

Influence of Life-History Parameters on Persistent Organic Pollutant Concentrations in Blubber of Eastern North Pacific Gray Whales (*Eschrichtius robustus*)

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Cite This: *Environ. Sci. Technol.* 2022, 56, 17119–17130



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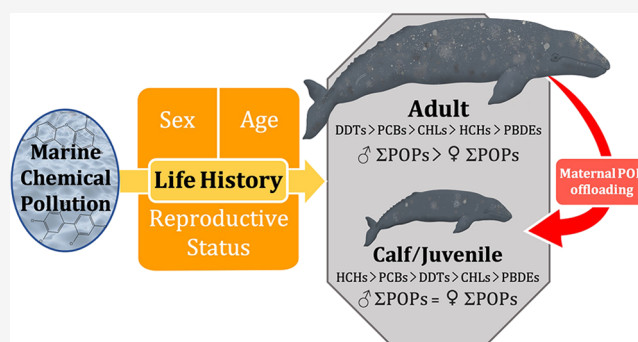
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ABSTRACT: Exposure to persistent organic pollutants (POPs) can significantly impact marine mammal health, reproduction, and fitness. This study addresses a significant 20-year gap in gray whale contaminant monitoring through analysis of POPs in 120 blubber biopsies. The scope of this substantial sample set is noteworthy in its range and diversity with collection between 2003 and 2017 along North America's west coast and across diverse sex, age, and reproductive parameters, including paired mothers and calves. Mean blubber concentrations of polychlorinated biphenyls (Σ PCBs), dichlorodiphenyltrichloroethanes (Σ DDTs), and chlordanes (Σ CHLs) generally decreased since previous reports (1968–1999). This is the first report of polybrominated diphenyl ethers (PBDEs) and select hexachlorocyclohexanes (HCHs) in this species. Statistical modeling of the 19 most frequently detected compounds in this dataset revealed sex-, age-, and reproductive status-related patterns, predominantly attributed to maternal offloading. Mean POP concentrations differed significantly by sex in adults (17 compounds, up to 3-fold higher in males) but not in immatures (all 19 compounds). Mean POP concentrations were significantly greater in adults versus immatures in both males (17 compounds, up to 12-fold) and females (13 compounds, up to 3-fold). POP concentrations were detected with compound-specific patterns in nursing calves, confirming maternal offloading for the first time in this species.

KEYWORDS: organochlorines, pollutants, gray whale, life-history, marine mammals



INTRODUCTION

The presence of persistent organic pollutants (POPs) in the marine environment is of global concern for marine mammal health and habitat quality.^{1,2} Marine mammals are particularly susceptible to bioaccumulating these lipophilic contaminants in their blubber³ and are at risk of associated effects including developmental dysfunction, endocrine system disruption, reproductive failure, and immunosuppression.^{4–11} Due to their toxicity and environmental persistence, the production and use of POPs, such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), chlordanes (CHLs), hexachlorocyclohexanes (HCHs), and polybrominated diphenyl ethers (PBDEs), have been prohibited or greatly limited since the 2001 Stockholm Convention on Persistent Organic Pollutants and its subsequent addendums. Despite this ban, POPs remain a threat to wildlife on account of their environmental persistence, long-range transport,

bioaccumulation, and continued production and use in nonsignatory countries.

Accumulation of POPs in marine mammals is dependent on biological factors including, but not limited to, trophic position, nutritional state, sex, age, and reproductive status.^{11–17} Maternal contaminant transfer is a known dominant factor contributing to marine mammal intraspecific variation of POP concentrations.^{3,18–24} Maternal offloading occurs primarily through lactation and, to a lesser extent, gestation.^{9,21,25} The influence of life-history parameters, such

Received: August 18, 2022

Revised: October 12, 2022

Accepted: October 24, 2022

Published: November 8, 2022



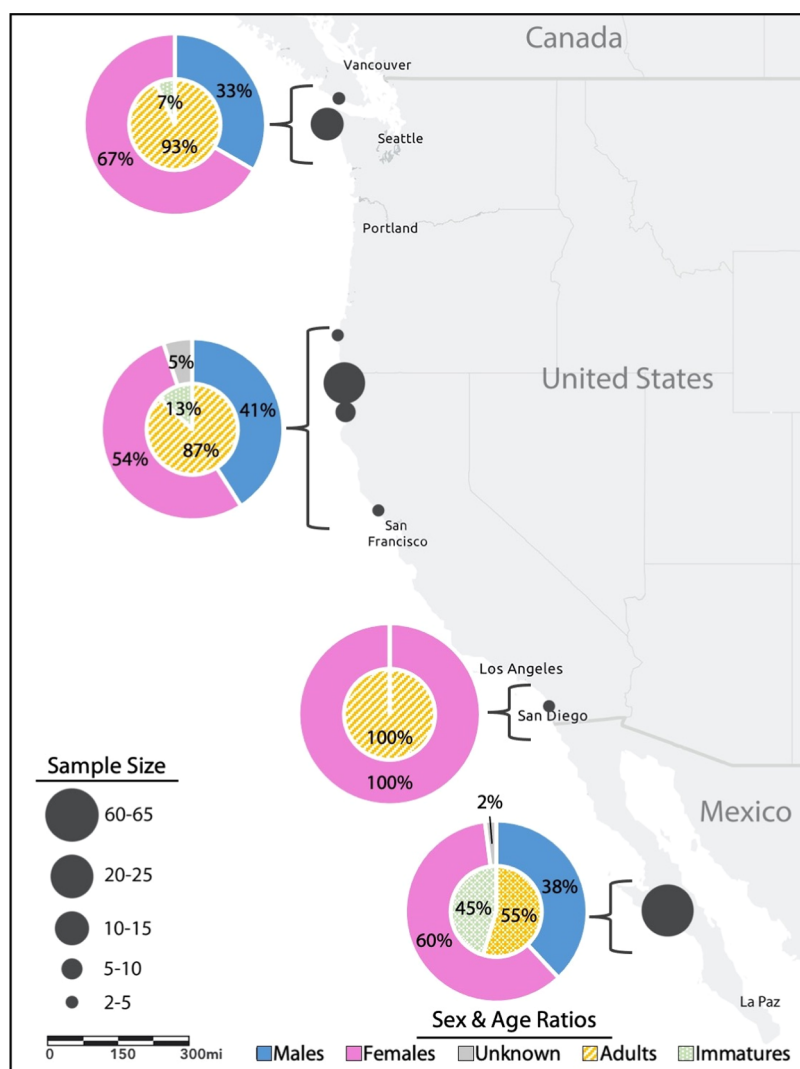


Figure 1. Biopsy sample collection locations with sex and age group (“immatures” includes juveniles and calves) composition of free-ranging gray whales between 2003 and 2017.

