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Jing Si	Date

Early-life exposure to persistent organic pollutants and health outcomes in common bottlenose dolphin (*Tursiops truncatus*) calves: a comparison of weighted exposure indices

By

Jing Si

Master of Public Health

Department of Environmental Health

Matthew Gribble, Ph.D. D.A.B.T.
Committee Chair

Melissa Smarr, Ph.D.
Committee Chair

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Bachelor of Medicine
Fudan University
2017

Thesis Committee Chair: Matthew Gribble, Ph.D. D.A.B.T.

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

Early-life exposure to persistent organic pollutants and health outcomes in common bottlenose dolphin (*Tursiops truncatus*) calves: a comparison of weighted exposure indices

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Objective: The aim of this study is to evaluate the association of maternal exposure to POPs with five health outcomes (calf length, calf weight, and three thyroid hormones) in bottlenose dolphin calves, comparing results from regression models using each of these methods of weighting POP exposures: simple sums, principal component analysis (PCA), and weighted quantile sum (WQS).

Method: Data for this study were from a research initiated in 1970 in Sarasota Bay, Florida by the Sarasota Dolphin Research Program. Complete exposure data including 69 different POPs in dolphin blubber from four moms and 32 calves, including 53 polychlorinated biphenyls (PCBs) congeners, five polybrominated diphenyl ethers (PBDEs) congeners, four organochlorine pesticides (OCPs) and five Dichlorodiphenyltrichloroethane (DDT) compounds. Health outcomes of interest are calf length, calf weight and three thyroid hormones of calves including triiodothyronine (T3), thyroxine (T4), and free T4 (fT4). We performed generalized linear regression, principal component analysis (PCA) and sparse principal component analysis regression and weighted quantile sum (WQS) regression to assess the associations. Root-mean-square error (RMSE) of each model were calculated to evaluate the model fit.

Results: Higher concentrations of total PCBs were positively and significantly associated with calf length and calf weight regardless of controlling for parity and calf age in WQS with associations constrained to be positive. We did not find an association between maternal exposure to POPs and any of the five health outcomes using simple sums method and PCA method with the statistically significant threshold of 0.0008. WQS method (positive or negative) had the root-mean-square error (RMSE) for all the models.

Conclusion: This study showed association between exposure to POPs, especially PCBs, with health endpoints of dolphin calves. Models using WQS showed stronger association than the simple sums and PCA methods, but the estimation is likely to be unstable due to small sample size.

Keywords: marine biology; ecotoxicology; One Health; infant health; epidemiologic methods

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Introduction

Persistent organic pollutants (POPs) persist long after their release into the environment and are widely distributed in the environment (Stockholm Convention). Most POPs are lipophilic and hydrophobic (Jones & De Voogt, 1999). When POPs enter biota, they tend to be stored in fat tissues and can be bioaccumulated and biomagnified through the food chain (Jones & De Voogt, 1999; World Health Organization, 2010). As a result, species at higher trophic levels tend to have higher concentrations of POPs in their bodies. Marine mammals, especially those such as cetaceans (including whales, dolphins and porpoises) that feed at higher trophic levels, have a large amount of fat tissue (blubber) that can store many kinds of environmental chemicals, and can accrue substantial body burdens of POPs (Yordy et al., 2010).

Common bottlenose dolphins (*Tursiops truncatus*) are apex predators (Balmer et al., 2011), and they have long lifespans and often inhabit long-term, year-round home ranges (Wells et al., 2004). As such, they are excellent sentinel species for coastal contamination as well as mammalian toxicological models for understanding the health implications of exposure to POPs. Dolphins can thus be used to assess not only the potential impacts of exposure to POPs on the health of their own species, but also give warning about the potential impacts on human health and the broader health of the whole coastal ecosystem ((Bossart, 2011; Wells et al., 2004).

Although veterinary environmental epidemiology in dolphin populations is valuable, conducting exposure and health assessments in dolphin populations is challenging. The process of sample collection from dolphins in the open ocean is logistically difficult (Yordy et al., 2010), and following dolphins over time to identify pregnant females and resulting offspring is even more complex and expensive. The consequently limited sample size of longitudinal dolphin population surveys complicates the epidemiological assessment of individual contaminants in relation to dolphin health endpoints. It is possible to glean insights from small sample size cohorts, but care must be taken when modeling the data and interpreting the findings (Octaria, Rebeiro, & Kainer, 2019).

The impact of POPs on the health of dolphin calves is of interest because calves may have substantial POP exposures. POPs can be transferred to offspring through placenta and breast milk (USEPA). Wells et al., (2005) found that the concentrations of POPs in females decline with successful calf rearing, while the concentrations in males will continue to increase throughout their lifetime. As females depurate the chemical load to their offspring, the first-born calves tend to receive the majority of their mother's original chemical load. Cockcroft, De Kock, Lord, & Ross, (1989) found that first-born calves of bottlenose dolphins receive almost 80 percent of the residue load of organochlorines from their mothers, which leads to a higher risk of negative health impacts on the calves.

Exposure to POPs such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), or dichlorodiphenyltrichloroethane (DDT) have been associated with multiple adverse health effects *in vivo*, including reproductive disorders, immune and endocrine system disruption, and some types of cancer (Stockholm Convention; USEPA; Yordy et al., 2010). Animal experiments and human studies both suggest that exposure to POPs can disrupt thyroid homeostasis (Berg et al., 2017; Boas, Feldt-Rasmussen, & Main, 2012); POPs have been shown to interfere with the regulation of thyroid hormones in marine mammals such as long-finned pilot whales (*Globicephala melas*) (Hoydal et al., 2016).

Therefore, thyroid hormones such as triiodothyronine (T3) and thyroxine (T4) are of interest as possible targets of POP toxicity in dolphin calves.

Studying the associations between multiple POP exposures and calf health in a small sample cohort is challenging, and requires adoption of appropriate statistical approaches (Octaria et al., 2019) such as suitable dimension reduction techniques (Johnstone & Lu, 2009; Y. Ma & Zhu, 2013).

Summing the total amount of each chemical within a class is a common way to consolidate data on multiple POPs into a single exposure index. Using this method, the individual chemicals are given equal weight, so the sum is a reflection only of their observed concentrations. This has several limitations. First, simply adding the concentrations of chemicals in a class assumes similar biologic activity across individual chemicals within the class, which may be biologically implausible. Second, there are often a greater number of chemicals in a class than can be feasibly measured in a study (e.g., PCBs typically have about 100 measurable congeners in marine mammal samples) and so an index based on an arbitrary subset of these may lack content validity as a measure of overall POP exposure patterns (Lawshe, 1975; Mokkink et al., 2010); Third, not all the individual chemicals included in the sum may actually be associated with the outcome of interest, and the association observed for the summed measure could be driven by some particular individual chemicals. Therefore, using the simple summed index in linear regression may add measurement error regarding the etiologically relevant exposure, and potentially reduce study power when a common exposure is related to the health outcome (de Bakker et al., 2005), attaching greater weight to chemicals that more closely correspond to the toxic exposures.

Principal components analysis (PCA) is a multivariate data analysis technique that transforms measured data on individual chemical biomarkers into a smaller number of uncorrelated variables while maximizing the variances of each chemical (Veyhe et al., 2015).Individual chemicals' contribution to the first principal component (PC)'s score is weighted based on their contribution to the maximum variance between study units (e.g., dolphins). PCs are calculated from the correlation or covariance between input (e.g., exposure) variables, regardless of their relationships to the outcome variables (Carrico, Gennings, Wheeler, Factor-Litvak, & statistics, 2015). Although PCA is widely used in high-dimensional data (S. Ma & Dai, 2011),PCA is limited when the number of variables is much larger than the number of observations (Johnstone & Lu, 2009). Sparse principal components analysis (SPCA) handles this data sparsity issue by selecting a subset of variables with largest variance, while constraining the majority of the variables' loadings to zero ((Erichson et al., 2018; Johnstone & Lu, 2009).

Weighted quantile sum (WQS) regression is an alternative approach for dimension reduction of exposure data that also incorporates data on the outcome of interest. In WQS regression, several chemicals with effects on an outcome that are assumed to be in the same direction (all effects positive or all effects negative), are used to calculate a body burden index (Czarnota, Gennings, Colt, et al., 2015; Czarnota, Gennings, & Wheeler, 2015). The individual chemicals are weighted into an index that allows for simultaneous estimation of a 'mixture' effect on the outcome in the WQS regression model, and identification of relatively important contributors to that overall effect estimated in the WQS regression model.

The objective of this study is to evaluate the association of maternal exposure to POPs with five health outcomes (calf length, calf weight, and three thyroid hormones) in bottlenose dolphin calves, comparing results from regression models using each of these methods of weighting POP exposures: simple sums, PCA, and WQS.

Methods:

Study population and analytic cohort

Data are from a cohort study initiated in 1970 on a multi-decadal, multi-generational resident community of individually recognizable bottlenose dolphins in Sarasota Bay, Florida by the Sarasota Dolphin Research Program, spanning up to five concurrent generations and including individuals up to 67 years of age (Wells, 2014; Yordy et al., 2010). Selected dolphins in small groups were encircled with a 500m-long seine net in shallow water (<2m deep) by a team of >70 trained handlers, biologists, and veterinarians (Wells et al., 2004). Individual dolphins were brought aboard a specially designed 9m-long veterinary processing vessel for a variety of samples and measurements. Full-thickness, 3 cm x 5 cm wedges of skin/blubber were obtained surgically under local anesthesia from a standard site approximately 10 cm caudal to, and 10 cm below, the trailing edge of the dorsal fin (Wells et al., 2005). Wedges were subsampled for different analyses before being placed in cryovials in liquid nitrogen for transport, and in a -80◦ C freezer for storage, prior to analyses by National Institute of Standards and Technology (NIST).

Blubber samples were analyzed by NIST using methods presented in detail elsewhere and summarized here (Litz et al., 2007). Briefly, from 0.5 g to 1 g of blubber was macerated while frozen and then added to a pressurized fluid extraction (PFE, ASE 200 Thermo Scientific) and extracted with dichloromethane. Prior to extraction a series of mass-labeled PCB congeners and organochlorine pesticides were added to the sample as internal standards. Sample extracts were reduced in volume and then cleaned up by size exclusion chromatography followed by solid phase extraction. Prior to cleanup, a weighed portion of the extract was taken and for gravimetric lipid determination. Final extracts were analyzed by capillary gas chromatography mass spectrometry (GC/MS; Agilent 6890/5973) for 85 PCB congeners, 2,4'- and 4,4' DDD, DDE and DDT, cis- and trans-chlordane and nonachlor, mirex, hexachlorobenze, hexachlorocyclohexanes, dieldrin and 26 PBDE congeners. Concentrations were determined using the method of internal standards based on a six-point calibration curve with individual calibrants processed as if they were samples. Calibration mixtures were prepared from NIST Standard Reference Materials (SRMs). At least one blank and one aliquot of SRM 1945 Organics in Whale Blubber were processed with each batch of samples. The limit of detection was determined as the mean blank value plus three standard deviations.

In this study, the exposures of interest are a large number of POPs (p=69) that are divided into four classes: PCBs (p=53), PBDEs (p=5), DDT (p=5) and OCPs (p=4), and the health outcomes of interest are three thyroid hormones of calves including triiodothyronine (T3), thyroxine (T4) and free thyroxine (fT4), as well as calf length and calf weight.

