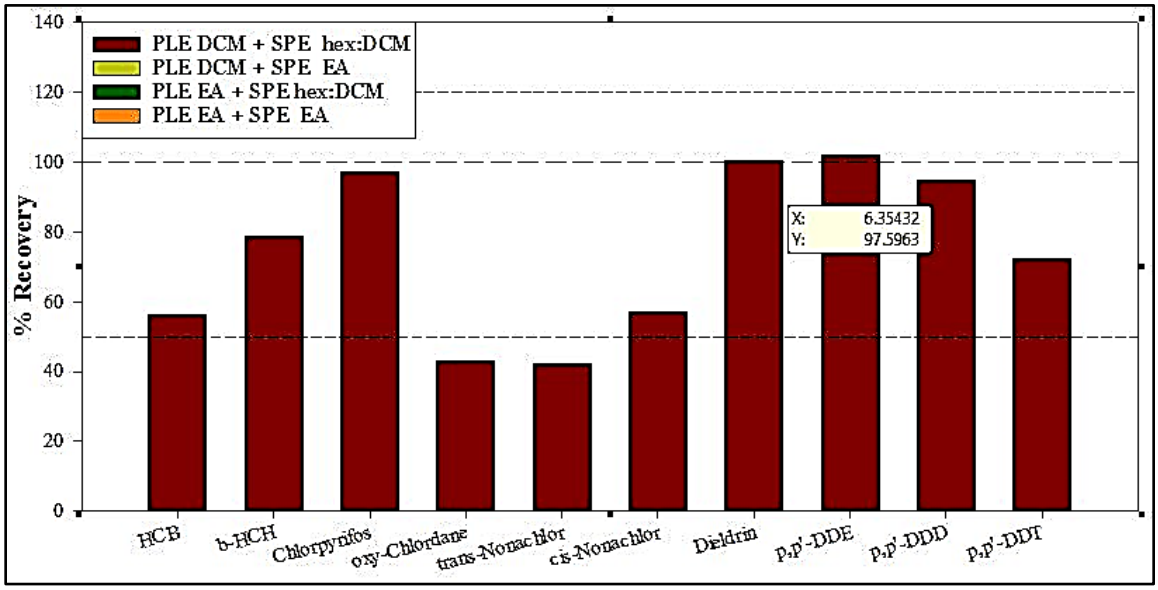

Figure 4: Spike recoveries of target compounds detected in whale scat fractions following PLE and SPE.

PLE-EA + SPE-hex:DCM fraction. This indicates that all of the detected compounds are highly nonpolar and that they can be quantified by GC analysis of the PLE-DCM + SPE hex:DCM fraction alone.

For the purpose of this study, target recoveries above 50% (the lower bound in Figure 4) were considered acceptable. Recoveries of two compounds found in gray whale scat, oxy-chlordane and trans-nonachlor, fell below the lower bound at 43% and 42%, respectively. A low recovery indicated the target compound was either not extracted during the extraction step, or was lost during the cleanup step. An improved extraction/cleanup method might increase the recoveries of these two compounds, but this was not done in this study due to limited sample availability.

A total of fifty compounds were analyzed in scat samples, forty of which were not detected in whale scat. Of these forty undetected compounds, twenty-six had successful spike recoveries above 50%, and fourteen had spike recoveries below 50% (see Table 1). Compounds with recoveries above 50% can be analyzed using the method described in this study. The rest of the compounds would need further modification to the method to

Compounds Analyzed but Not Detected in Whale Scat		
Spike Recovery ≥ 50%		**Spike Recovery <50%**
Trifluralin	Simazine	Aldrin
α-HCH	Atrazine	Heptachlor epoxide
γ-HCH	Diazinon	trans-chlordane
δ-HCH	Methyl parathion	Endosulfan I
Triallate**	Alachlor	cis-chlordane
Heptachlor	Malathion	Endrin aldehyde
Dacthal	Metolachlor	Ethion
Endrin	o,p-DDE	Parathion*
Endosulfan II	o,p-DDD	Carbaryl
Endosulfan sulfate	o,p-DDT	Cyanazine*
Mirex	Methoxychlor	Triallate**
Propachlor		EPTC
Atrazine desethyl		Etridiazole
Carbofuran		Atrazine desisopropyl