as sex, age, and reproductive status, on contaminant concentrations is well known for pinnipeds and toothed whales but unconfirmed in many baleen whales due to the absence of captive animals and challenges associated with sampling in the wild. Differences in reproductive ecology, bioenergetics, diet, and/or milk composition among whales warrant species-specific POP studies that support population-wide health assessments, especially in understudied species in ecotoxicology such as the gray whale (*Eschrichtius robustus*).

POPs had been reported in eastern North Pacific gray whales (1960s–1990s), but data on contemporary levels and across life-history parameters were lacking^{26–30} and are now even more relevant due to the ongoing 2019–2022 Unusual Mortality Event along the west coast of North America.³¹ The first objective of this study was to provide a large and comprehensive assessment of contemporary POP concentrations in gray whale blubber between 2003 and 2017 (post Stockholm Convention POP ban), across a wide range of their coastal migration in Mexico, USA, and Canada. This study was a collaborative effort among universities, nonprofit entities, and governments representing Mexico, USA, Canada, and the Makah Tribe. Additional objectives were to elucidate variations of POP concentrations across sex, age, and reproductive

parameters to inform interpretation and monitoring of contaminants in this protected species. Maternal offloading was expected to be a key factor influencing patterns of lipophilic POP concentrations in gray whales, particularly since their milk has one of the highest percent fat content (~53%) reported in cetaceans.³² This study is noteworthy in its scope with a substantial sample size (120 biopsies) for a marine mammal study including a rare collection of repetitively sampled individuals and the first analysis of POPs in gray whale mothers and calves (including matched pairs), allowing for direct assessment of maternal offloading. Additionally, this study reports the most extensive POP panel, including the first report of PBDEs and select HCHs, in this species.

MATERIALS AND METHODS

Sample Collection. Blubber biopsies ($n = 120$) were collected from free-ranging gray whales in 2003 ($n = 2$), 2010–2012 ($n = 51$), and 2015–2017 ($n = 67$) along the eastern North Pacific gray whale range in Mexico (San Ignacio Lagoon breeding ground), USA, and Canada (Figure 1 and Table S1). Skin and blubber were collected from the dorsal region using sterilized biopsy darts (specific methods varied across collectors). Samples were stored below -20 °C (short-term)

or $-80\text{ }^{\circ}\text{C}$ (long-term) until analyzed. Biopsies were collected from mothers ($n = 23$) and calves ($n = 32$) including 19 paired samples of related mothers and calves (one pair sampled twice). Related pairs were sampled only while isolated from other individuals to ensure familial relation.

Whale Identification. All individuals were identified photographically³³ using collaborative ID catalogs (UABCS, Cascadia Research Collective, the Makah Tribe, and VE Enterprises) and/or genetic analyses (SWFSC) (Table S1). Calves were classified by small size and close association with their mother. Juveniles were classified by known birth year or estimated body size as individuals who had weaned from mothers but expected to be reproductively immature (less than 6 years old).³⁴ “Immatures” refer to both calves and juveniles. Females were classified as “mothers” if observed with a calf at the time of sampling. SWFSC samples were sexed by amplification of the zinc finger (ZFX and ZFY) genes.³⁵ UABCS samples were sexed at Purdue University by analyzing two sex-linked (ZFY_288 and ZFY_342) single-nucleotide polymorphisms.³⁶ Ten repetitively sampled individuals were selected from tissue archives for longitudinal assessment of contaminant concentrations.

Chemical Analysis. Blubber samples were analyzed for POPs as previously described.³⁷ Briefly, samples (0.2–0.7 g) were mixed with drying agents (sodium sulfate and magnesium sulfate) and then extracted with dichloromethane using accelerated solvent extraction. Extraneous polar and biogenic compounds (i.e., neutral lipids) were removed using gravity-flow silica/alumina columns followed by high-performance size-exclusion liquid chromatography. Concentrated sample extracts (2 μL) were analyzed on a GC/MS system equipped with a 60 m DB-5 GC capillary column. Analyte levels were quantified using up to 10 multilevel GC/MS calibration standards of known concentrations. Total percent lipid was determined gravimetrically.

The POPs reported included hexachlorobenzene (HCB), mirex, endosulfan I; α -, β -, and γ -HCH ($\sum\text{HCHs}$); *cis*-chlordane (α -Chlor), *cis*-nonachlor (*c*-Nona), heptachlor epoxide (HPE), nonachlor III, oxychlordane (oxychlor), *trans*-nonachlor (*t*-Nona) ($\sum\text{CHLs}$); *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, and *p,p'*-DDT ($\sum\text{DDTs}$); 45 PCB congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 205, 206, 208, and 209 ($\sum\text{PCBs}$); and PBDE congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183 ($\sum\text{PBDEs}$).

Quality Assurance. Performance-based quality assurance (QA) guidelines were followed for measuring POPs and percent lipid.³⁸ Quality assurance samples including a solvent (dichloromethane) method blank and a National Institute of Standards and Technology Standard Reference Material (SRM 1945, Organics in Whale Blubber) were analyzed with each set of samples. Surrogate standard (PCB 103) recoveries for field and quality assurance samples (67–119%) met the QA program criteria (60–130%), as did all other program measures. The lower limits of quantitation (LOQ) of individual analytes, calculated based on sample mass and the analyte areas in the lowest-level calibration standard, ranged from <0.38 to <2.5 ng/g, wet weight.

Data Analysis. All contaminant data are reported as lipid-normalized unless otherwise noted. Arithmetic means (here on out referred to as means) were calculated to allow for

comparisons with similarly reported previous gray whale and other cetacean studies. These means included all compounds and values < LOQ were replaced with zero (Tables 1 and 2).

