Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Victoria M. Kennerley Date

Evaluating Statistical Approaches to Model Multi-Pollutant Mixtures on Common Bottlenose Dolphin (*Tursiops Truncatus*) Health Along the Eastern Coast of Florida and South Carolina

By

Victoria M. Kennerley

Degree to be awarded: Master of Science in Public Health

Department of Biostatistics and Bioinformatics

_____

Howard H. Chang, PhD

Thesis Advisor

_____

Adam M. Schaefer, MPH

Reader

Evaluating Statistical Approaches to Model Multi-Pollutant Mixtures on Common Bottlenose Dolphin (*Tursiops Truncatus*) Health Along the Eastern Coast of Florida and South Carolina

By

Victoria M. Kennerley

Bachelor of Science

University of Florida

2018

Thesis Advisor: Howard H. Chang, PhD

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Science in Public Health

in Biostatistics

2020

Abstract

Evaluating Statistical Approaches to Model Multi-Pollutant Mixtures on Common Bottlenose Dolphin (*Tursiops Truncatus*) Health Along the Eastern Coast of Florida and South Carolina

By Victoria M. Kennerley

Background: Bottlenose dolphins (*Tursiops truncatus*) are the most common cetacean species found in coastal and estuarine ecosystems along the southeastern coast of the United States. Their widespread distribution and role as apex predators make them an ideal sentinel species for monitoring pollutants. Previous studies have revealed associations between individual chemical pollutants and pathophysiological endpoints in dolphins. However, the reality is that dolphins are exposed to a large number of pollutants simultaneously and single-pollutant models do not capture the mixture and potential interplay of combined exposures. In recent years, an increased number of studies have implemented more sophisticated statistical methods to assess the relationship between multi-pollutant mixtures and health outcomes in humans. These methods have not been previously applied to marine mammal research.

Methods: In this study, we focus on combining the application of principal component analysis and Bayesian kernel machine regression to evaluate the association between environmental exposure mixtures and absolute counts of MHCII+ cells in Atlantic bottlenose dolphins while simultaneously examining the impact of missing values using random forest imputation and multiple imputation.

Results: Multiple imputation resulted in the highest average pollutant concentrations. A statistically significant association was found between absolute counts of MCHII+ and the first and second principal components primarily made up of 1) ∑PFCs, ∑PFCAs, and ∑PFSAs and 2) ∑Pesticides, ∑PCBs, and ∑PBDEs across all methods for handling missing data. Bayesian Kernel Machine Regression with hierarchical variable selection identified PFCAs as most influential. Principle components 1 and 2 were still found to be significant in Bayesian Kernel Machine Regression analyses with principle components as predictors.

Conclusions: Of the methods presented, Bayesian Kernel Machine Regression with hierarchical variable selection yielded the most straightforward results by identifying a single predictor with the most influence. Analyses conducted with different methods for handling missing data yielded similar results across all three methods.

Evaluating Statistical Approaches to Model Multi-Pollutant Mixtures on Common Bottlenose Dolphin (*Tursiops Truncatus*) Health Along the Eastern Coast of Florida and South Carolina

By

Victoria M. Kennerley

Bachelor of Science

University of Florida

2018

Thesis Advisor: Howard H. Chang, PhD

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Science in Public Health

in Biostatistics

2020

Acknowledgements

I am so grateful for my experiences as a graduate student at Emory University and the opportunity to submit this work but even more so for all of those that I have worked with along the way that have made this possible.

Thank you to the Department of Biostatistics and Bioinformatics for providing not only a wealth of valuable knowledge and skills but also an incredibly warm community that I have been thoroughly grateful for over the past two years. I am always amazed to have encountered so many brilliant educators and researchers that I have continually witnessed go above and beyond for myself and my peers.

I'd like to especially thank my research advisor, professor, and mentor, Howard Chang. Thank you for your kindness, for never making me feel like I couldn't ask questions, and for always pushing me academically, professionally, and in leadership.

Thank you to Adam Schaefer for taking me on as an intern and providing the opportunity and guidance to work on this project. I never could have guessed that my career as a statistician would allow me to combine my passions for biostatistics and marine life, let alone study a population of dolphins in a lagoon that I grew up visiting and I am so grateful.