Table 1: Compounds analyzed using the method described in this study. Recoveries ≥ 50% were considered successful, while recoveries < 50% were not. *Compounds had 0% recovery. **Triallate was analyzed in both EI and NCI modes. Its recovery was below 50% in EI mode, but above 50% in NCI (likely due to matrix interferences).

improve their percent recoveries. This is especially true for the chlordanes, which all but one had low recoveries, and for parathion and cyanazine, which were not detected in any of the spiked recovery fractions. All compounds detected in NCI mode on the mass-spectrometer eluted in the PLE-DCM + SPE-hex:DCM fraction, and all compounds detected in EI mode eluted in either the PLE-DCM + SPE hex:DCM or PLE-DCM + SPE-EA fractions; thus all targets with successful spike recoveries can be quantified in the two PLE-DCM fractions.

Gray Whale Scat Samples

The developed method was used to analyze scat samples from three whales, two females of at least 22 years old, and one male of unknown age. The two females are identified as S2F1 and S2F2, and the male is identified as S5F6-A1. Lipid analysis performed on these samples was unsuccessful, because of low lipid mass in 300 mg dw of scat. As a result, concentrations of OCPs are given in ppb normalized by the dry

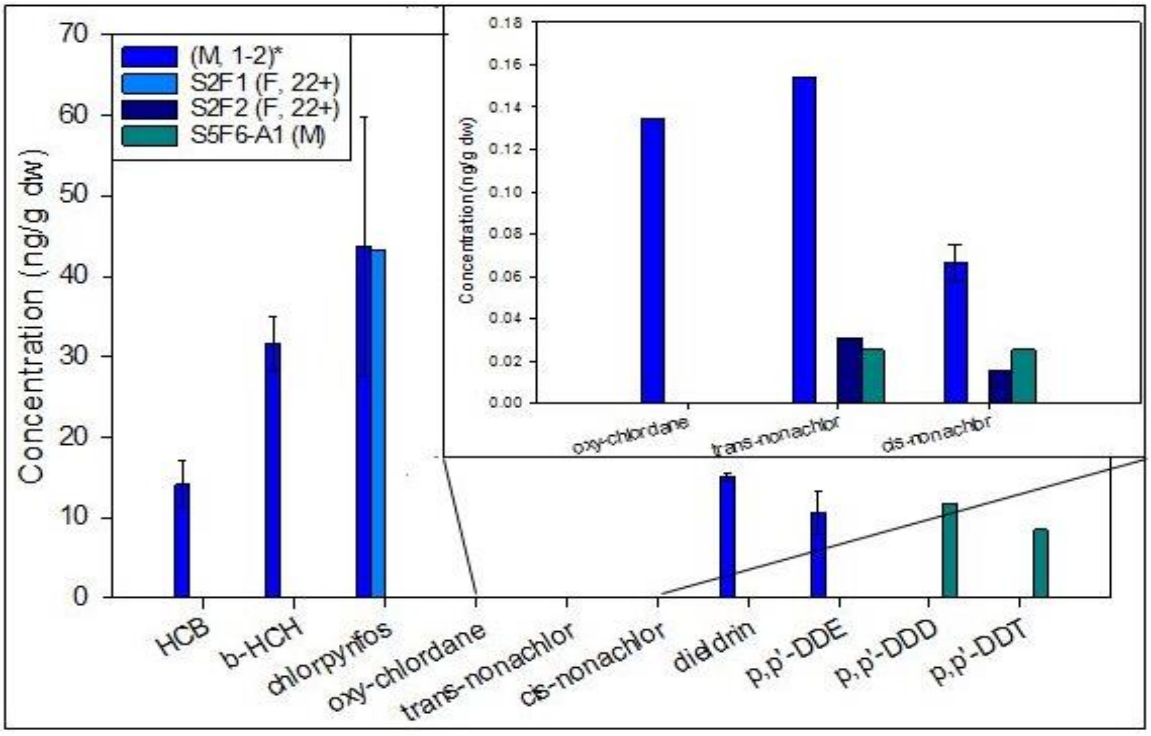

Figure 5. Pesticides detected in gray whale scat. Error bars = standard error. *Stranded whale.

weight (dw) of the samples instead of their lipid weights. Pesticides detected in these samples included chlorpyrifos, trans- and cis-nonachlor, p,p-DDT, and p,p-DDD. Figure 5 shows quantities of the compounds detected in the three live whale scat samples, as well as in the sample collected from the stranded male that was used for method development. Detection of historic-use pesticides in the scat samples indicates these compounds persist in the marine environment despite their status as banned products in most countries.