Since females would give the majority of their chemical burden to their first-born calf, and dolphins up to 2 years old are getting most of their nutrition from their mothers (Wells et al., 2005).we used exposure data from sampled females (two from primiparous females, prior to calving, two from pregnant females) and calves sampled 0-2 years of age. Using both maternal exposure (n=4) and postnatal exposure (n=35) data, we had early-life exposure data for a cohort of 39 calves. Samples with complete exposure and outcome data were included in the present analysis (n=36), more specifically, three calves' samples were excluded due to missing data on PCB15, PCB56, PCB92, PCB119, PCB130, PCB137, PCB146, PCB157, PCB163, PCB167, PCB172, PCB174, PCB176, PCB178, PCB185, PCB189 and PCB197. Outcome data are from 36 calves (including 32 original calves and four resulting calves from four mothers).

Statistical analysis

Data Pre-Processing

All concentrations of chemicals below the limit of detection (LOD) were deterministically imputed as LOD/√2. Specific POP chemicals were excluded from the analysis if > 30% of the dolphin samples were below the LOD for that chemical. 53 out of 55 individual PCBs congeners (PCB 18, 28+31, 44, 49, 52, 66, 74, 87, 92, 95, 99, 101, 105, 110, 118, 119, 128, 130, 137, 138, 146, 149, 151, 153/132, 154, 156, 157, 158, 163, 167, 170, 172, 174, 176, 177, 178, 180/193, 183, 185, 189, 194, 195, 197, 199, 200, 201, 202, 203/196, 206, 207, 208, 209), all five PBDEs congeners (47, 99, 100, 153, 154), four organochlorine pesticides (cis-chlordane, trans-chlordane, cis-nonachlor, AND trans-nonachlor), five DDTs (2,4′- and 4,4′-DDE, 2,4′- DDD, 4,4′-DDT and 2,4′-DDT+ 4,4′-DDD) were detected in more than 70% of the samples and therefore were included in the present analyses.

Regression Analysis

First, linear regressions were performed using summed PCB, summed PBDE, summed organochlorine pesticides and summed DDT as independent variables modeled separately for each outcome. Second, PCA were conducted separately for chemicals in classes of OCPs, PBDEs and DDT, and the first principal component (PC1) was saved for subsequent regression analysis. SPCA was performed for PCBs, because there were more individual PCBs than observations. PC1 from the SPCA of PCBs was multiplied by -1 for clarity of interpretation since all nonzero PCB loadings were negative; the resulting analytic variable corresponds to increases in PCB exposure. The PC1 for each chemical class (or negative PC1 for PCBs) was included as predictor in a separate linear regression model fitted to each chemical class-calf outcome pair. Finally, weighted quantile sum regressions were performed only with the class of PCBs. The other chemicals were not suited for WQS because EQS has a step of bootstrap to select the individual chemicals (Carrico et al., 2015), but there are only four or five individual chemicals in the other three classes. Since the WQS model constrains the direction of the association between the outcome and the WQS index in one direction, both positive and negative WQS models were fitted for each outcome. Calf age (continuous) and parity (primiparous, 1 older calf, and ≥ 2 older calves) were adjusted for in all regression models as confounders.

In WQS models, the mixed variables was ranked in dichotomies (q=2), the number of bootstrap samples used in parameter estimation was 50 (b=50), to validate the model, we set validation = 0, which means the test dataset was used as validation set.

Evaluation of Model Performance

To evaluate how well each model fit, histogram of residuals from each model were graphed. Additionally, Root-mean-square error (RMSE) of each model were calculated.

Software

All statistical analyses were performed using Rstudio (version 1.1.463 1). R packages used for analyses included “sparsepca” (N. Benjamin Erichson, 2018) and “gWQS” (Stefano Renzetti & 2018

)

Results:

Contamination data were reported as wet-mass normalized (**Appendix Table S1)**. PCB153+132 had the highest median concentration among the PCBs (3, 010 ng/g), followed by PCB 138 (1,132 ng/g) and PCB 187 (987 ng/g). PCB 200 has the lowest median concentration among the PCBs (6.85 ng/g). PCB 70 and PCB 56 were excluded from this study due to a rate of detection below 70% in all 43 samples. BDE 47 and BDE153 have the highest (408 ng/g) and lowest (9.87 ng/g) median concentration among the PBDEs, respectively. The highest median concentration of organochlorine pesticides was trans-nonachlor (3,737 ng/g) and the lowest was trans-chlordane (168 ng/g). Among five DDT chemicals, 4,4'-DDE has the highest median concentration (5, 766 ng/g), and 2,4'-DDD has the lowest median concentration (26.0 ng/g).

Correlations between individual chemicals within each class are shown in **Appendix Figures S1 - S4**. the intragroup correlations in PCBs had a range from -0.180 (between PCB 28+31 and PCB 203+196) to 0.994 (between PCB 128 and PCB 138), the lowest absolute correlation coefficient was 0.004 between PCB 194 and PCB 28+31. The highest correlation coefficient in PBDEs was 0.879 between BDE 153 and BDE 154, the lowest correlation coefficient in PBDEs was 0.390 between BDE 100 and BDE 154. The strongest correlation among OCPs was between cis.chlordane and cis.nonachlor (r =0.972), the weakest correlation among OCPs was between trans.nonachlor and trans.chlordane (r = 0.085). The highest correlation coefficient in DDTs was 0.886 between 4,4′-DDE and 2,4′-DDT+ 4,4′-DDD, the lowest correlation coefficient in DDTs was 0.676 between 2,4′-DDE and 2,4′-DDD.

Demographic and health characteristics of the dolphin calves including calf length, calf weight, T3, T4, fT4, Parity and calf age are shown in **Table 1**. The mean length of studied calves was 195cm (SD = 10cm), and the mean weight of studied calves was 86kg (SD = 14kg). Among the three thyroid hormones, T3 had the largest range from 0.84ng/ml to 213.00ng/ml and median value of the T3 level was 1.69ng/ml. Among all 36 samples included in the present analyses, 30 dolphins were 2 years old and 6 dolphin calves were 1.5 years old.

Weights from the simple sums and PCA methods assigned to individual chemicals for PCBs (congeners selected by sparse PCA), PBDEs, OCPs and DDTs are shown in **Table 2**. All of the weights assigned in the simple sums method of the individual chemicals were equal to 1. PCB-153+132, PCB-138 and PCB-187 comprised PC1 for the SPCA, and the weights estimated for those three congeners in PC1 were -1.10, -0.06 and -0.04. To reorient this axis such that greater PCB exposures corresponded to more positive index values, we multiplied these weights by -1. PC1 from PCA of PBDEs assigned highest weights to BDE-47 (weight=0.48) and BDE-153 (0.48), followed by BDE-99 (0.46), BDE-100 (0.43) and BDE-154 (0.40). PC1 from PCA of organochlorine pesticides (OCPs) assigned highest weight to cis-nonachlor (weight = 0.58) followed by cis-chlordane (0.57), trans-nonachlor (0.56) and trans-chlordane (0.16). PC1 from PCA of DDT compounds assigned highest weight to 2,4'-DDT+4,4'-DDD (weight = 0.47), followed by 4,4'-DDE (0.46), 4,4'-DDT (0.45), 2,4'-DDE (0.43) and 2,4'-DDD (0.43). **Figure 1** shows the weights of PCBs contributing to the ‘PCB exposure index' under each method, including the outcome-specific, direction-of-association-constraint-specific WQS results.

Associations between POPs and five calf health outcomes are presented in **Table 3 (unadjusted)** and **Table 4 (adjusted for parity and age).** In WQS with associations constrained to be positive, PCBs were significantly and positively associated with calf length and calf weight. After controlling for parity and calf age, in WQS with associations constrained to be positive, PCBs were still significantly associated with calf length and calf weight. There were not significant associations between POPs and any of the five health outcomes with the simple sums method and PCA method under the statistical significance threshold of 0.0008.

When examining how well the models performed, **Appendix Table S2** and **Appendix Table S3** show the root-mean-square error (RMSE) of each model for each chemical class (relevant histograms are also shown in supplement materials). For PCBs, models with T3 as the outcome had the highest RMSE among all the five health outcomes. Of the three methods applied, WQS method (positive or negative) had the lowest RMSE for all the models, more specifically, WQS negative method had the lowest RMSE for models with T3 as the outcome and WQS positive method had the lowest RMSE for models with the other four outcomes. For the other three chemical classes, model fit was similar between the simple sums method and the PCA method. **Appendix Figures (S5- S29)** shows the distribution of residuals in each model by outcome in each chemical class.

Discussion:

In this paper, we reported the possible association between maternal exposure to four classes of POPs and five health outcomes of dolphin calves with three ways of weighting individual chemicals. More specifically, in our results, PCBs were significantly and positively associated with calf length and calf weight in WQS with associations constrained to be positive. After controlling for parity and calf age, PCBs were still significantly associated with calf length and calf weight in WQS with associations constrained to be positive. There were not significant associations between POPs and any of the five health outcomes with the simple sums method and PCA method under the statistical significance threshold of 0.0008.

Findings from human studies about associations between exposure to POPs and birth weight is inconsistent. A cross-sectional study based on a Swedish cohort showed prenatal exposure to PCBs was associated with higher birth weight while prenatal exposure to PBDEs was associated with lower birth weight (Lignell et al., 2013). A meta-analysis within 12 European birth cohorts showed PCBs was significantly associated with lower birth weight (Govarts et al., 2011). In this study, we found PCBs were significantly associated with calf length and calf weight with WQS positive method.

Several studies have shown that exposure to POPs is associated with the disruption of thyroid hormones in cetaceans: Schwacke et al., (2011) found a negative association between concentrations of PCBs measured in blubber and T4, fT4 and T3 levels in bottlenose dolphins from Georgia, USA. Additionally, negative associations between PBDEs with fT3, T4 and fT4 were also found in a study of white whales (*Delphinapterus leucas*) from Svalbard, Norway.(G. Villanger et al., 2011). Studies have also shown the disruption of thyroid hormones under the exposure to POPs happened in other marine mammals such as walruses, seals and polar bears ((Routti et al., 2010; Routti et al., 2019; G. D. Villanger et al., 2011). We did not find any statistically significant association between exposure to POPs and calf thyroid hormones. This result may be biased since female dolphins that have the highest concentration of POPs burden may not be able to successfully reproduce (Alonso Farré et al., 2010). We did not account for this competing risk in our analysis.

The three methods gave different weights on individual chemicals. The simple sums method gave every individual the same weight of 1, so the chemicals with higher detected concentrations will be weighted more in this method. In PCBs class, PCB 153+132, PCB 138 and PCB 187 had the top three highest median concentration, so they were weighted more in the simple sums methods. These three PCBs congeners were also selected by sparse PCA and were included in the first PC that entered the PCA models with the same rank of weight, so the coefficient results based on these two methods were similar in terms of the direction and significance. Table 2 also showed the weights assigned to individual chemicals within PBDEs, OCPs, and DDT class are close to each other, which is similar to the weights assigned by the simple sums method, the results of these two methods were also similar.