Statistical analysis (using Stata MP version 17.0) of POPs across life-history parameters included concentration data for the 19 most frequently detected compounds in this dataset (see Table S2 for % detection >LOQ), including 9 PCB congeners (52, 95, 99, 101, 118, 138, 149, 153, and 187), two DDTs (*p,p'*-DDD and *p,p'*-DDE), five CHLs (α -Chlor, *c*-Nona, HPE, oxychlor, *t*-Nona), and three HCHs (HCB, α -HCH, and β -HCH). Analytes with $\geq 65\%$ of their values < LOQ were excluded from the analysis. Concentration data were log-transformed and values < $\log(\text{LOQ})$ were imputed as follows. When a sample- and compound-specific LOQ was below the 10th percentile of all observed measurements above LOQ for that analyte, the value < $\log(\text{LOQ})$ was replaced with the $\log(\text{LOQ})/(\text{square root of } 2)$. When a sample- and compound-specific LOQ was above the 10th percentile of all observed measurements above LOQ for that analyte, the value < $\log(\text{LOQ})$ was imputed using multiple imputation with chained equations (MICE)³⁹ to reflect its broader range of plausible values. Imputations of contaminant log-concentrations using MICE were based on the following predictor variables: log-transformed concentrations of the 19 included compounds, reproductive status, sex, adulthood, date, locality (state, country), sample weight, lipid percent, and internal standard (PCB 103) percent recovery. Any imputed value that exceeded the sample-specific log-transformed LOQ was replaced by that $\log(\text{LOQ})$. This process yielded 70 imputed datasets that were the basis for statistical analysis of the 19 log-transformed compound concentrations using multiply imputed linear regression models.⁴⁰ Regression coefficients and robust 95% confidence intervals were then exponentiated to estimate geometric mean ratios (GMR) of contaminant concentrations across life-history parameters (Table 3). Robust standard errors were used since some observations (i.e., repeated observations, mother/calf pairs) were dependent.^{41,42} A multiple imputation postestimation 1-degree-of-freedom F-test was used to evaluate whether the GMR was significantly different from 1,^{43–45} to identify associations that were Bonferroni-significant at $\alpha_{\text{Bonferroni}} = 0.0003$.⁴⁶

RESULTS AND DISCUSSION

Overall Results and Historical Perspective. Overall mean POP concentrations ranked as follows: $\sum\text{DDTs} > \sum\text{PCBs} > \sum\text{CHLs} > \sum\text{HCHs} > \sum\text{PBDEs}$ (Table 1). The highest analyte concentrations detected were 2430 ng/g for *p,p'*-DDE, and from 250 to 370 ng/g for HCB, PCB 153, *t*-Nona, and β -HCH. PBDE 85, PBDE 183, and endosulfan I were not detected above LOQ. The most prevalent compound classes were CHLs and HCHs which were detected above LOQ in 98% of samples, followed by DDTs (93%), PCBs (83%), and PBDEs (46%).

POP concentrations were generally lower than previous gray whale reports (1968–1999)^{26–30} though this is the first report of PBDEs and select HCHs in this species (Table 2). The observed decline of some POP classes could indicate decreased exposure due to the 2001 Stockholm Convention on Persistent Organic Pollutants and other regulatory efforts. Decreased levels of $\sum\text{DDTs}$, $\sum\text{PCBs}$, $\sum\text{HCHs}$, and $\sum\text{PBDEs}$ have been observed in Arctic marine biota⁵⁴ including marine mammals.^{50,55,56} However, $\sum\text{PBDEs}$ also were reported to have increased in Arctic marine animals,⁵⁷ including belugas

Table 1. POP Concentrations (Mean (ng/g Lipid Weight) \pm Standard Error) and Percent Lipid in Blubber Biopsies According to Age Group and Sex of Gray Whales Collected between 2003 and 2017 along the West Coast of North America^{a,b,c}

age group	sex	n	% lipid	\sum PCBs	\sum DDTs	\sum CHLs	\sum HCHs	\sum PBDEs
overall		120	23 \pm 1	190 \pm 20 (100) [120 (ND-1940)]	220 \pm 30 (112) [60 (ND-2630)]	90 \pm 7.0 (118) [60 (ND-460)]	70 \pm 5.0 (118) [60 (ND-270)]	30 \pm 5.0 (55) [ND (ND-350)]
adult	M	30	24 \pm 2	370 \pm 70 [290 (30-1940)]	390 \pm 90 [190 (10-2630)]	170 \pm 20 [170 (30-460)]	130 \pm 10 [110 (30-270)]	50 \pm 14 (20) [20 (ND-350)]
	F	52	22 \pm 2	170 \pm 30 (43) [120 (ND-970)]	220 \pm 50 (44) [50 (ND-1660)]	70 \pm 7.0 (50) [50 (ND-220)]	50 \pm 4.0 (50) [40 (ND-120)]	40 \pm 7.0 (27) [7.0 (ND-150)]
juvenile	U	3	25 \pm 9	260 \pm 90 [340 (80-360)]	400 \pm 190 [570 (20-610)]	70 \pm 30 [90 (10-100)]	60 \pm 10 [70 (40-80)]	120 \pm 60 (2) [170 (ND-180)]
	M	2	32 \pm 7	60 \pm 1.0 [60 (60-60)]	50 \pm 20 [50 (30-60)]	50 \pm 10 [50 (40-60)]	70 \pm 10 [70 (60-80)]	8.0 \pm 8.0 ^d (1) [8.0 (ND-20)]
	F	1	37	240 ^d	270 ^d	80 ^d	110 ^d	70 ^d
calf	M	13	20 \pm 3	20 \pm 6.0 (7) [20 (ND-60)]	20 \pm 2.0 [20 (10-30)]	30 \pm 3.0 [40 (20-50)]	50 \pm 4.0 [40 (30-80)]	ND
	F	19	24 \pm 2	60 \pm 20 (14) [40 (ND-230)]	50 \pm 20 [20 (10-310)]	50 \pm 7.0 [40 (20-160)]	60 \pm 8.0 [50 (30-190)]	10 \pm 6.0 (4) [0.0 (ND-110)]

^aNumber of values detected above LOQ, if different from n, are listed in parentheses after mean. Median (minimum–maximum) concentrations listed in brackets. ^bND = not detected above LOQ. ^cM = male, F = female, U = unknown sex. ^dReported as observed concentration ng/g (n = 1).