Though other compounds were not detected in gray whale scat, it is unlikely they are completely absent from the whales themselves. Compounds with low spike recoveries (see Table 1) are most susceptible to falling below the instrument's detection limit, as well as compounds that are more affected by matrix effects than others. Variations between sample values could exist due to variations in the age and sex classes between individual whales. Discrepancies can also be explained by the formation of metabolites that were not analyzed in the developed method, such as chlorpyrifos-oxon, a derivative of chlorpyrifos. Future studies would benefit from increasing the compound list to include common POP metabolites, especially for scat analysis.

Scat vs. Tissue Analysis

Analysis of lipids in tissues from these whales would be beneficial in order to identify a relationship, if one exists, between OCPs in lipids versus scat. Although lipid analysis was unsuccessful for most of the gray whale samples, the sample from the stranded whale had a lipid content of 0.8% w/w dry weight. In comparison to published literature values[14], concentrations of OCPs in scat are on the same order of magnitude on a lipid weight basis. Figure 6 compares pesticide concentrations found in scat in this

study to concentrations found in muscle and liver tissues collected from gray whales in the Bering Sea[14]. All samples were collected from individuals in the Eastern North Pacific population, which migrates from the Bering Sea to the west coast of Mexico[24] (the blue-shaded region in Figure 7). Scat and tissue values are from animals in the same population. The OCP concentrations fall within a wide range, even among individuals within the same population. OCP concentrations also vary with age, sex, and pregnancy status[6]. This variation is one of the reasons for the large standard deviations indicated by the error bars in Figure 6. OCP data would ideally be reported to reflect these individual classifications to facilitate pattern recognition, but this method of reporting would require a larger number of samples from each classification to properly represent the respective group. Because tissue collection has many limitations, such as difficulty, cost, and availability, large sample numbers are not feasible for tissue analysis.

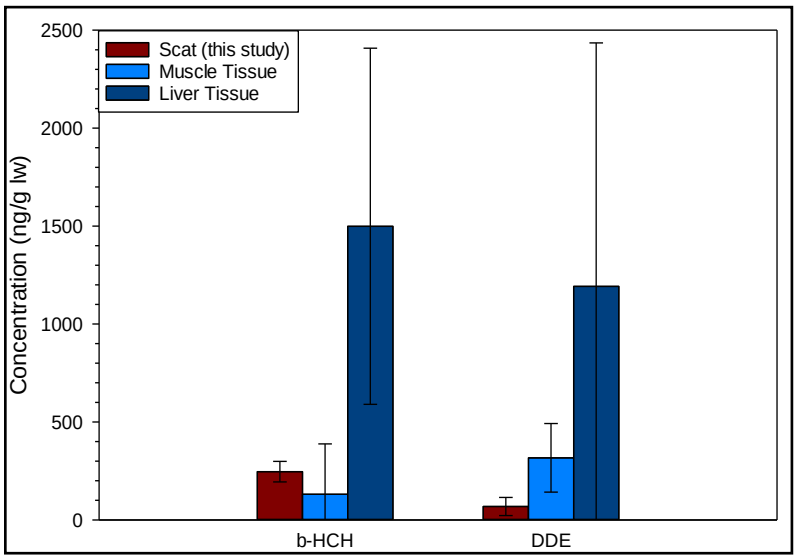

Figure 6: OCP concentrations in gray whale scat, muscle[14], and liver[14] tissues. Standard deviation is shown with error bars.