Lastly, I would like to acknowledge my friends and family for being an incredible support system through this entire journey. Special thanks to my grandmother for raising me to love the ocean and still taking me to drink lattes and look for dolphins on the Indian River Lagoon every time I visit home.

Lucky is an understatement and I am so thankful for all of you.

1. INTRODUCTION

Bottlenose dolphins (*Tursiops truncatus*) are the most common cetacean species found in coastal and estuarine ecosystems along the southeastern coast of the United States (Bossart, 2006). Their widespread coastal distribution and role as apex predators make them an ideal sentinel species for monitoring ocean and human health (Bossart, 2011). In 2003, the Atlantic Bottlenose Dolphin Health and Environmental Risk Assessment (HERA) Project was initiated in order to evaluate individual and population health of bottlenose dolphins in Charleston, South Carolina (CHS) and the Indian River Lagoon, Florida (IRL) (Murdoch et al., 2006). Because these dolphins are exposed to a number of chemical pollutants, various studies have attempted to quantify these environmental exposures as well as their associations with pathophysiological effects.

Dolphins from CHS have been found to have higher concentrations than IRL dolphins of legacy persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and dichloro-diphenylethanes (DDTs), as well as emerging contaminants such as polybrominated diphenyl ethers (PBDEs) and perflouroalkyl acids (PFAAs) (Reif et al., 2017). Decreased lymphocyte response has been previously observed to be associated with increased concentrations of PCBs and DDT in peripheral blood in free-ranging bottlenose dolphins (Lahvis et al., 1995). Perfluoroalkyl compounds (PFCs) have been observed to be associated with increases in a number of immunological parameters such as absolute numbers of MHCII+ cells, CD19+ immature B cells, and CD2+ T cells. Additionally, several PFC analyte groups were suggested to have an effect on immune, hematopietic, kidney, and liver function by previous results such as a positive association with gamma-glutamyltransferase (GGT) and negative association with cholesterol levels. (Fair et al., 2013).

Concentrations of mercury (Hg) in the blood and skin of IRL dolphins have been found to be among the highest reported worldwide (Reif et al., 2015). An increase in blood and skin total Hg (THg) concentrations was observed to be associated with a decrease in total thyroxine (T4) and triiodothyronine (T3) suggesting an effect on endocrine function, an increase in GGT suggesting an effect on liver function, and an increase in blood urea nitrogen (BUN) suggesting an effect on renal function. Increased blood and skin THg were also associated with various hematological parameters such as decreases in the absolute number of lymphocytes, eosinophils, and platelets. (Schaefer et al., 2011).

Though the single-pollutant models utilized in previously described research have revealed many important results and associations, the reality is that dolphins are exposed to a large number of pollutants simultaneously and single-pollutant models may fail to capture adverse health effects of the mixture and potential interplay of this combined exposure (Reif et al., 2017).Furthermore, the impact of missing values exposure measurements has not been accounted for in these analyses despite an often occurrence. This use of only complete cases can result in discarding much of the data collected as well as potentially bias results (Gelman & Hill, 2006).

In recent years, an increased number of studies have implemented more sophisticated statistical methods to assess the relationship between multi-pollutant mixtures and health outcomes in humans (Billionnet et al., 2012; Stafoggia et al., 2017). In a review of these approaches, Stafoggia et al. propose a classification of these methods into three categories: (1) dimension reduction methods such as principal component analysis, positive matrix factorization, and partial least squares regression, (2) variable selection methods such as Bayesian kernel machine regression, LASSO, and Bayesian model averaging, and (3) grouping

of observations methods such as Bayesian profile regression, classification and regression trees, and groups based on score.

In this study, we focus on combining the application of principal component analysis (PCA) (Anderson, 1985) and Bayesian kernel machine regression (BKMR) (Bobb et al., 2014) to evaluate the association between environmental exposure mixtures and absolute counts of MHCII+ cells in Atlantic bottlenose dolphins while simultaneously examining the impact of missing values using two different imputation methods (Stekhoven & Bühlmann, 2012; van Buuren & Groothuis-Oudshoorn, 2011). Though other studies of the impact of multi-pollutant mixtures on human health have implemented PCA (Agay-Shay et al., 2015; Richards et al., 2016) and BKMR (Kim et al., 2019; Valeri et al., 2017) individually as well as in the same study to compare (Chiu et al., 2018; Kim et al., 2019; Li et al., 2019), this is the first to combine the two as one method. Exploration of the association between multi-pollutant mixtures and health outcomes has not previously been investigated in marine mammal research.