(*Delphinapterus leucas*),⁵⁵ suggesting this more recently regulated POP class may still bioaccumulate until more degradation or sequestration occurs. The extensive decline (120–300x lower) of \sum PCBs, \sum DDTs, \sum CHLs, and HCB observed in this study compared to the highest reported concentrations in gray whales from 1988 to 1991²⁹ likely reflects decreased exposure as well as varying analytical/quantification methodologies, sampling design, tissue qualities, and animal conditions. Previous gray whale studies used GC/ECD^{26–30} or HPLC/PDA²⁶ for POP quantification as opposed to the contemporary GC/MS methodology presented here. While this study also included more analytes, individual congener concentrations were low and thus unlikely to have masked the extent of the observed decline. Most notably, only one prior gray whale POP study included biopsied samples (n = 38)²⁶ and moreover from individuals of unreported sex and age groups, while the others included samples from stranded (n = 47)^{26,27,29} and harvested (n = 40)^{28,30} whales. Blubber composition can be influenced significantly by degradation or leaching of lipids before or during necropsy and varies across blubber depths sampled.⁵⁸ Additionally, utilization of lipids for energy when a whale is sick, injured, or otherwise stressed can lead to contaminants concentrating in remaining blubber or mobilizing into the circulatory system. Indeed, lower mean lipid content and higher \sum PCBs and \sum DDTs blubber concentrations have been detected in stranded versus hunted gray whales, with differences attributed to greater lipid mobilization and associated concentrated contaminants in the remaining blubber, as well as lipid leaching in decomposing stranded whales.²⁸ Importantly, the gray whale study²⁹ (1988–1991) reporting the highest detected mean POP concentrations did feature animals with very low lipid levels (<1–16%) including some that decomposed on the beach for up to a month before sample collection. Additional differences such as sampling timing and locations across migration (and feeding and fasting periods) could also have influenced lipid content and associated POP levels across gray whale studies.^{28,29} In the previous gray whale studies, around 70% of samples (and all biopsies) were collected in Washington, with the rest from Alaska, California, and the Bering Sea, while the current study sampled between Baja California, Mexico, and Southern British Columbia, Canada (Figure 1). Assessing geographic and seasonal influence on lipid and POP concentrations, while beyond the scope of this paper due to limited sample sizes of adults across location and timing of migration, would provide valuable information for gray whale biology. However, the overall observed trends of contemporary POP concentrations were consistently lower than previous reports regardless of whether calculated on a wet weight or lipid weight basis.

Interspecies Comparisons and Implications. Gray whales, known for their unique diet of benthic invertebrates,^{59,60} had mean POP concentrations within the range reported for other Northern Hemisphere baleen whales during similar timeframes (Table 2). This range reflects overall low trophic levels and varying feeding strategies and geographical areas. Gray whale POP concentrations were substantially lower than in toothed whales which are susceptible to higher trophic level contaminant biomagnification.^{49,61–64} Interpretations of relatively low contemporary gray whale POP concentrations remain challenging due to significant data gaps concerning cetacean organochlorine metabolism, toxic effects thresholds, and health impacts.^{61–65} Toxic health effects thresholds for PCBs have been developed for marine mammals (using seals,

Table 2. POP Concentrations (Mean ± Standard Error, Unless Otherwise Noted) and Percent Lipid in Blubber of Gray Whales across Time and in Other Baleen Whale Species between the Same Sampling Period and in the Northern Hemisphere^{a,b,f}

year (ocean)	n	% Lipid	∑PCBs	∑DDTs	∑CHLs (<i>Eschrichtius robustus</i>)	∑HCHs	∑PBDEs	HCB	Reference
2003–2017 (N. Pacific) ¹	120	23 ± 1	190 ± 20 {50 ± 6}	220 ± 30 {60 ± 10}	90 ± 10 {20 ± 3}	70 ± 5 {20 ± 2}	30 ± 5 {20 ± 1}	100 ± 7 {25 ± 3}	this study
1999 (N. Pacific) ²	24	12 ± 3	8200 ± 1400	3100 ± 510				4600 ± 730	26
1996–1998 (N. Pacific) ¹	38	10 ± 1	2100 ± 190	1200 ± 140				600 ± 40	
1994 (N. Pacific) ³	17	48 ± 5	1400 ± 130	330 ± 50	320 ± 40			530 ± 80	26, 28
1988–1991 (N. Pacific) ²	22	9 ± 4	56,000 ± 26,000	26,000 ± 15,000	22,000 ± 14,000			21,000 ± 14,000	26, 29
1976 (N. Pacific) ²	1		{230} ^c	{470} ^c					27
1968–1969 (N. Pacific) ³	23		ND	{70}					30
2017 (Indian) ¹	87	42	2.0 ± 3.0 ^d	8.0 ± 4.0 ^d	Humpback Whale (<i>Megaptera novaeangliae</i>) 4.0 ± 3.0 ^d	0.3 ± <0.1 ^d	0.4 ± 0.1 ^d	70 ± 20 ^d	47
2014–2015 (S. Pacific) ¹	59	40	2.0 ± 1.0 ^d	20 ± 20 ^d	4.0 ± 3.0 ^d	0.6 ± 0.7 ^d	0.4 ± 0.1 ^d	40 ± 20 ^d	
2010–2011 (Indian) ¹	25	37	3.0	10	8.0	3.0	2.0	30	48
2005–2006 (N. Atlantic) ¹	20	30	7700 ^c	3500 ^e	1500 ^e	35 ^e	890 ^e		49
2003–2004 (N. Pacific) ¹	67	23	340 ^e	930 ^e	230 ^e	230 ^e	90 ^e		
2006–2015 (N. Pacific) ³	71	73	{70} ^e	{50} ^e	Bowhead Whale (<i>Balaena mysticetus</i>) {70} ^e	{90} ^e	{2.0} ^e	{60} ^e	50
2014–2018 (Arctic) ¹	18	47 ± 11 ^d	150 ± 70 ^d	240 ± 170 ^d	Blue Whale (<i>Balaenoptera musculus</i>) 100 ± 50 ^d	10 ± 5 ^d	8.0 ± 5 ^d	50 ± 20 ^d	51
2011, 2013 (N. Pacific) ¹²	38		150	50			8.0	20	52
2014–2018 (Arctic) ¹	12	44 ± 10 ^d	290 ± 140 ^d	380 ± 200 ^d	Fin Whale (<i>Balaenoptera physalus</i>) 150 ± 80 ^d	20 ± 10 ^d	30 ± 20 ^d	70 ± 30 ^d	51
2006 (N. Pacific) ³	27	38	2000	2600	Minke Whale (<i>Balaenoptera acutorostrata</i>) 290	530	160	120	53

^aConcentrations reported as ng/g lipid weight or {wet weight}. ^bCollection type: ¹biopsied, ²stranded, ³harvested. ^cReported as observed concentration ng/g (*n* = 1). ^dStandard deviation. ^eGeometric mean. ^fND = not detected above LOQ in any samples.