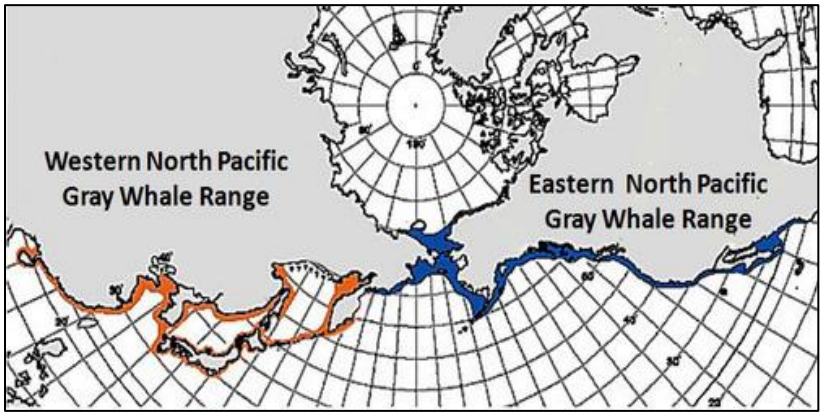

Figure 7. Gray whale population migration patterns[24].

The concentrations of OCPs in gray whale scat from this study can be compared to those found in Orca whale scat from Lundin, et al.[6], though caution should be taken in comparing whales of two different species. The concentration of p,p-DDE found in sub-adult Orca whale scat samples[6] (n = 22) compared to the scat sample collected from the sub-adult gray whale in this study is shown in Table 2. Orcas reach sexual maturity at 10 years of age, compared to 5 years in gray whales; as a result, it is expected that juvenile Orca whales would have higher OCP concentrations than gray whales. Furthermore, Orca prey, specifically salmon, is high in fatty tissue and thus contains higher POP concentrations than gray whale prey. These considerations account for the difference in p,p-DDE concentrations between the two species.

	Gray whales, sub-adult (this study, n=1)	**Orca whales, sub-adult (Lundin, et al.[6], n = 22)**
p,p-DDE (ng/g lw)	79.8	291.9

Table 2: Mean concentration of p,p-DDE in sub-adult scat samples of Orca and gray whales.

Scat collection is simpler than tissue collection, so scat samples are more readily available to researchers than are tissue samples. For example, Lundin, et al.[6], collected over 260 scat samples from the SRKW population over the course of four years. Researchers at the Hatfield Marine Science Center were able to collect 80 scat samples from gray whales off the Oregon coast in the summer of 2017 alone. Collection of scat samples allows researchers to maintain an appropriate distance from the whales, limits disturbances to the whales, and has fewer regulatory hurdles. Analysis of OCP concentrations in scat can be performed with larger sample sizes, so scat provides a more comprehensive means of monitoring individuals in a population. Furthermore, scat analyses would allow study of subsets of populations that share similar characteristics, such as sex or maturity status.

However, in order to replace tissue analysis with scat analysis, the relationship between the two must be characterized. The relationship between scat and blubber for p,p'-DDE was determined by Lundin, et al.[6] to be roughly a 1:1 ratio log[lipid]:log[scat], indicating results from the two types of analysis are on the same order of magnitude. However, relationships for other OCPs between scat and tissue have not been identified. This brings into question the issue of stored versus transient OCPs. Compounds that accumulate in the organism over the course of its lifetime are "stored" compounds, whereas those that pass through the organism along with waste are "transient" compounds. While lipids stored in the body do occasionally exit through fecal matter, there is no way of differentiating between stored OCPs in lipids and transient OCPs obtained through diet. However, analysis of OCPs in the whale's prey and sediment consumed during feeding could be used to quantify OCPs in the diet. This may provide a rough estimate of dietary versus stored OCPs, and is thus one of the future endeavors of this research project.

To identify and quantify the relationship between OCP concentrations in scat and tissues, samples of both types would have to be taken from the same individual. Each type of sample collection should occur in the same year to correct for the continuous accumulation of stored OCPs from the diet. Scat samples that are collected at different times than tissue samples might contain some lipid- associated OCPs that do not correspond to the concentrations of stored OCPs in the tissue samples. Scat samples collected at the same time as tissue samples would contain lipid-associated OCPs that better reflect the concentrations of stored OCPs in tissues. Concurrent collection and

analysis of these sample types eliminates the variable of time, hence allowing a more accurate determination of the relationship between stored and transient OCPs.