WQS method gave individual chemicals weights based on each outcome, so for each outcome, the weight rank of PCBs was different, which allow us to interfere the possible higher toxic individual chemicals to each outcome, and the inference is set in a specific direction (positive or negative). Among the three methods, WQS showed higher frequencies of statistically significant associations between exposure to POPs and the five health outcomes, the simple sums method and PCA method performed similarly with regards to the direction and the statistical significance in the associations. Based on our results, WQS method has the lowest RMSE all the models, which indicated WQS method provided a stronger association between PCBs and calf outcomes than other methods (Carrico et al., 2015). However in this study, we used the entire dataset for WQS rather than using a training vs. validation datasets due to a small sample size, and the models using WQS were overfitting the data. The estimation of models with WQS might be unstable. We also found chemicals in each class were highly correlated with each other (see **Appendix Figures S1 - S4**), which might also decrease the estimation stability of the WQS method since a cluster of highly associated individual chemicals tend to have lower weights (Carrico et al., 2015). The limitation based on small sample size would also affect the estimation stability of models using the simple sums method and PCA method. Another limitation of this study is that all the models were only adjusted for parity and calves'age, mother's condition such as mother's size would also be a confounder for the health endpoints of the calves (Robinson et al., 2017). Studies showed that the trends of concentration of POPs in the biota is decreasing (Rigét, Bignert, Braune, Stow, & Wilson, 2010; Vorkamp, Rigét, Glasius, Muir, & Dietz, 2008), the levels of thyroid hormones will also change on a seasonal basis (Suzuki et al., 2018), which indicated that the sample timing could also be a influence factor when study the association, we did not account for the sample timing in this present study.

Statistical methods to analyze exposure to mixtures of environmental toxicants such as POPs are of higher concern in environmental health research in recent years (Lazarevic, Barnett, Sly, & Knibbs, 2019). The coefficient results from the simple sums method is easier to interpret but it gives more weight on the chemicals with higher detected concentration, which may not be the most toxic chemicals to the outcome (Braun, Gennings, Hauser, & Webster, 2016). The PCA method is known for its reduction of high dimensionality and collinearity of mixtures of data, but it is hard to interpret since it is based on the difference of variance of chemicals (Forns et al., 2016). This study indicated the difference between WQS with the traditional analysis methods but it was effected by the small sample size of current study, which indicated further direction for future studies in environmental health and veterinary epidemiology area.

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Tables

Table 1. Characteristics of the dolphin calves included in the study

	Mean ± SD	Median	Range
Length(cm)	195 ± 10	194	175, 214
Weight (kg)	86 ± 14	84	62, 121
T3(ng/ml)	19.48 ± 49.41	1.69	0.84, 213.00
T4(mcg/dl)	15.76 ± 2.33	15.37	11.71, 22.40
fT4(ng/dl)	2.91± 0.81	3.16	1.20, 4.13
Parity	2.7 ± 1.9	2.0	1.0, 8.0
Calf age	2 ± 0	2.0	1.5, 2

Table 2. Weights per compound from PCA and SPCA. The first principal component from SPCA had negative or zero values for all loadings, so was multiplied by negative 1 and nonzero loadings presented as positive for ‘SPCA weights’. PCA weights are the loadings onto the first principal component.

	SPCA weights		**PCA weights**		**PCA weights**		**PCA weights**
PCBs		**PBDEs**		**OCPs**		**DDTs**	
PCB153+132	1.10	BDE_47	0.48	Trans-chlordane	0.18	2,4'-DDE	0.43
PCB138	0.06	BDE_99	0.46	Cis-chlordane	0.57	4,4'-DDE	0.46
PCB187	0.04	BDE_100	0.43	Trans-nonachloride	0.56	2,4'-DDD	0.43
		BDE_153	0.48	Cis-nonachloride	0.58	2,4'-DDT+4,4'-DDD	0.47
		BDE_154	0.40			4,4'-DDT	0.45

Table 3. Unadjusted associations between POPs and dolphin calf health outcomes.

POPs (ng/g wet mass)		Length		Weight		T3		T4		fT4	
		coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p value
PCBs											
	Sum	0.00	0.96	0.00	0.40	0.00	0.63	0.00	0.35	0.00	0.16
	PCA	0.00	0.90	0.00	0.44	0.00	0.70	0.00	0.33	0.00	0.20
	WQS+	**23.59**	**3.29E-04**	**30.88**	**8.90E-05**	38.39	0.115	3.97	8.93E-04	1.49	7.57E-03
	WQS-	0.94	0.85	1.20	0.78	-37.35	0.06	-0.63	0.55	-0.04	0.94
PBDEs											
	Sum	0.00	0.47	0.01	0.13	-0.03	0.21	0.00	0.56	0.00	0.31
	PCA	0.52	0.54	1.36	0.19	-2.61	0.41	0.14	0.45	0.09	0.25
OCPs											
	Sum	0.00	0.29	0.00	0.34	0.00	0.58	0.00	0.30	0.00	0.11
	PCA	-0.57	0.55	-0.50	0.67	-2.95	0.40	-0.15	0.47	-1.11	0.19
DDT											
	Sum	0.00	0.20	0.00	0.01	0.00	0.68	0.00	0.17	0.00	0.11
	PCA	0.89	0.26	2.38	0.01	-1.80	0.54	0.16	0.35	0.15	0.08

Table 4. Associations between exposure of POPs and five health outcomes, adjusted for parity and calf age.

POPs (ng/g wet mass)		**Length**		**Weight**		**T3**		**T4**		**fT4**	
		coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p value
PCBs											
	Sum	0.00	0.75	0.00	0.52	0.00	0.25	0.00	0.55	0.00	0.57
	PCA	0.00	0.69	0.00	0.45	0.00	0.32	0.00	0.61	0.005	0.61
	WQS+	**28.70**	**1.57E-04**	**26.46**	**2.63E-04**	43.54	0.162	3.78	1.21E-02	1.04	8.93E-02
	WQS-	-2.89	0.69	-3.53	0.65	-55.81	0.01	-2.35	0.06	-0.74	0.16
PBDEs											
	Sum	0.00	0.58	0.00	0.62	-0.06	0.03	0.00	0.36	0.00	0.85
	PCA	0.25	0.84	0.27	0.84	-7.82	0.08	-0.23	0.35	0.05	0.63
OCPs											
	Sum	-0.00	0.34	0.00	0.37	0.00	0.60	-0.00	0.27	0.00	0.13
	PCA	-0.52	0.59	-0.48	0.66	-3.10	0.40	-0.17	0.38	-0.10	0.19
DDT											
	Sum	0.00	0.13	0.00	0.07	0.00	0.32	0.00	0.91	0.00	0.51
	PCA	1.29	0.22	1.98	0.09	-4.56	0.26	-0.08	0.70	0.08	0.46

Figures

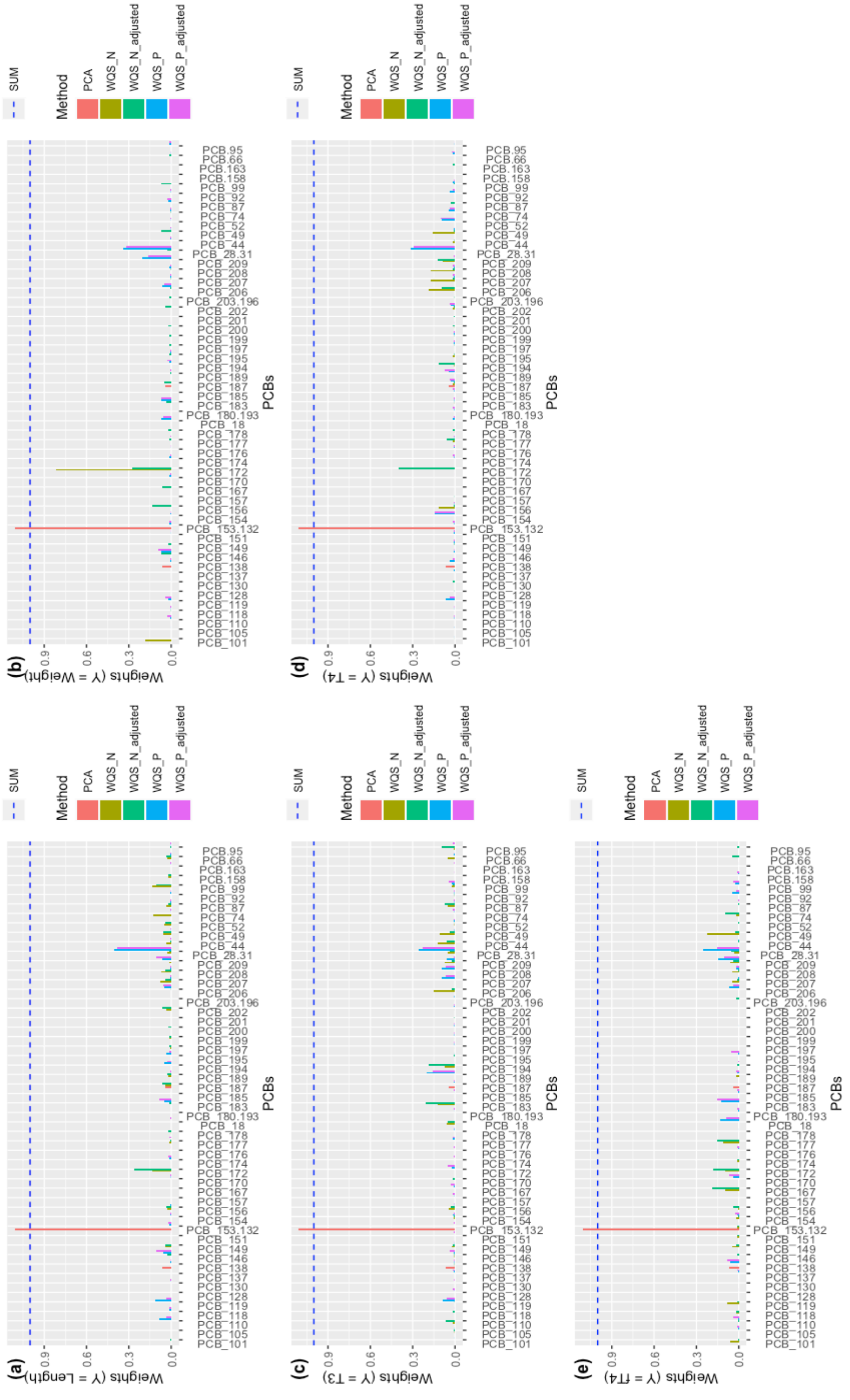


Figure 1. Weights of PCBs in different methods by each outscome

Appendix

Tables

Table S1 Characteristics of POPs included in the study

Compound	Mean ± S.D.	Median	Range		Number of values below LOD (%)
PCB_18	27.77 ± 14.28	28.957	1.414	57.461	3(6.98%)
PCB_28+31	33.17 ± 17.55	30.639	4.729	80.915	0
PCB_44	24.98 ± 11.63	25.688	1.653	55.01	0
PCB_49	73.48 ± 29.43	74.893	6.434	128.545	0
PCB_52	331.41 ± 248.37	272.88	8.529	1067.63	0
PCB_56	9.47±5.96	11.55	1.414	20.762	12 (27.91%)
PCB_66	82.89 ± 29.75	85.861	4.977	145.163	0
PCB_70	7.13±13.93	1.414	1.414	86.55	23 (53.49%)
PCB_74	80.45 ± 37.66	89.244	2.546	151.81	0
PCB_87	24.70 ± 10.74	23.66	1.414	51.14	1(2.33%)
PCB_92	139.74 ± 109.06	123.542	2.136	548.911	0
PCB_95	189.06 ± 194.86	180.509	1.414	910.278	10 (23.26%))
PCB_99	957.59 ± 746.14	758.742	18.765	3400.39	0
PCB_101	359.33 ± 152.64	343.423	14.365	811.901	0
PCB_105	136.25 ± 69.34	128.935	3.242	357.623	0
PCB_110	19.16 ± 9.06	15.883	3.7	36.088	0
PCB_118	503.42 ± 255.62	465.389	12.035	1190.15	0
PCB_119	30.07 ± 19.25	27.509	1.414	103.683	2(4.65%)
PCB_128	205.53 ± 163.72	163.454	3.161	757.635	0
PCB_130	65.70 ± 43.26	54.78	1.414	193.745	1(2.33%)
PCB_137	46.64 ± 35.73	32.555	1.414	122.6	1(2.33%)
PCB_138	1475.54 ± 1281.57	1132.08	23.51	5932.35	0
PCB_146	441.82 ± 288.64	371.305	8.776	1457.19	0
PCB_149	618.70 ± 512.72	517.116	14.023	2644.84	0
PCB_151	250.52 ± 237.03	185.234	4.437	1162.4	0
PCB_153+132	3986.80 ± 3,167.65	3009.81	64.329	14681.62	0
PCB_154	115.09 ± 74.80	100.881	2.61	323.613	0
PCB_156	41.18 ± 17.38	42.857	1.453	93.277	0
PCB_157	19.16 ± 11.66	18.417	1.414	49.1	2(4.65%)