Table 3. Geometric Mean Ratios (and 95% CI) of POP Concentrations in Blubber Biopsies of Gray Whales across Biological Variables Including Sex, Age, and Reproductive Parameters as well as Calving Season (January–April Assumes Continuous Trend between Months)^{a,d}

	adult M vs F <i>n</i> = 82	immature M vs F <i>n</i> = 35	M adult vs immature <i>n</i> = 45	F adult vs immature <i>n</i> = 72	mothers vs resting F <i>n</i> = 52	mothers vs calves <i>n</i> = 55	January–April mothers <i>n</i> = 21	January–April calves <i>n</i> = 29
PCB52	1.99 ^b (1.49–2.65)	0.79 (0.58–1.09)	3.85 ^c (2.89–5.13)	1.54 ^b (1.12–2.11)	0.54 ^c (0.37–0.79)	1.24 (0.88–1.75)	1.24 (0.85–1.80)	1.20 (0.95–1.52)
PCB95	1.96 ^b (1.45–2.66)	0.79 (0.55–1.12)	3.91 ^c (2.86–5.35)	1.57 ^b (1.11–2.21)	0.58 ^b (0.37–0.89)	1.31 (0.88–1.96)	1.31 (0.85–2.04)	1.15 (0.90–1.48)
PCB99	2.35 ^b (1.65–3.35)	0.72 (0.48–1.08)	6.13 ^c (4.37–8.62)	1.87 ^b (1.23–2.82)	0.40 ^c (0.26–0.63)	1.36 (0.92–2.00)	1.26 (0.83–1.90)	1.24 (0.91–1.69)
PCB101	1.85 ^b (1.41–2.43)	0.74 (0.54–1.03)	4.31 ^c (3.27–5.68)	1.73 ^b (1.26–2.37)	0.53 ^c (0.37–0.77)	1.43 ^b (1.03–1.99)	1.22 (0.84–1.76)	1.18 (0.94–1.48)
PCB118	2.00 ^b (1.43–2.81)	0.72 (0.48–1.06)	5.53 ^c (3.96–7.71)	1.98 ^b (1.34–2.94)	0.39 ^c (0.26–0.60)	1.42 (0.96–2.08)	1.26 (0.84–1.89)	1.23 (0.92–1.64)
PCB138	2.09 ^b (1.47–2.98)	0.69 (0.46–1.05)	6.60 ^c (4.71–9.26)	2.20 ^b (1.45–3.33)	0.36 ^c (0.23–0.54)	1.53 ^b (1.06–2.21)	1.20 (0.85–1.70)	1.22 (0.93–1.59)
PCB149	2.30 ^b (1.63–3.23)	0.72 (0.48–1.07)	6.39 ^c (4.51–9.07)	2.00 ^b (1.36–2.93)	0.44 ^c (0.29–0.67)	1.51 ^b (1.05–2.19)	1.23 (0.83–1.80)	1.21 (0.91–1.60)
PCB153	2.22 ^b (1.54–3.20)	0.68 (0.45–1.04)	7.38 ^c (5.17–10.55)	2.27 ^b (1.49–3.45)	0.34 ^c (0.22–0.52)	1.55 ^b (1.07–2.24)	1.17 (0.84–1.65)	1.22 (0.92–1.62)
PCB187	1.95 ^b (1.29–2.96)	0.68 (0.42–1.10)	7.49 ^b (4.80–11.71)	2.60 ^b (1.66–4.09)	0.40 ^c (0.24–0.66)	1.93 ^b (1.21–3.09)	1.20 (0.75–1.92)	1.20 (0.88–1.63)
<i>p,p'</i> -DDD	2.03 ^b (1.39–2.98)	0.73 (0.50–1.08)	4.92 ^c (3.53–6.86)	1.77 ^b (1.16–2.70)	0.34 ^c (0.21–0.55)	1.18 (0.80–1.73)	1.21 (0.81–1.80)	1.12 (0.88–1.43)
<i>p,p'</i> -DDE	2.87 ^b (1.61–5.12)	0.62 (0.36–1.05)	11.72 ^c (7.35–18.68)	2.51 ^b (1.35–4.70)	0.12 ^c (0.07–0.23)	1.07 (0.70–1.65)	1.10 (0.79–1.53)	1.18 (0.90–1.55)
α -CHLOR	1.08 (0.73–1.59)	0.91 (0.62–1.35)	1.06 (0.71–1.59)	0.90 (0.62–1.30)	2.33 ^b (1.38–3.93)	1.47 (0.97–2.23)	1.14 (0.73–1.80)	1.08 (0.80–1.44)
<i>c</i> -Nona	2.15 ^b (1.60–2.88)	0.81 (0.58–1.13)	3.74 ^c (2.72–5.13)	1.42 ^b (1.04–1.92)	0.58 ^c (0.40–0.84)	1.16 (0.86–1.57)	1.15 (0.83–1.59)	1.16 (0.91–1.48)
HPE	1.73 ^b (1.36–2.21)	0.87 (0.68–1.11)	1.73 ^b (1.32–2.26)	0.87 (0.70–1.08)	0.84 (0.63–1.11)	0.86 (0.67–1.11)	1.04 (0.79–1.37)	1.09 (0.92–1.30)
oxychlor	3.11 ^b (2.13–4.54)	0.73 (0.49–1.09)	5.17 ^c (3.56–7.51)	1.21 (0.81–1.81)	0.40 ^c (0.25–0.64)	0.85 (0.57–1.28)	1.09 (0.75–1.58)	1.16 (0.82–1.65)
<i>t</i> -Nona	2.40 ^c (1.79–3.23)	0.85 (0.64–1.12)	3.88 ^c (2.87–5.25)	1.37 ^b (1.04–1.80)	0.67 ^b (0.49–0.93)	1.19 (0.93–1.53)	1.13 (0.85–1.50)	1.20 (0.97–1.48)
HCB	1.49 (0.98–2.26)	1.10 (0.73–1.68)	0.92 (0.58–1.47)	0.68 ^b (0.48–0.98)	1.35 (1.00–1.80)	0.74 ^b (0.57–0.97)	0.93 (0.78–1.10)	1.19 (0.98–1.44)
α -HCH	0.92 (0.64–1.34)	1.03 (0.78–1.36)	0.65 ^b (0.44–0.95)	0.72 ^b (0.55–0.94)	1.34 (0.93–1.91)	0.84 (0.59–1.19)	0.97 (0.68–1.38)	1.06 (0.89–1.26)
β -HCH	2.48 ^c (1.91–3.22)	0.84 (0.63–1.10)	2.57 ^c (1.96–3.35)	0.86 (0.66–1.12)	0.57 ^c (0.43–0.74)	0.71 ^b (0.56–0.89)	0.98 (0.81–1.19)	1.18 (0.93–1.49)