A gap between scat and tissue collection is especially important in the contexts of age and pregnancy status. In regard to pregnancy status, females offload their stored toxins when they bear their offspring. Scat and tissue samples should therefore be collected and analyzed as closely as possible in time until the relationship between their OCP concentrations is determined. Sample collection for this type of analysis is beyond the scope of this study, but it would be necessary for the transition from tissue to scat analysis.

CONCLUSIONS

Historic- and current-use pesticides were detected and quantified in multiple scat samples in this study, indicating that gray whale scat may be suitable for analysis of OCPs in gray whales. The results of this study confirmed the presence of HCHs, HCB, chlorpyrifos, DDTs, dieldrin, endrin, nonachlors, and chlordanes in scat samples. Detection of these ubiquitous compounds suggests that, with larger sample sizes and improved detection limits, other organochlorine pesticides might also be detected in whale scat. These results open up new possibilities for the analysis of toxicants in gray whales, other cetaceans, and potentially many species of concern worldwide. Scat can be collected in large quantities without invasive measures or harm to the animal, thereby permitting unrestricted sample collection for analysis. As a result, more comprehensive analyses of toxicants and their quantities in whales can replace more difficult methods that require tissue and blood collection. The potential benefits of this type of analysis

may also extend to studies of other species, especially other endangered arctic and marine species that are disproportionately affected by residual toxins in aquatic environments.

For scat analysis to become more widespread, the relationship between scat and tissue toxicant quantities must be established. Repeated analysis of tissue and scat concurrently collected from the same animal would be necessary to identify this connection and is suggested as a future course of action to validate this approach. This is beyond the scope of this study; however, OCP intake determined through analysis of prey, sediment, and seawater consumption could be compared to fecal samples to estimate the impact of transient, dietary compounds on scat analysis. Analysis of prey, water, and sediment in areas where gray whales feed is a future application of the method developed in this study.

The recoveries of some pesticides, primarily chlordanes and nonachlors, were low in this study, and could likely be improved through adjustments to the extraction method. The clean-up method may also need to be adjusted to improve detection limits in larger samples, where lipids reach concentrations that interfere with instrumental analysis. This is a concern only when samples larger than 300 mg dry weight are analyzed, which is not often feasible, since the typical sample mass collected from the whales is small. Analyzing samples in triplicate is also recommended for significant results.

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APPENDICES

Table A: NCI SIM Windows and Quantifying Ions

			Quantifying Ions		
	Quantifying Compound	**SIM Window**	**Q1***	**Q2**	**Q3**
Internal Standard:					
^{13}C-PCB-180		10	405.9	407.9	409.9
Surrogates:					
d_{14}-Trifluralin	^{13}C-PCB-180	1	349.2	350.2	319.2
^{13}C-HCB	^{13}C-PCB-181	2	291.8	293.8	289.8
d_6-γ-HCH	^{13}C-PCB-182	2	72	74	262.9
d_4-Endosulfan I	^{13}C-PCB-183	7	377.9	375.9	373.9
d_4-Endosulfan II	^{13}C-PCB-184	9	411.9	413.9	409.9
Target Compounds:					
Trifluralin	d_{14}-Trifluralin	1	335.1	336.1	305.1
HCB	^{13}C-HCB	2	283.8	285.8	281.8
α-HCH	d_6-γ-HCH	2	71	73	70
β-HCH	d_6-γ-HCH	2	71	73	70
γ-HCH	d_6-γ-HCH	2	71	73	70
δ-HCH	d_6-γ-HCH	3	71	252.9	254.9
Triallate	d_6-γ-HCH	3	160.1	161.1	104.1
Heptachlor	d_6-γ-HCH	4	265.9	267.9	299.9
Dacthal	d_6-γ-HCH	5	332	330	334
Aldrin	d_6-γ-HCH	5	237	239	329.9
Chlorpyrifos	d_6-γ-HCH	5	313	315	214
Heptachlor epoxide	d_4-Endosulfan I	6	389.8	387.8	391.8
Oxy-chlordane	d_4-Endosulfan I	6	423.9	425.9	351.9
Trans-chlordane	d_4-Endosulfan I	7	409.9	407.9	411.9
Endosulfan I	d_4-Endosulfan I	7	403.9	371.9	369.9
Cis-chlordane	d_4-Endosulfan I	7	265.9	263.9	267.9
Trans-nonachlor	d_4-Endosulfan I	7	443.9	445.9	441.9
Dieldrin	d_4-Endosulfan I	8	345.9	347.9	379.9
Endrin	d_4-Endosulfan II	8	345.9	347.9	379.9
Endosulfan II	d_4-Endosulfan II	9	405.9	407.9	371.9
Cis-nonachlor	d_4-Endosulfan II	9	443.9	445.9	441.9
Endrin aldehyde	d_4-Endosulfan II	9	379.9	381.9	345.9
Endosulfan sulfate	d_4-Endosulfan II	10	385.9	387.9	421.8
Mirex	d_4-Endosulfan II	10	403.9	405.9	438.9