2. METHODS

2.1 Study Population

Dolphins were captured, sampled, and released using previously described techniques at IRL during the months of June and July and in CHS in August of 2003-2011 (Murdoch et al., 2006). Samples were restricted to a total of 125 individual dolphins from the IRL (n = 65) and CHS (n = 60) with complete data for age, sex, and chemical concentration of each exposure. The research was approved under National Marine Fisheries permit no. 998-1678 and by the Harbor Branch Oceanographic Institutional Animal Care and Use Committee.

Blood samples were drawn from the periarterial rete in the flukes during the first ten minutes of capture with a 19-guage, 1.9-cm, butterfly catheter. Vacutainer tubes containing lithium herapin, ethylenediamine tetraacetic acid or serum separator gel were used to collect samples for hematology (Murdoch et al., 2006). Samples were analyzed by the Cornell University Veterinary Diagnostic Laboratory in Ithaca, NY, USA after being stored in an insulated cooler and shipped overnight. Extraction of a tooth under local anesthesia using an injection of 3% mepivacaine was used in order to determine age by counting postnatal dentine layers as described by Hohn et al (Hohn et al., 1989).

2.2 Exposure Assessment and Handling of Missing Values

Blood THg concentration was determined by thermal desorption/amalgamation/atomic absorption spectrophotometry using a direct mercury analyzer (DMA-80; Milestone Inc., Shelton, CT) (Schaefer et al., 2011). Concentrations of PBDEs, PCBs, and pesticides were measured in blubber biopsy samples using gas chromatography and mass spectrometry (Fair et al., 2010). Concentrations of PFCs, perfluoroalkyl sulfonates (PFSAs), and perfluorocarboxylates (PFCAs) were determined by methods described by Fair et al (Fair et al., 2013).

Measured congeners were excluded if there were less than 25% complete observations. After exclusion, PFCAs (92.4% complete) included PFOA, PFNA, PFDA, PFUA, PFDoA, and PFTA. The PFCs (88.24% complete) included PFDA, PFDS, PFDoA, PFHxS, PFNA, PFOA, PFOS, PFOSA, PFTA, and PFUA. The PCB (97.83% complete) congeners included 1, 101/90, 105, 106/118/123, 107/108, 110, 114, 119, 12, 123, 126, 128/167, 130, 132/153/168, 141, 146, 149, 15, 151, 154, 156, 157, 159, 169, 170/190, 172, 174, 177, 18, 180, 183, 187, 188, 189, 193, 194, 195, 2, 20, 200, 201, 202, 206, 207, 209, 26, 28/31, 29, 3, 37, 33, 35, 44, 45, 48, 49, 5/8, 50, 52, 56/60, 61/74, 63, 66, 69, 70/76, 77, 82, 84, 87/115, 88/95, 89, 9, 92, and 99. The PFSAs

(82% complete) included PFOS, PFOSA, PFDS, and PFHxS. The PBDE congeners (99.67% complete) included 17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 183, and 190. Pesticides (100% complete) included 2,4'-DDD, 2,4'-DDE, 2,4'-DDT, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, and Aldrin.

We consider three types of analyses to handle missing values: (1) not accounting for missing data, (2) imputing missing values using a random forest algorithm, and (3) imputing missing values using multiple imputation. After implementation of each method for handling missing congeners, congener sums were calculated for each of the previously mentioned pollutant groups. Details of this process are summarized in **Figure** 1. When not accounting for missing data, missing congener values were treated as zeros in the sum calculations.