^aGray whales sampled between 2003 and 2017 along the west coast of North America. All related and nonrelated mothers and calves were included. Resting female refers to adult female without a calf and expected to be not pregnant. Immature refers to calves and juveniles. ^bNominal significance corresponds to CI excluding 1 ($p < 0.05$). ^cBonferroni significant based on 1-degree-of-freedom *F* test for $(e^b) - 1 = 0$ ($p < 0.0003$). ^dM = male, F = female.

river otters, and mink) based on effects related to immune system suppression, hepatic function, and endocrine system alterations and a variety of biomarkers including (though not exhaustively) retinol and the thyroid and aryl hydrocarbon receptors.^{66–68} In the current study, mean \sum PCB concentrations (190 ng/g) were well below three frequently referenced toxic health effects thresholds for marine mammal blubber including 17,000 ng \sum PCBs/g,⁶⁶ and more recently, 9000 ng \sum PCBs/g,⁶⁸ and 1300 ng \sum PCBs/g.⁶⁷ Only one whale (adult male) in the current study had \sum PCB concentrations (1940 ng/g) between the two lower thresholds. However, these three thresholds are PCB-specific and would likely be lower if additive or synergistic effects of contaminant

mixtures were factored into realistic exposure scenarios.⁷ Additionally, some individuals may be more susceptible to effects such as those in sensitive phases of development (the very young and old) or facing additional stressors such as reproduction, fasting, migration, or illness/injury.^{69,70} More research is needed toward defining species-specific toxicological risks and thresholds for a wider range of compounds, chemical mixtures, and across biological parameters.

Sex and Age Influence on POP Concentrations. All sex- and age-based patterns of the 19 modeled compounds are reported as ratios of geometric mean concentrations (Table 3). Males comprised 37% of adults and 43% of immatures. Geometric mean concentrations were significantly greater (up