PCB.158	92.48 ± 71.22	66.959	1.414	281.443	1(2.33%)
PCB_163	475.00 ± 367.05	366.652	6.807	1788.19	0
PCB_167	42.58 ± 22.78	39.939	1.414	97.93	1(2.33%)
PCB_170	383.08 ± 309.27	300.497	7.104	1254.67	0
PCB_172	52.12 ± 28.77	45.711	2.811	115	0
PCB_174	119.24 ± 106.59	86.976	1.604	472.639	0
PCB_176	28.22 ± 23.62	18.956	1.414	88.836	1(2.33%)
PCB_177	241.82 ± 207.32	166.399	4.731	952.01	0
PCB_178	207.62 ± 169.41	154.934	3.93	808.58	0
PCB_187	1191.13 ± 885.34	987.314	26.611	4387.09	0
PCB_180+193	879.71 ± 690.82	681.054	8.074	2844.18	0
PCB_183	296.40 ± 223.42	228.666	6.886	913.411	0
PCB_185	21.61 ± 17.91	14.151	1.414	72.438	2(4.65%)
PCB_189	14.44 ± 13.26	10.22	1.414	76.2	2(4.65%)
PCB_194	192.41	169.997	9.854	394.165	0
PCB_195	44.64 ± 26.83	38.313	1.814	105.84	0
PCB_197	16.21 ± 9.54	13.546	1.414	40.382	1(2.33%)
PCB_199	275.33 ± 156.77	238.589	8.569	602.209	0
PCB_200	8.33 ± 5.72	6.849	1.414	21.273	2(4.65%)
PCB_201	49.27 ± 27.14	45.225	2.194	106.39	0
PCB_202	149.17 ± 93.40	135.639	5.853	455	0
PCB_203+196	299.80 ± 484.50	208.195	13.742	3190	0
PCB_206	72.48 ± 36.65	66.285	10.906	203.889	0
PCB_207	12.93 ± 6.74	12.189	1.414	34.3	1(2.33%)
PCB_208	42.22 ± 22.87	40.282	1.414	127	1(2.33%)
PCB_209	9.41 ± 10.12	8.374	1.414	62.469	11 (25.58)
BDE_47	407.83 ± 204.76	407.763	9.171	947.279	0
BDE_99	41.18 ± 33.19	33.61	2.095	212.98	0
BDE_100	100.96 ± 63.21	101.225	1.414	241.855	1(2.33%)
BDE_153	12.23 ± 8.32	9.873	1.414	50.118	1(2.33%)
BDE_154	31.33 ± 16.30	24.825	2.987	70.955	0
trans.chlordane	153.56 ± 114.47	168.398	1.831	397.79	0
cis.chlordane	408.16 ± 469.39	361.609	19.963	3043.61	0
trans.nonachlor	5995.08 ± 9261.01	3737.39	50.093	57540.92	0

cis.nonachlor	1034.12 ± 928.37	928.094	20.037	5910.07	0
2,4′-DDE	43.18 ± 20.02	41.617	1.439	88.535	0
4,4′-DDE	7675.95 ± 5702.25	5765.84	180.935	23969.1	0
2,4′-DDD	35.33 ± 23.56	26.015	1.414	89.671	1(2.33%)
2,4′-DDT+ 4,4′-DDD	592.99 ± 331.15	493.825	20.554	1467.13	0
4,4′-DDT	96.19 ± 60.33	81.536	1.414	304.007	1(2.33%)

Table S2 Root-mean-square error (RMSE) of each regression model of POPs exposures and dolphin calf health outcomes

Outcome	SUM	PCA	WQS+	WQS-
PCBs				
Length	9.57	9.57	7.9	9.57
Weight	11.65	11.67	9.36	11.76
T3	35.2	35.25	34.04	33.55
T4	2	1.99	1.72	2.01
fT4	0.79	0.8	0.73	0.82
PBDEs				
Length	9.5	9.52	-	-
Weight	11.39	11.48	-	-
T3	34.51	34.96	-	-
T4	2.01	2	-	-
fT4	0.81	0.8	-	-
OCPs				
Length	9.41	9.52	-	-
Weight	11.61	11.74	-	-
T3	35.17	34.96	-	-
T4	1.99	2.01	-	-
fT4	0.79	0.8	-	-
DDTs				
Length	9.34	9.39	-	-
Weight	10.7	10.7	-	-
T3	35.23	35.13	-	-
T4	1.97	2	-	-
fT4	0.79	0.78	-	-

Table S3 Root-mean-square error (RMSE) of each adjusted regression model of POPs exposures and dolphin calf health outcomes

Outcome	**SUM**	**PCA**	**WQS+**	**WQS-**
PCBs				
Length	9.04	9.03	7.57	9.47
Weight	10.28	10.26	9.21	10.59
T3	33.88	34.08	33.88	31.77
T4	1.86	1.86	1.73	1.8
fT4	0.73	0.73	0.7	0.72
PBDEs				
Length	9.01	9.05	-	-
Weight	10.31	10.35	-	-
T3	32.17	32.89	-	-
T4	1.84	1.84	-	-
fT4	0.74	0.73	-	-
OCPs				
Length	8.92	9.01	-	-
Weight	10.22	10.32	-	-
T3	34.47	34.22	-	-
T4	1.83	1.84	-	-
fT4	0.71	0.71	-	-
DDTs				
Length	8.72	8,83	-	-
Weight	9.81	9.89	-	-
T3	34.08	33.9	-	-
T4	1.87	1.86	-	-
fT4	0.73	0.73	-	-