Random forest imputation was conducted using the nonparametric method described by Stekhoven *et al.* (2012). An initial imputation was first made for each missing value using mean imputation. Variables with missing values were then sorted by the number of missing observations. For each variable with missing values, a random forest was fit using observed values of that variable as the response and corresponding values of all other variables as predictors. The trained random forest was then applied to predict the missing values of that variable and iterated until a stopping criterion is met. For this analysis, 100 trees were grown in each forest and 10 iterations were performed given that the stopping criterion was not met first. Performance was assessed by out-of-bag (OOB) error estimates.

Multiple imputation was implemented using Multivariate Imputation by Chained Equations (MICE) (van Buuren & Groothuis-Oudshoorn, 2011). Predictive mean matching with 50 iterations was used as the imputation model for each variable to create five imputed datasets.

Subsequent analyses were repeated with each of the five datasets and final point estimates of interest were combined using Rubin's rules (Campion & Rubin, 1989).

2.3 Statistical Analysis

Summaries of dolphin demographic characteristics were summarized as percentages for binary variables or with means and standard deviations for continuous variables. Summary statistics were calculated for ∑PFCAs, ∑PFCs, ∑PCBs, ∑PFSAs, ∑PBDEs, and ∑Pesticides to compare across methods for handling missing data. In addition to pollutant concentrations, age and sex were selected to be included *a priori* in all analyses as potential confounders. Separate regression analyses were conducted between the absolute count of MHCII+ cells and each pollutant concentration to determine significant single-pollutant associations for the purpose of comparison to the effects estimated by other methods explored in this analysis. Absolute count of MHCII+ was log-transformed as necessary to meet normality assumptions for all three methods.

2.3.1 Principal Component Analysis and Regression

Principal component analysis (PCA) is a dimension reduction method which identifies independent linear combinations of the predictors that capture the most variance across predictors (Anderson, 1985). It does not take into account the outcome of interest in calculation of components. After implementation of PCA, regression analysis models were run with the scores from the first three components as predictors for 82 dolphins with complete observations for absolute count of MCHII+ cells.

2.3.2 Bayesian Kernel Machine Regression with Variable Selection

Bayesian Kernel Machine Regression (BKMR) is a recently proposed method which utilizes a smooth function to flexibly model the relationship between exposures and an outcome of interest (Bobb et al., 2014). It allows incorporation of non-linear effects and or interactions

among exposures, as well as flexibility in both choice of kernel function and optional variable selection. The model is given by

$$Y_i = h(\sum \text{PFC}_i, \sum \text{PFCA}_i, \sum \text{PFSA}_i, \sum \text{Pesticide}_i, \sum \text{PCB}_i, \sum \text{PBDE}_i, \text{Blood THg}_i) + \mathbf{x_i}^{\mathbf{T}}\boldsymbol{\beta} + \epsilon_i$$

where Y_i is the absolute count of MHCII+ cells for dolphin i ($i = 1, \dots, n$), $h()$ is the exposure-response function of pollutants to be estimated, $\mathbf{x_i}$ is composed of covariates age and sex, and ϵ_i is the random error term assumed to be independent and identically normally distributed with mean 0 and common variance. The Gaussian kernel function used to represent the exposure-response function in our analysis can be expressed as

$$K(\mathbf{z}, \mathbf{z}') = exp\left\{-\sum_{m=1}^{7} r_m(z_m - z_m')^2\right\}$$

where **z** and **z'** denote vectors of pollutants for two different dolphins and $r_m \geq 0$ represents the tuning parameter that controls the smoothness of the exposure-response function as a function of pollutant m.

Of the two methods for variable selection that can be incorporated with BKMR, our analysis focused on hierarchical variable selection as component-wise variable selection may fail for highly correlated exposures as is the case with the pollutant concentrations of interest. For hierarchical variable selection, exposures are partitioned into groups such that highly correlated pollutants are grouped together and correlation across groups is low. No more than one pollutant from a group is able to enter the model at a time. For this analysis, ∑PCBs, ∑PBDEs, and ∑Pesticides were retained as individual pollutants. A combined group was formed for ∑PFCAs, ∑PFCs, and ∑PFSAs. All analyses were conducted running the MCMC sampler with a burn-in

of 25,000 iterations and 25,000 iterations for inference. Trace plots were inspected for model convergence.