Q1* = identifying ion

Table B: EI SIM Windows and Quantifying Ions

			Quantifying Ions		
	Quantifying Compound	**SIM Window**	**Q1***	**Q2**	**Q3**
Internal Standards:					
d_{10}-Acenapthene		2	164	162	
d_{10}-Fluoranthene		8	212	213	
Surrogates:					
d_{14}-EPTC	d_{10}-Acenapthene	1	128.1	132.1	189.1
d_5-Atrazine	d_{10}-Acenapthene	4	205	220	
d_{10}-Diazinon	d_{10}-Acenapthene	5	314	138	
d_{11}-Acetochlor	d_{10}-Fluoranthene	6	173	245	
d_{13}-Alachlor	d_{10}-Fluoranthene	6	200	251	
d_6-Methyl parathion	d_{10}-Fluoranthene	6	269	115	
d_7-Malathion	d_{10}-Fluoranthene	7	174	131	
d_{10}-Parathion	d_{10}-Fluoranthene	7	115		
d_8-p,p'-DDE	d_{10}-Fluoranthene	9	326	324	
d_8-p,p'-DDT	d_{10}-Fluoranthene	9	243	245	
Target Compounds:					
EPTC	d_{14}-EPTC	1	128.1	132.1	189.1
Etridiazole	d_{14}-EPTC	2	210.9	212.9	182.9
Propachlor	d_5-Atrazine	3	120.1	176.1	93.1
Atrazine desisopropyl	d_5-Atrazine	3	173	175	158
Atrazine desethyl	d_5-Atrazine	3	172	174	187.1
Carbofuran	d_5-Atrazine	4	164.1	149.1	131
Simazine	d_5-Atrazine	4	201.1	203.1	186.1
Atrazine	d_5-Atrazine	4	200.1	202.1	215.1
Diazinon	d_{10}-Diazinon	5	179.1	199.1	304.1
Acetochlor	d_{11}-Acetochlor	6	146.1	162.1	223.1
Methyl parathion	d_6-Methyl parathion	6	263	125	109
Alachlor	d_{13}-Alachlor	6	188.1	160.1	237.1
Carbaryl	d_6-Methyl parathion	6	144.1	115.1	116.1
Malathion	d_7-Malathion	7	173.1	158	127
Metolachlor	d_{13}-Alachlor	7	162.1	238.1	240.1

Table B (continued)			Quantifying Ions		
Target Compounds (cont.)	**Quantifying Compound (cont.)**	**SIM Window**	**Q1***	**Q2**	**Q3**
Parathion	d_{10}-Parathion	7	291	155	109
Cyanazine	d_5-Atrazine	7	225.1	227.1	240.1
o,p-DDE	d_8-p,p'-DDE	8	318	316	320
p,p-DDE	d_8-p,p'-DDE	9	317.9	315.9	319.9
o,p-DDD	d_8-p,p'-DDE	9	235	237	165.1
p,p-DDD	d_8-p,p'-DDE	9	235	237	165.1
Ethion	d_{10}-Parathion	9	231	384	153
o,p-DDT	d_8-p,p'-DDT	9	235	237	165.1
p,p-DDT	d_8-p,p'-DDT	9	235	237	165.1
Methoxychlor	d_8-p,p'-DDT	10	227.1	228.1	274.1

Q1* = identifying ion