Figures

Figure S1 Correlation plot of individual chemicals in PCBs

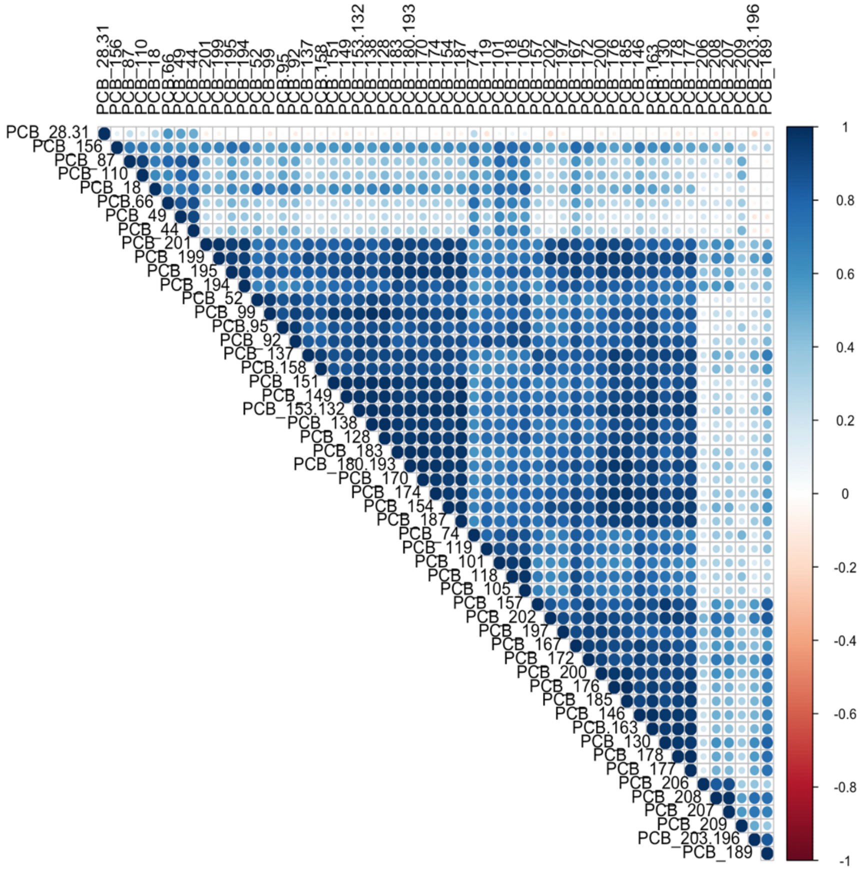


Figure S2 Correlation plot of individual chemicals in PBDEs

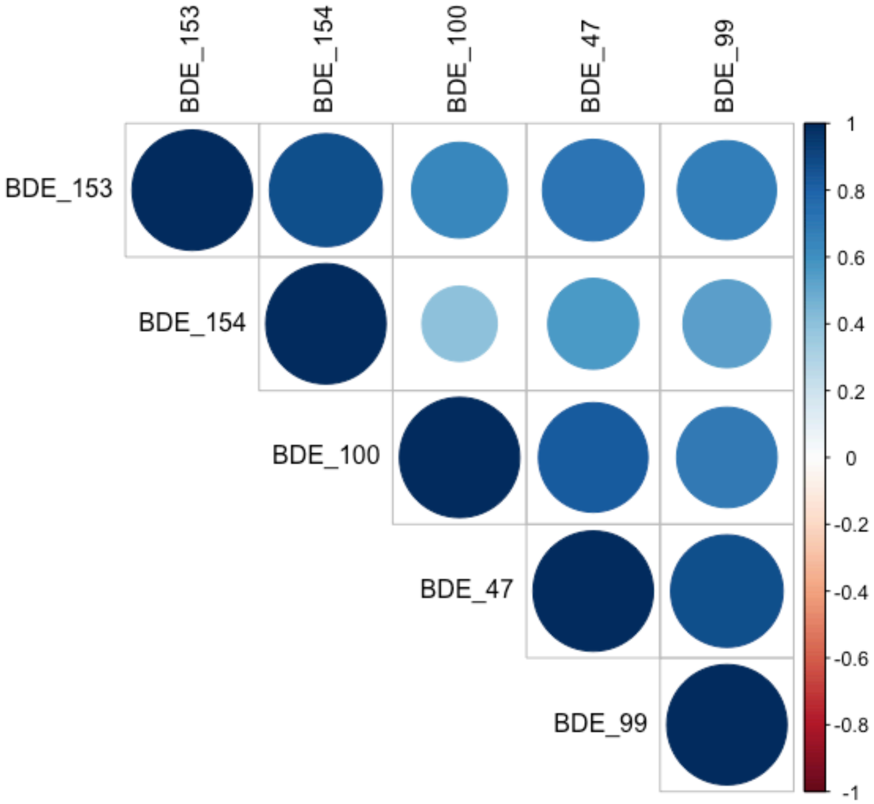


Figure S3 Correlation plot of individual chemicals in OCPs

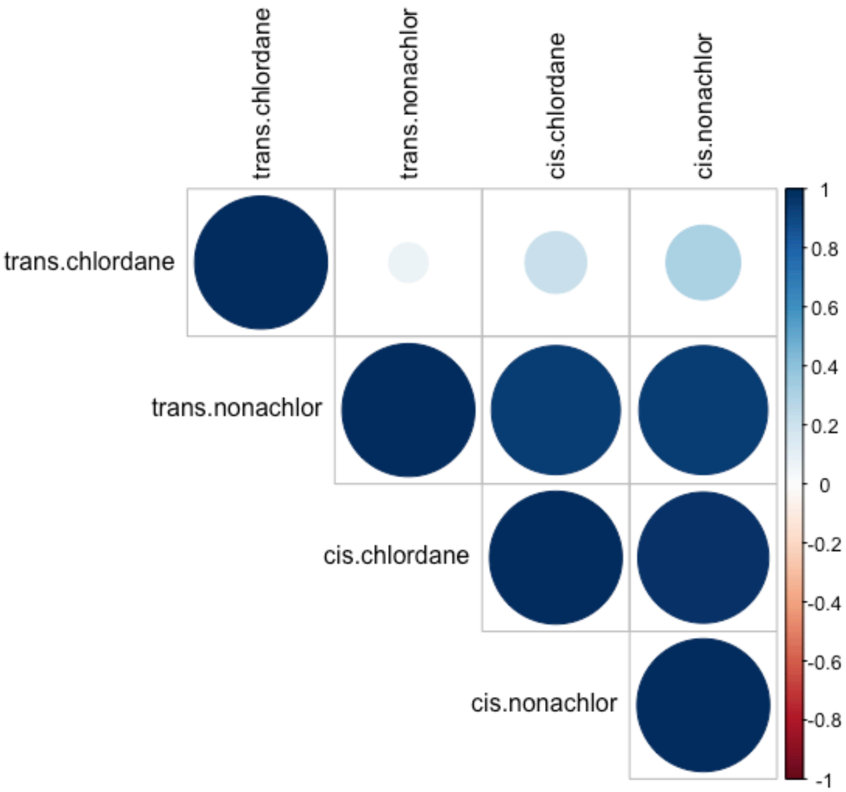


Figure S4 Correlation plot of individual chemicals in DDTs

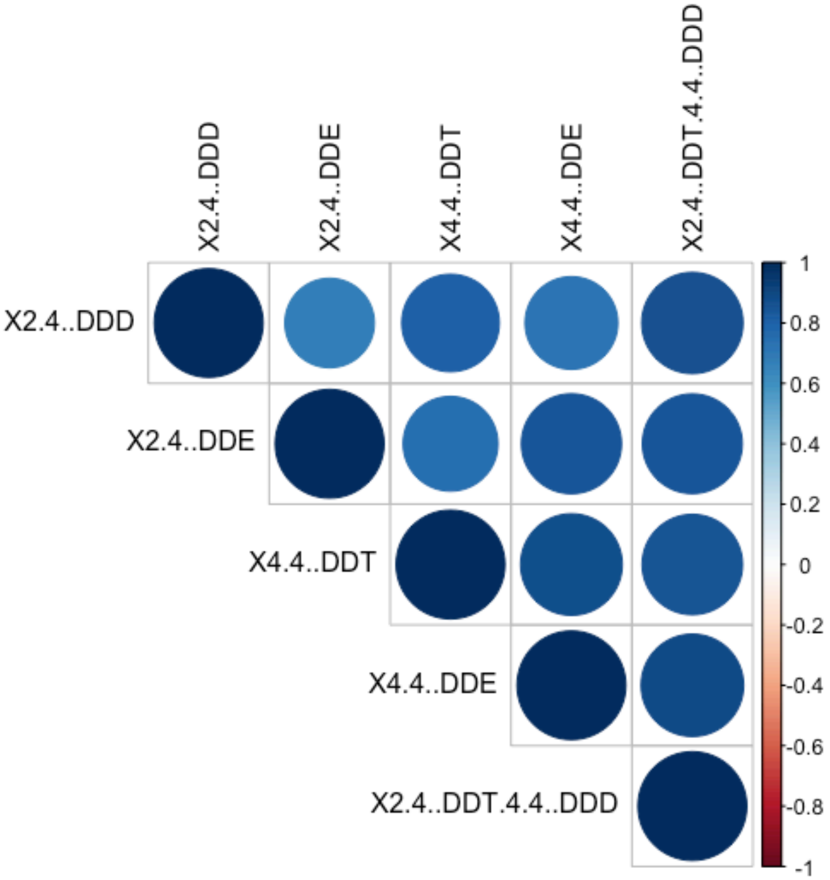


Figure S5 Patterns of residuals in regression models of PCBs exposures and dolphin calf length

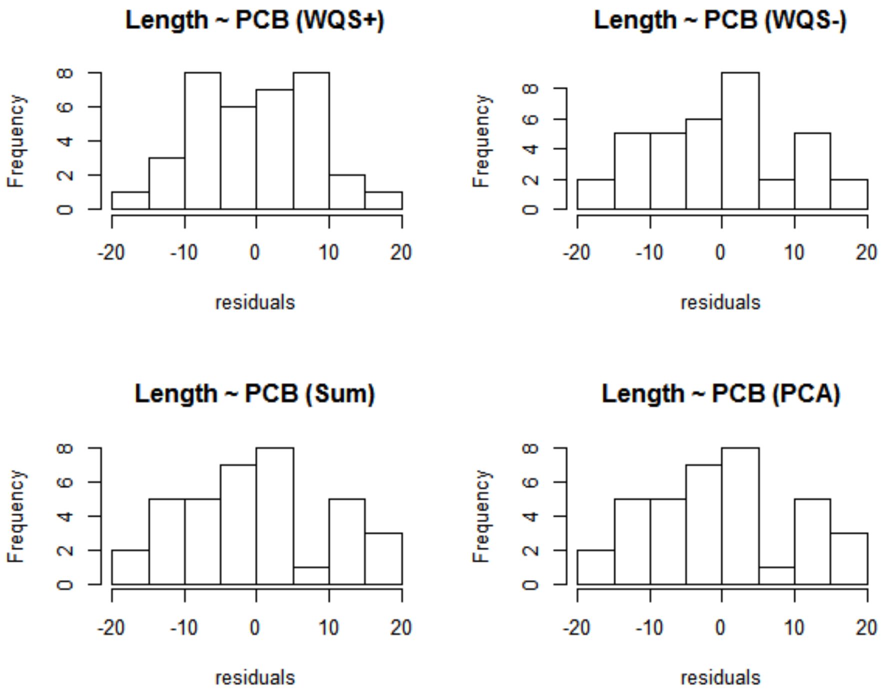


Figure S6 Patterns of residuals in adjusted regression models of PCBs exposures and dolphin calf length

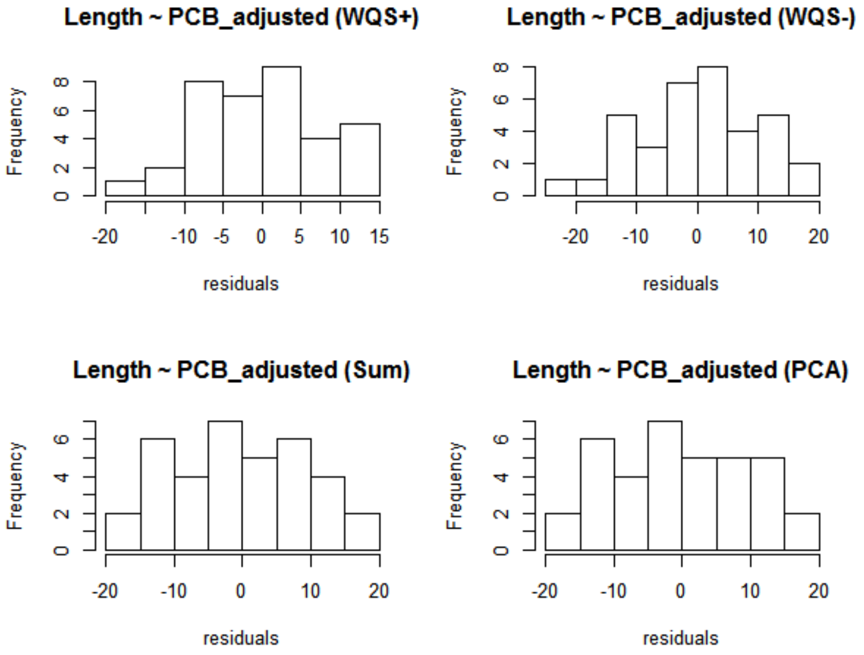


Figure S7 Patterns of residuals in regression models of PCBs exposures and dolphin calf weight

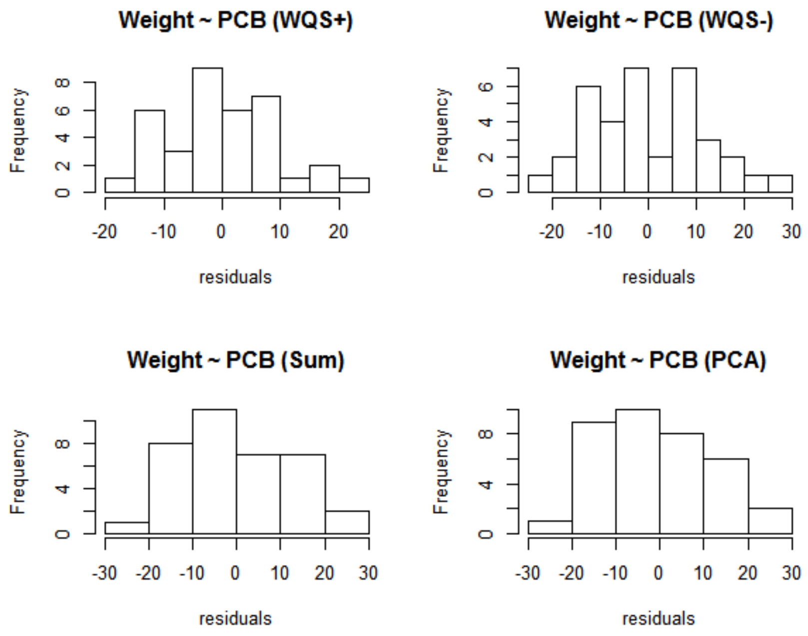

Weight ~ PCB (WQS+)
Weight ~ PCB (WQS-)
Weight ~ PCB (Sum)
Weight ~ PCB (PCA)

Figure S8 Patterns of residuals in adjusted regression models of PCBs exposures and dolphin calf weight

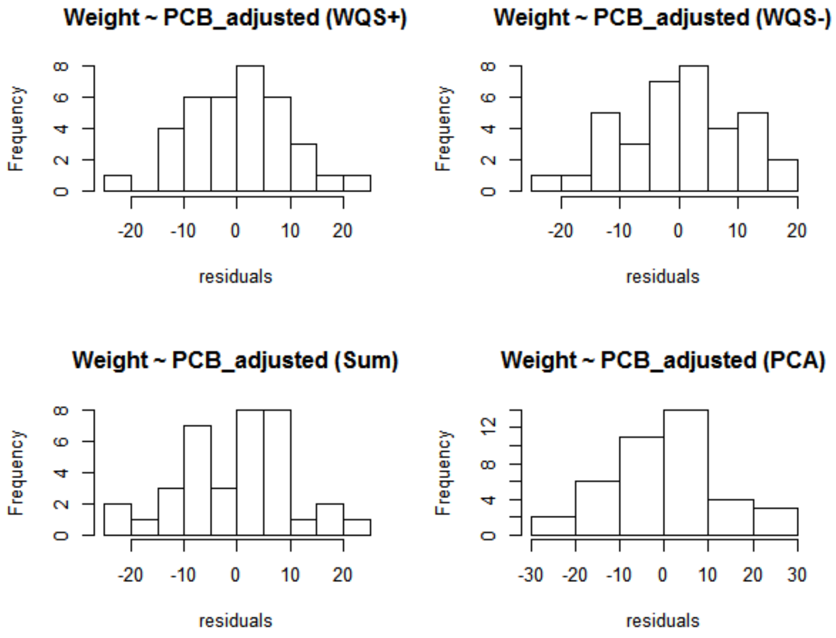

Weight ~ PCB_adjusted (WQS+)
Weight ~ PCB_adjusted (WQS-)
Weight ~ PCB_adjusted (Sum)
Weight ~ PCB_adjusted (PCA)