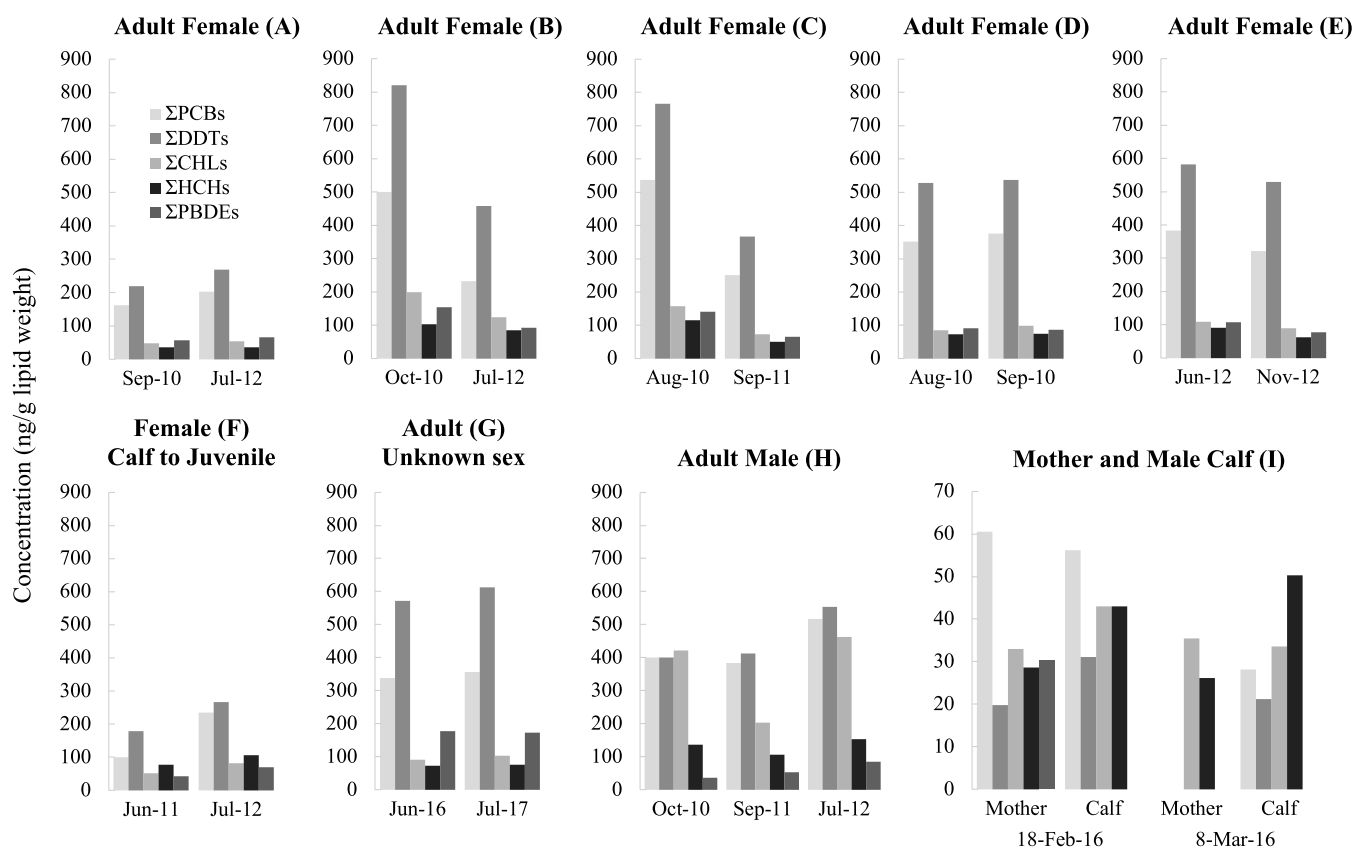


Figure 2. Temporal patterns of Σ POP class concentrations (ng/g lipid weight) in blubber of individual gray whales repetitively sampled across months and/or years. Note differing scale of y-axis in panel (I). Values below LOQ were imputed as zero.

to 3-fold) in adult males compared to adult females for 17 of the modeled compounds. A previous gray whale study also found adult males ($n = 2$) had generally higher concentrations of Σ PCBs, Σ DDTs, and HCB than adult females ($n = 6$).²⁶ In another gray whale study, adult males ($n = 6$) had generally higher concentrations of Σ DDTs than adult females ($n = 2$) but no distinct differences were observed across sexes for Σ PCB, Σ CHLs, or HCB.²⁹ The sex-specific POP patterns observed in adult gray whales are consistent with established accumulation patterns in cetaceans.^{15,24,71} Geometric mean POP concentrations in immatures (32 calves and 3 juveniles) did not differ significantly between sexes, suggesting similar POP accumulation before reaching reproductive maturity, as previously reported for juvenile gray whales²⁶ and other cetaceans.^{16,18,19,24,72}

Geometric mean POP concentrations were significantly higher in adults compared to immatures for both males (17 compounds, up to 12-fold) and females (13 compounds, up to 3-fold), as commonly reported in cetacean literature.^{19,24,72,73} Concentrations of *p,p'*-DDE (a DDT metabolite) and higher chlorinated PCB compounds had the greatest fold difference between adults and immatures of both sexes. In addition to bioaccumulation with age, this pattern could reflect greater metabolism capacity in adults and/or past exposure events experienced only by older individuals.^{19,21} In contrast, geometric mean POP concentrations of select CHLs and HCHs were similar or lesser (down to 0.7 \times) in adults versus immatures in both sexes. A previous gray whale study found no significant age-specific patterns for either sex though featured a small sample size.²⁶ While less common, this pattern of decreasing POP concentrations with age in males had been

reported previously, for CHLs and HCHs among other classes, in cetaceans and attributed to growth dilution, metabolism, and/or decreased environmental POP levels.^{25,74}

One adult male (H) was sampled across three consecutive years in Northern California (Figure 2). Σ PCB, Σ DDTs, Σ CHLs, Σ HCHs, Σ PBDEs concentrations were higher in 2012 than in 2010, exhibiting overall POP accumulation. However, Σ PCB, Σ CHLs, and Σ HCHs concentrations were lowest in 2011. This observed POP trend was mirrored by the corresponding biopsy blubber percent lipid values (48, 34, and 54%) of this male across the three years. Overall, this suggests inter-annual fluctuations possibly due to varying energetic stressors (fasting, migration, injury, disease, etc.) and associated mobilization and/or reaccumulation of lipids and lipophilic contaminants.^{12,75}

One female (F) sampled across two life stages (calf, 18% lipid, and juvenile, 37% lipid) exhibited up to a 2-fold increase of Σ POP class concentrations and percent lipid (Figure 2). The number of analytes detected above LOQ also increased from calf (16 compounds) to juvenile (41 compounds, see Tables S4 and S5 for congener-specific concentrations). The observed trend supports POP accumulation across immature age groups in females and reflects the broader range of compounds acquired while feeding on prey versus nursing as a calf.

The age- and sex-related POP patterns observed in this study indicate significant compound-specific maternal contaminant offloading, as further described below.^{18,19,21,24,76–79}

Maternal Offloading. All reproduction-based patterns of the 19 modeled compounds are reported as ratios of geometric mean concentrations (Table 3). Cetacean mothers expend

substantial lipid reserves during reproduction,^{80,81} resulting in associated lipophilic contaminant mobilization into the Adult Female (E) bloodstream and subsequent placental or lactational transfer to calves.^{17,82} Geometric mean POP concentrations were statistically similar in mothers and calves or higher in calves for 15 modeled compounds. This confirms significant maternal contaminant offloading as all calves were sampled during nursing season and expected to be feeding exclusively on milk. Cetacean literature indicates that during gestation and lactation contaminants with a $\log K_{ow}$ (octanol-water partition coefficient) below 6.5 tend to readily transfer to calves while those with a higher $\log K_{ow}$ are preferentially retained in the mother.^{17,22,83} This is consistent with reports that low-molecular-weight and less lipid-soluble compounds were more readily transferred.^{18,84–86} Indeed, in the current study, concentrations of PCB52, PCB95, PCB99, DDD, α -CHLOR, *c*-Nona, HPE, oxychlor, HCB, α -HCH, and β -HCH ($\log K_{ow} < 6.5$) and PCBs 138, 149, 153, and 187 ($\log K_{ow} > 6.5$) followed expected partitioning. However, PCB 101 ($\log K_{ow} < 6.5$) and PCB118, DDE, and *t*-nona ($\log K_{ow} > 6.5$) did not, suggesting additional chemical properties and processes (i.e., biotransformation) influence transfer. Maternal offloading exposes calves to contaminants during sensitive phases of development while the associated mobilization of contaminants can also result in redistribution to sensitive organs in the mother.^{75,87} Both could increase risks for endocrine system disruption, reproductive failure, immunosuppression, or other toxicity.^{4–10}