Figure S9 Patterns of residuals in regression models of PCBs exposures and dolphin calf T3 level

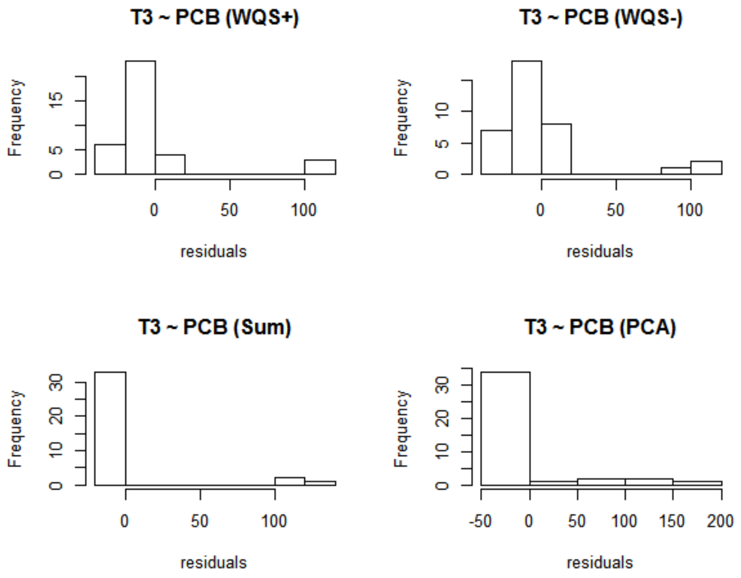


Figure S10 Patterns of residuals in adjusted regression models of PCBs exposures and dolphin calf T3 level

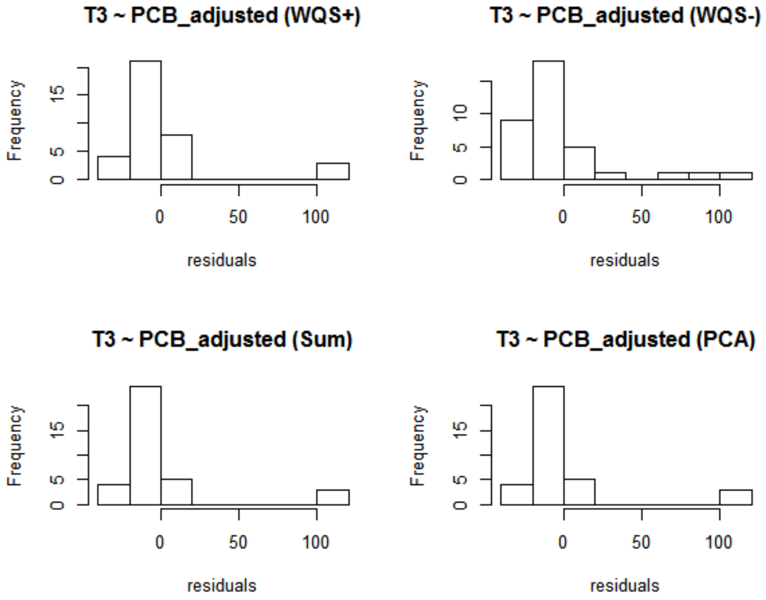


Figure S11 Patterns of residuals in regression models of PCBs exposures and dolphin calf T4 level

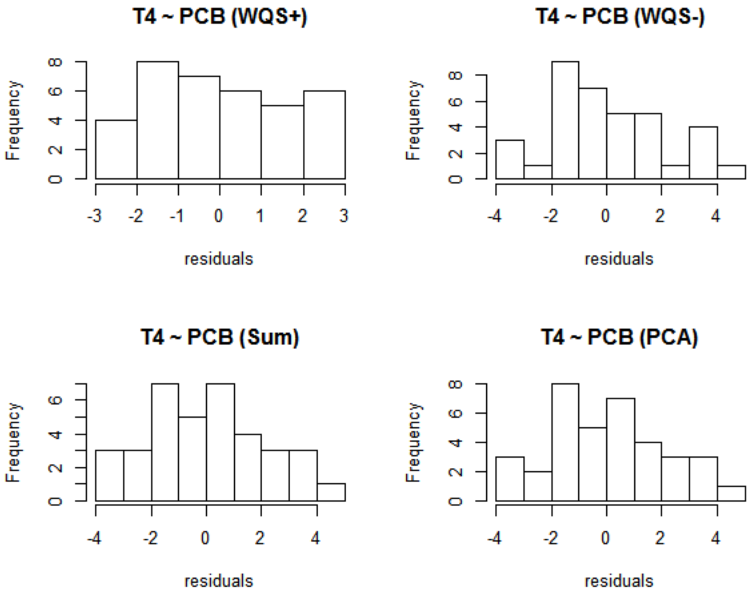


Figure S12 Patterns of residuals in adjusted regression models of PCBs exposures and dolphin calf T4 level

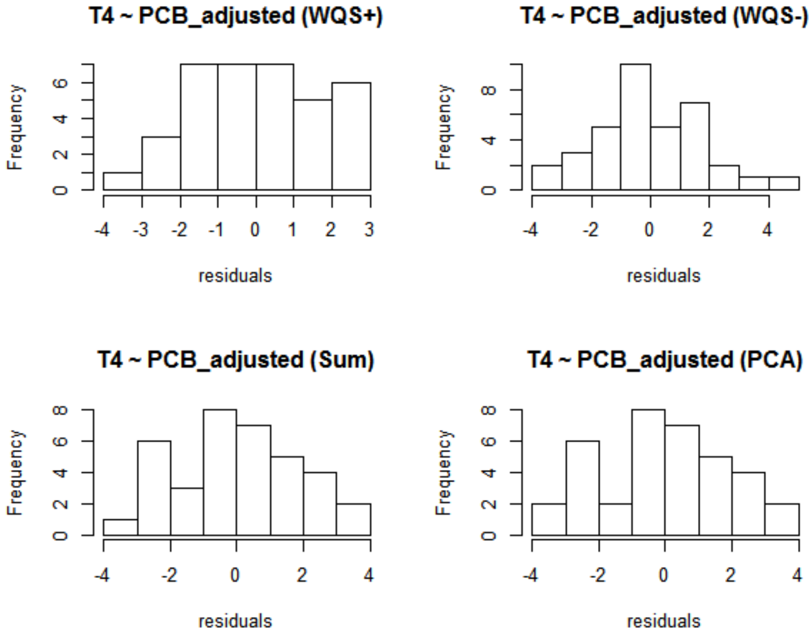


Figure S13 Patterns of residuals in regression models of PCBs exposures and dolphin calf fT4 level

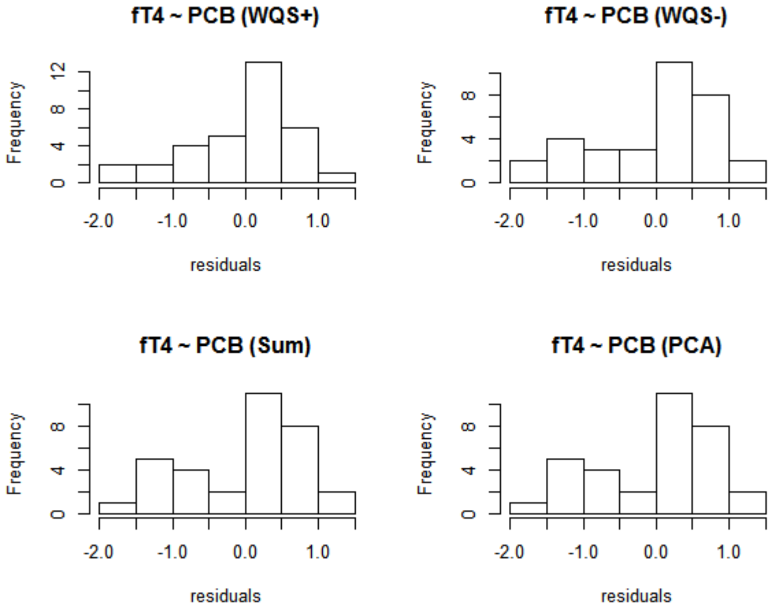


Figure S14 Patterns of residuals in adjusted regression models of PCBs exposures and dolphin calf fT4 level

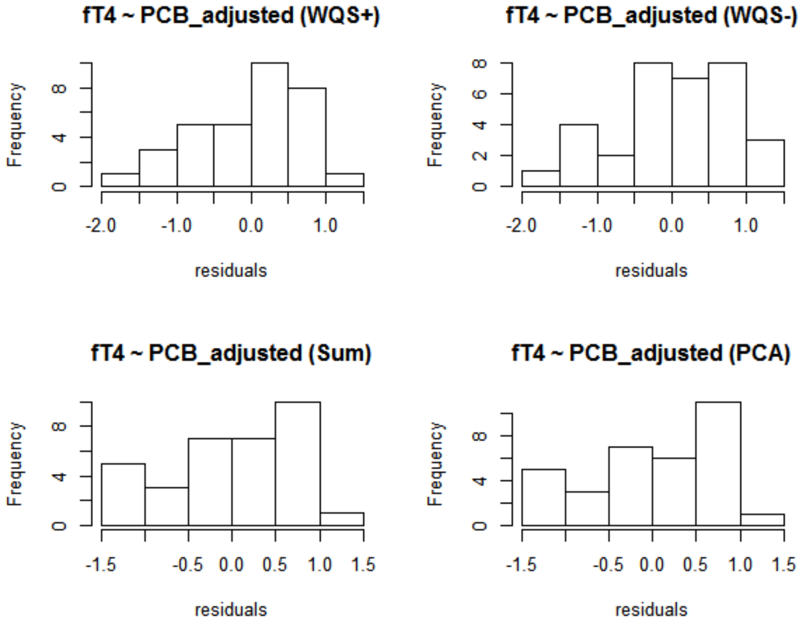


Figure S15 Patterns of residuals in regression models of PBDEs exposures and dolphin calf length

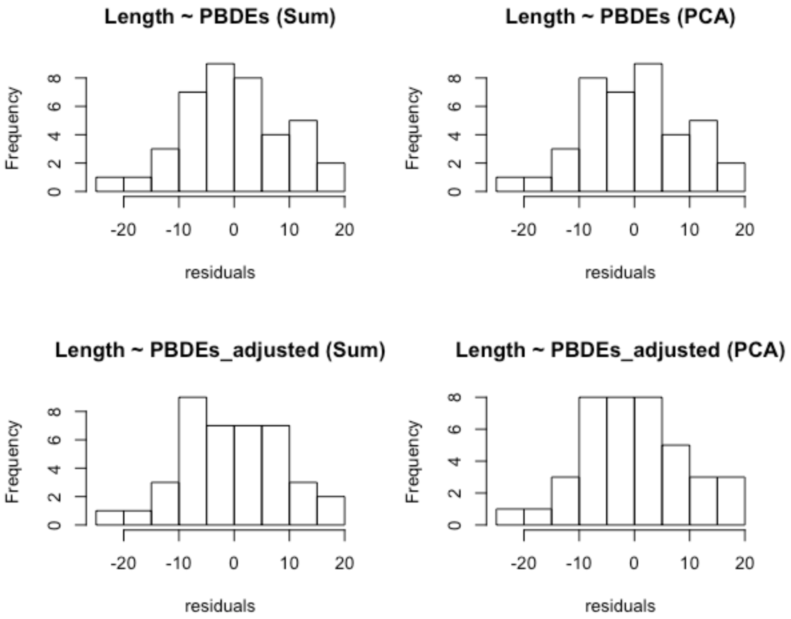


Figure S16 Patterns of residuals in regression models of PBDEs exposures and dolphin calf weight

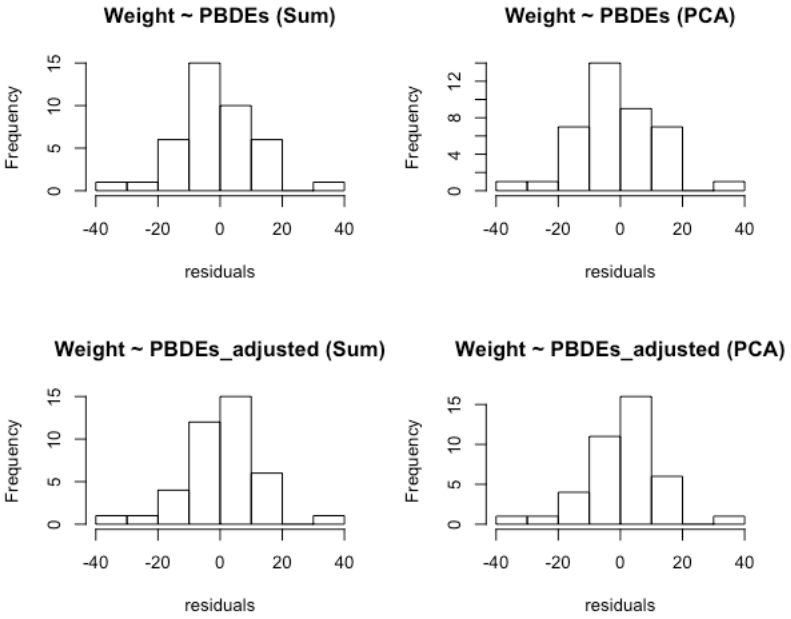


Figure S17 Patterns of residuals in regression models of PBDEs exposures and dolphin calf T3 level

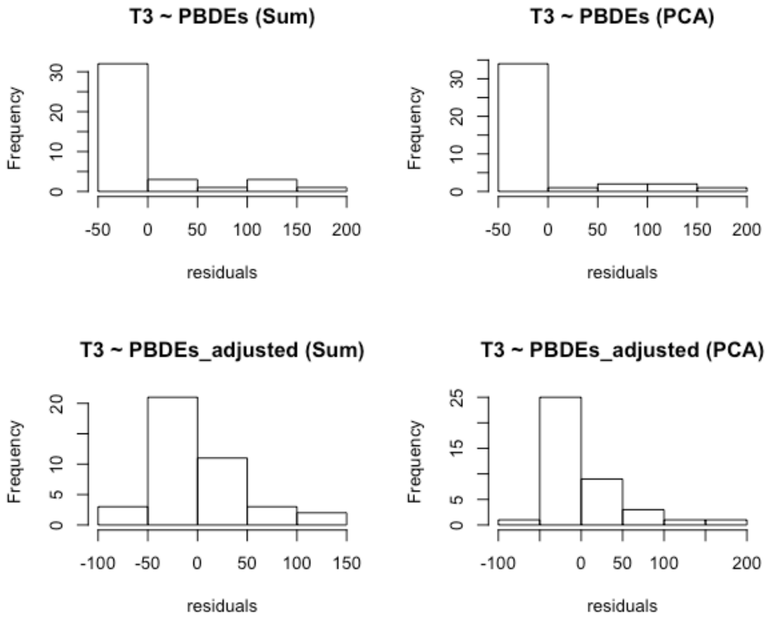


Figure S18 Patterns of residuals in regression models of PBDEs exposures and dolphin calf T4 level

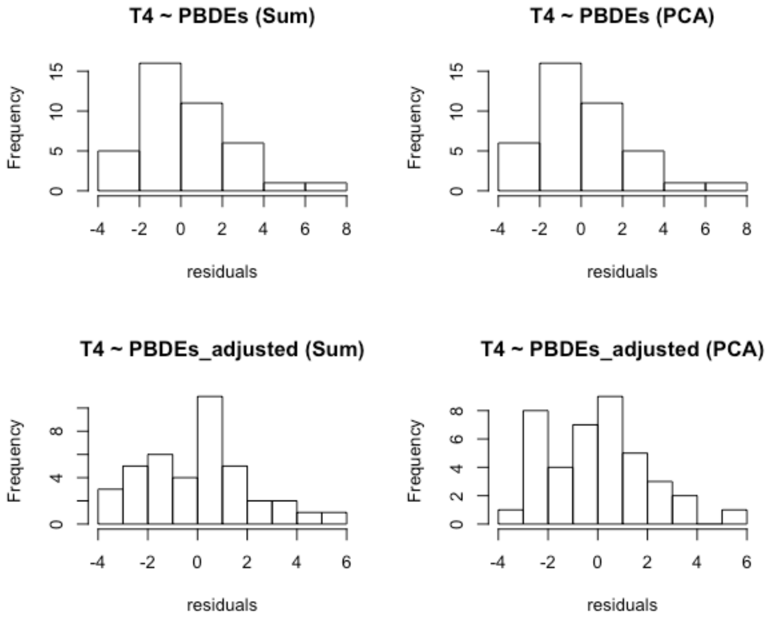


Figure S19 Patterns of residuals in regression models of PBDEs exposures and dolphin calf fT4 level

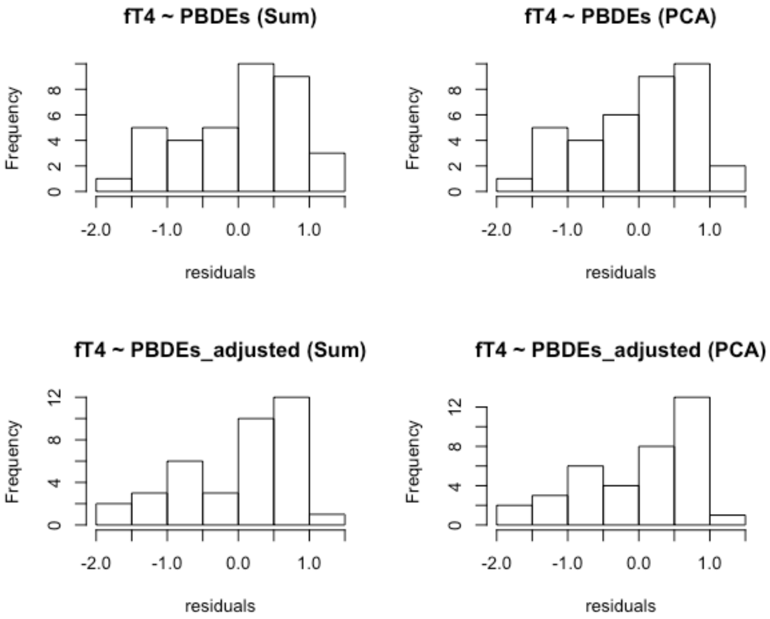


Figure S20 Patterns of residuals in regression models of OCPs exposures and dolphin calf length

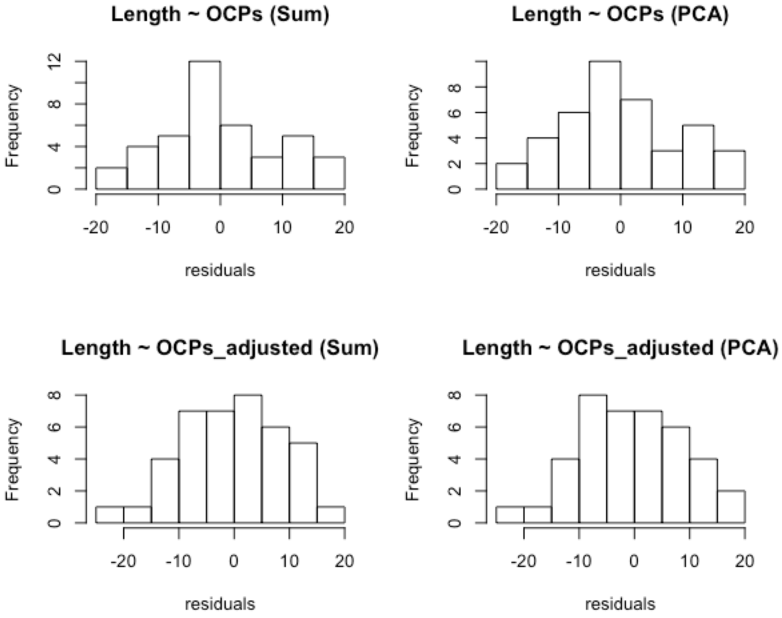
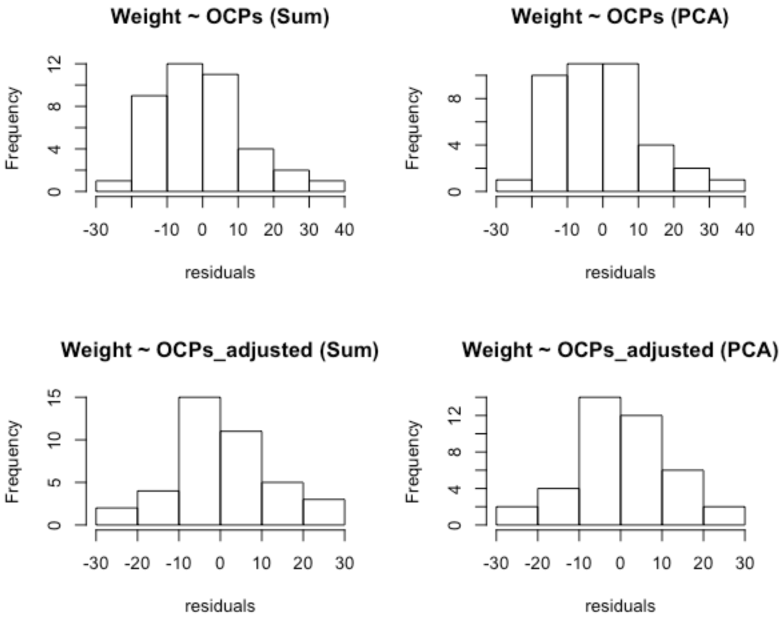


Figure S21 Patterns of residuals in regression models of OCPs exposures and dolphin calf weight



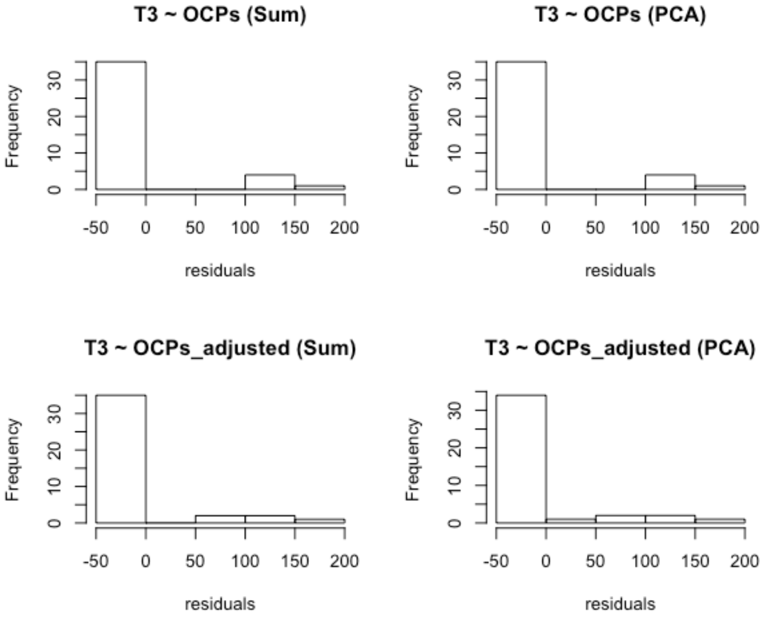
Weight ~ OCPs (Sum)