Geometric mean POP concentrations in mothers and calves did not appear to change significantly across the early nursing season (January–April, assuming a continuous trend). This was unexpected as offloading rates in cetaceans have been reported to be highest at the beginning of the several month³⁴ nursing period.^{18,24} However, it is possible that staggered birth dates of the sampled calves obscured the accumulation trend. Gray whale calves are born between December and February with a mean calving date between January 10 and 27.^{34,88,89} While exact birth dates are challenging (near impossible) to obtain, mean sampling dates of mothers and calves during the 4-month period included in the model were February 25 and March 3, respectively. A few calf samples collected outside the 4-month period were excluded from this model to avoid confounding outliers. Additional samples across a larger range of the nursing period would be needed to characterize maternal offloading rates. The observed steady POP trends across the nursing season in calves could also be the result of growth dilution as total lipids increase rapidly, which has been reported in other cetaceans.^{22,25,74,90}

A mother and calf pair were sampled twice across the 2016 nursing season, on February 18 and March 8. Concentrations of overall \sum POPs decreased in both individuals across the 19 days but trends differed across compound classes (Figure 2, I; see Table S4 for congener-specific concentrations). Concentrations in the mother decreased by 100% for \sum PCBs, \sum DDTs, and \sum PBDEs, and 8% for \sum HCHs, but increased by 7% for \sum CHLs. The absolute decline of \sum PCBs, \sum DDTs, and \sum PBDEs is unlikely to be due exclusively to offloading or other lipid mobilization (maternal lipid percent decreased from 18 to 6%) in such a short period. This could alternatively be explained by a shallow biopsy (resulting in a greater proportion of connective tissue) or lipid leaching. Concentrations in the calf decreased by 50, 32, and 22% for \sum PCBs, \sum DDTs, and \sum CHLs, respectively, while \sum HCHs increased by 14%.

PBDEs were never detected above LOQ in the calf. The calf's lipid levels also decreased (30–26%) which could indicate a decline in nutritional status and subsequent lipid utilization.

Geometric mean concentrations of 15 modeled compounds were statistically lower in mothers compared to reproductively resting females. This pattern substantiates POP offloading as well as reaccumulation of POPs between reproductive events in gray whales. Interestingly, geometric mean concentrations of HPE, HCB, α -HCH, and α -CHLOR, were either similar across reproductive statuses or higher in mothers. Overall, POP concentration differences between mothers and resting females were not explained fully by $\log K_{ow}$ or molecular weights. The timing of sampling across the duration of reproductive or resting periods, undetected or unsuccessful reproductive events, and calves' birth order (firstborn offspring generally receiving greatest offload)^{16,18,21,24,71,82} could have contributed to these patterns but were unknown, limiting further interpretation.

Repetitively sampled adult females described longitudinal POP patterns, though interpretation remains speculative as their reproductive histories were unknown (Figure 2). Female A had consistently low \sum POP concentrations at years 1 and 3, possibly indicating calving prior to each sampling. This presumes reproduction at the 2-year minimum interval for gray whales⁹¹ and sampling around weaning, hence after significant offloading. \sum POP concentrations in Females B and C decreased across years, suggesting reproduction between sampling events. \sum POP concentrations were expectedly stable across one month in Female D and decreased slightly across six months in Female E (possibly indicating pregnancy).

Geometric mean ratios of POP concentrations across sex, age, and reproductive parameters were further analyzed by controlling for years across the 15-year sampling period (Table S3). Overall patterns remained the same for 90–100% of modeled compounds, except when comparing mature versus immature females and mothers versus resting females, where the influence of maturity and reproductive status became less apparent. However, controlling by year was likely limited by data sparsity and therefore relied more heavily on model extrapolation. Accordingly, and since 98% of samples were collected within 7 years (2010–2017), analyses controlled by year were not further pursued.

Implications and Future Directions. This study fills a 20-year data gap in gray whale blubber contaminant monitoring, providing key information for population health assessment of this protected species. This update is particularly relevant considering the ongoing 2019–2022 gray whale UME along the west coast of North America and the associated need for contemporary pre-UME contaminant data. These results add to the limited published data on chemical contaminants in gray whales including the first featuring blubber biopsy samples from individuals of known sex and age groups and from mothers and calves. \sum DDTs, \sum PCBs, and \sum CHLs concentrations were lower in these contemporary gray whales than in previous reports for this population, possibly due to regulatory efforts, while PBDEs and select HCHs were measured for the first time. Continued monitoring of legacy and novel contaminants in gray whales is needed as climate change and increased human activities could affect contaminant transport and exposure and as toxicity risk in cetaceans is poorly understood, particularly for individuals in sensitive life stages or experiencing additional stressors. Maternal offloading,

described for the first time in gray whales, was highlighted as the underlying biological basis for significant intraspecific POP patterns across life-history parameters. These data substantiate the importance of considering life history for the design and interpretation of individual and population-wide health monitoring and contaminant risk assessment of this protected species.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.2c05998>.

Sample IDs and summed POP concentrations; percent detection (>LOQ) of compounds included in statistical analyses; geometric mean ratios of POP concentrations controlled by year; POP concentrations in repetitively sampled Female F and Mother (I) and Calf (I); and PCB and PBDE congener-specific concentrations (PDF)

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Notes

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■ ACKNOWLEDGMENTS

This work was conducted under the National Marine Fisheries Service (NMFS) Office of Protected Resources' Marine Mammal Health and Stranding Response Program (MMHSRP) permits 932-1905-MA-009526 and 18786, with assistance from Teri Rowles (NMFS, Silver Spring, MD); Mexico-SGPA/DGVS/00283/16, CITES-17US082589/9, CITES-MX89451; and SWFSC-affiliated researchers' permits. The Northwest Fisheries Science Center, NMFS West Coast Regional Office, American Cetacean Society (San Francisco Chapter), Emory SCORE (U54AG062334), and Texas Tech University provided funding. Many thanks to all sample contributors and laboratory personnel: Laguna San Ignacio Ecosystem Science Program, Ocean Foundation, Makah Tribe, Humboldt State University Vertebrate Museum and Marine Mammal Stranding Program, NMFS-MMHSRP, Dr. Kelly Robertson (SWFSC), Dr. Jim Dines (Natural History Museum—Los Angeles), Dr. John Ford and Linda Nichol (Fisheries and Oceans Canada), and Dar Boyd, Jonelle Gates, and Dr. Irv Shultz (NWFSC).

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