Weight ~ OCPs (PCA)

Weight ~ OCPs_adjusted (Sum)

Weight ~ OCPs_adjusted (PCA)

Figure S22 Patterns of residuals in regression models of OCPs exposures and dolphin calf T3 level



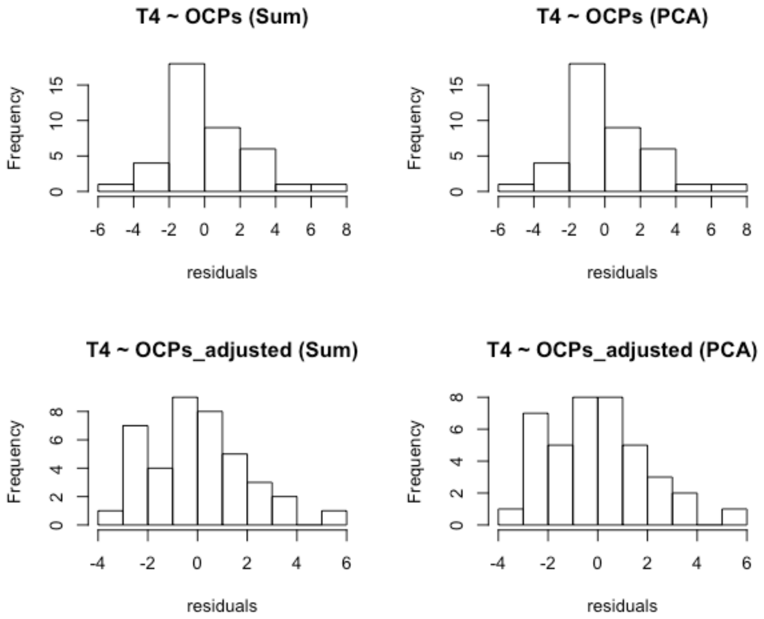
T3 ~ OCPs (Sum)

T3 ~ OCPs (PCA)

T3 ~ OCPs_adjusted (Sum)

T3 ~ OCPs_adjusted (PCA)

Figure S23 Patterns of residuals in regression models of OCPs exposures and dolphin calf T4 level

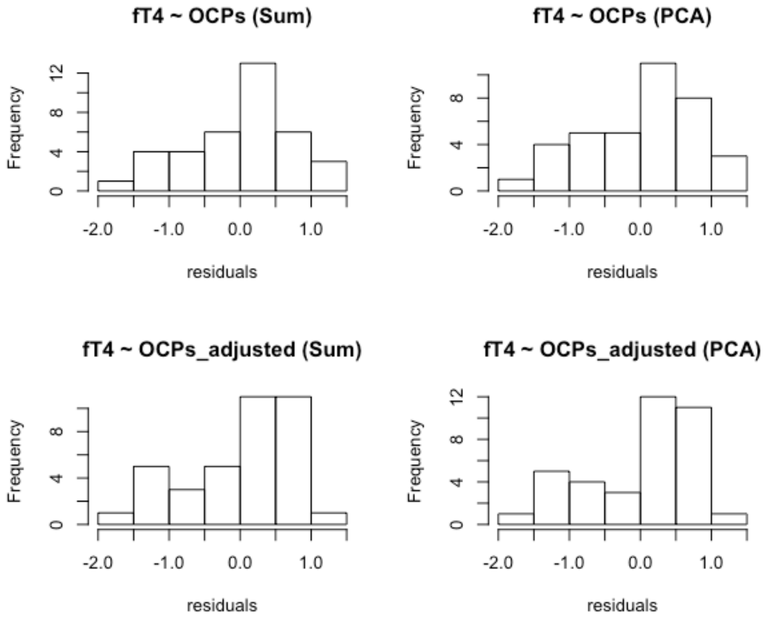

T4 ~ OCPs (Sum)

T4 ~ OCPs (PCA)

T4 ~ OCPs_adjusted (Sum)

T4 ~ OCPs_adjusted (PCA)

Figure S24 Patterns of residuals in regression models of OCPs exposures and dolphin calf fT4 level


fT4 ~ OCPs (Sum)

fT4 ~ OCPs (PCA)

fT4 ~ OCPs_adjusted (Sum)

fT4 ~ OCPs_adjusted (PCA)

Figure S25 Patterns of residuals in regression models of DDTs exposures and dolphin calf length

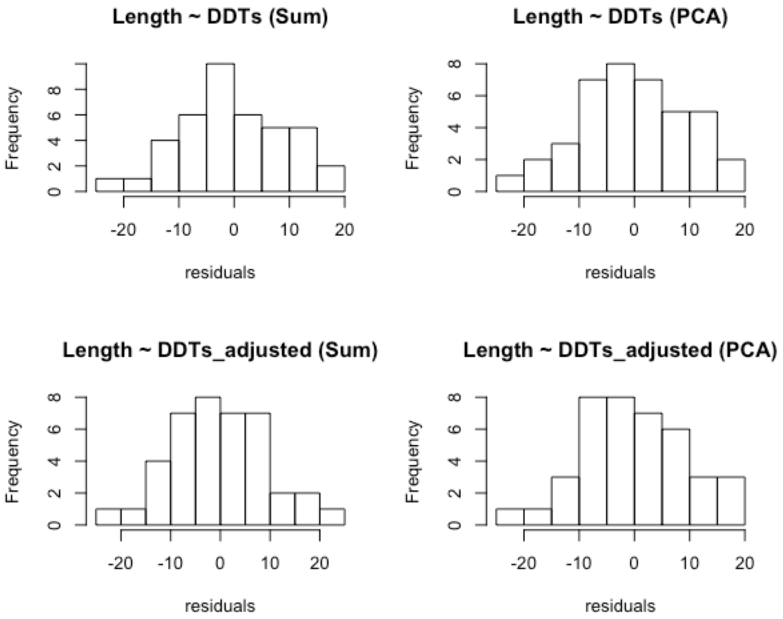


Figure S26 Patterns of residuals in regression models of DDTs exposures and dolphin calf weight

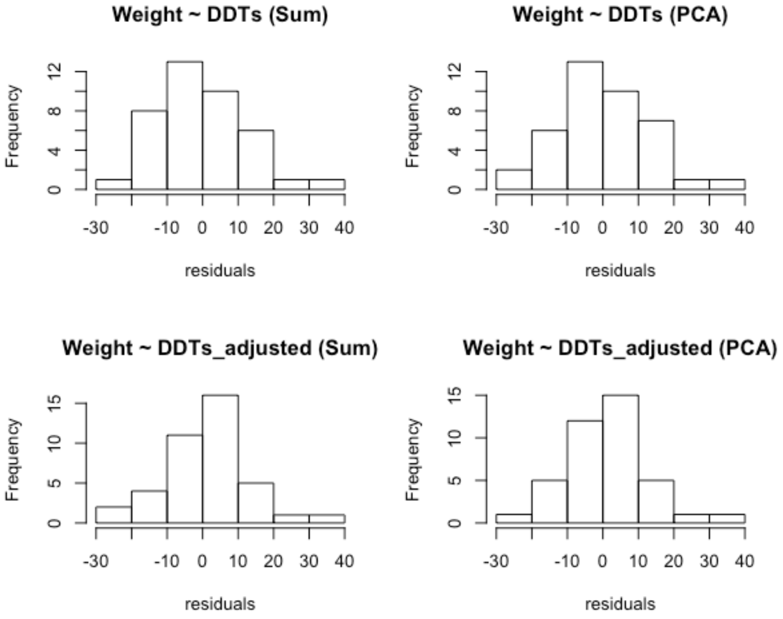


Figure S27 Patterns of residuals in regression models of DDTs exposures and dolphin calf T3 level

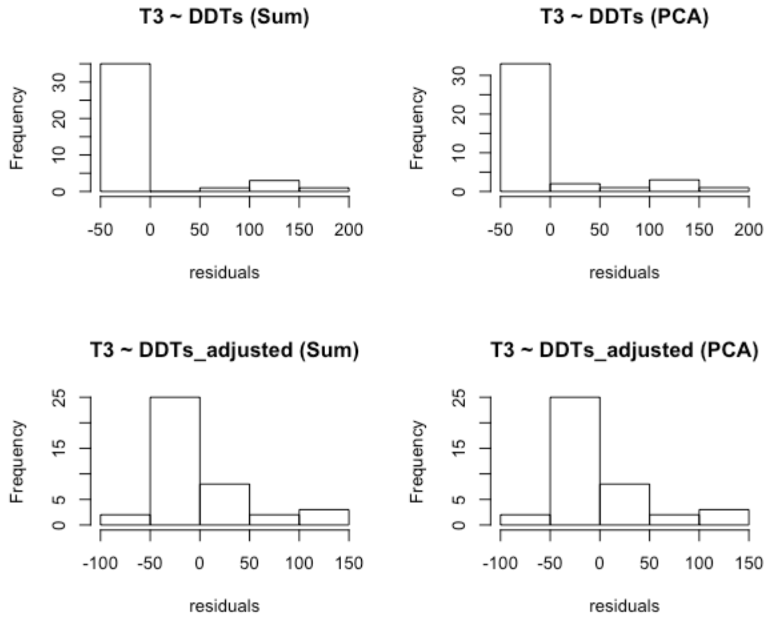


Figure S28 Patterns of residuals in regression models of DDTs exposures and dolphin calf T4 level

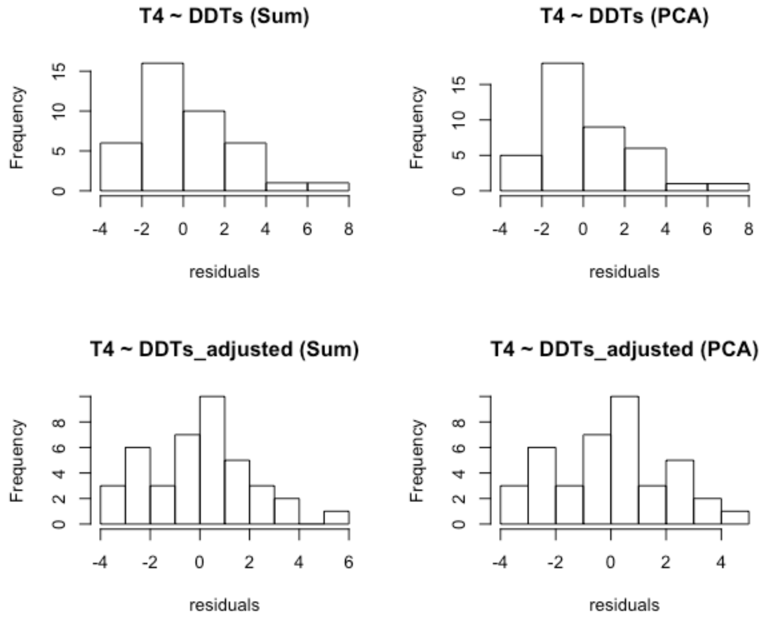


Figure S29 Patterns of residuals in regression models of DDTs exposures and dolphin calf fT4 level