2.3.3 Bayesian Kernel Machine Regression with Principal Components

Running BKMR without variable selection in this analysis would face issues with multicollinearity due to high correlation between pollutants, a typical issue in multi-pollutant analyses. One feature of PCA is the independence between resulting components. In this section, we also consider the application of BKMR without variable selection but with principal components as predictors instead of individual pollutants.

To improve convergence, modifications were made to default tuning parameters by specifying the standard deviation of the proposal distribution of the kernel parameters $r_1, \ldots, r_7$ for each pollutant so the acceptance rate is around 40. We also considered setting the kernel parameter to be identical for all three principle component predictors. All statistical analyses were conducted in R (version 3.5.1; R Foundation for Statistical Computing).

3. RESULTS

Summary statistics of dolphin characteristics are presented in **Table 1**. The mean age was 14.63 (± 8.19) for CHS dolphins and 11.55 (± 5.10) for IRL dolphins. Of the dolphins sampled from IRL, 72% (N = 47) were male compared to only 58% (N = 35) sampled from CHS. A comparison of pollutant concentration summary statistics across methods for handling missing data are presented in **Table 2**. Multiple imputation resulted in the highest average pollutant concentrations. Higher average concentrations were expected because otherwise missing values were treated as zero concentration when computing the sums. For all three methods for handling missing data, the single-pollutant regression model showed statistically significant associations

between absolute counts of MHCII+ and ∑PFCs, ∑PFSAs, and ∑PCBs (**Supplementary Table 1**).

3.1 Principal Component Analysis

We selected 3 principle components from the pollutants. The derived principal components and the weights of the pollutants that make up each component across methods for handling missing data are summarized in **Table 3**. The analysis identified the percentage of total variation described by the first three components as 83.58% for pollutant sums calculated without congener imputation, 83.42% for those calculated after random forest imputation on congeners, and an average of 83.35% across the 5 imputed datasets for those calculated after MICE imputation. Across methods for imputation, each of the principle components were primarily made up of the following pollutants: 1) ∑PFCs, ∑PFCAs, and ∑PFSAs, 2) ∑Pesticides, ∑PCBs, and ∑PBDEs, and 3) blood THg. A statistically significant association was found between absolute counts of MCHII+ and the first and second principal components across all methods for handling missing data (**Supplementary Table 2**).

3.2 Bayesian Kernel Machine Regression

The univariate relationship between each pollutant and absolute counts of MCHII+ where all other exposures are fixed to their 50th percentile are plotted in **Figure 2** for each imputation method. From **Figure 2A**, we see that increasing values of PFCAs were associated with a decrease in the log absolute counts of MCHII+ cells. Panels **B, C,** and **D** of **Figure 2** all show decreasing values log absolute counts of MCHII+ cells for increased values of principle component 1.

Figure 3 shows the joint (multi-pollutant) exposure-response function when all predictors are at a particular percentile as compared to when all of them are at their 50th percentile (reference level). Panel **A** exhibits a positive trend in contrast to panels **B**, **C**, and **D** which exhibit negative

trends. This difference can be explained as the weights of the primary pollutants of principle components 1 and 3 being negative.

Figure 4 shows the effect of a single exposure at its 75th percentile compared to its 25th percentile while the remaining exposures are fixed to a particular percentile (25^{th}, 50^{th}, or 75^{th}) is plotted in **Figure 4.** This plot is useful for examining complex interactions between exposures. Higher values of PFCAs (panel **A**) as well as lower values of principle components 1 and 2 (panels **B**, **C**, and **D**) were associated with higher values of the exposure-response function. In addition, panels **B**, **C**, and **D** suggest an interaction between all three components because the effects vary slightly when other exposures were fixed at different levels.

In Figures 2,3 and 4, panels **B**, **C**, and **D** show similar results in analyses conducted without accounting for missing data, with random forest imputation and with multiple imputation.

4. DISCUSSION

Bottlenose dolphins are the most common cetacean species found in coastal and estuarine ecosystems along the southeastern coast of the United States and an ideal sentinel species for monitoring ocean and human health due to their widespread coastal distribution and role as apex predators. Previous studies of bottlenose dolphin health have investigated the relationship between single pollutants and health outcomes. In this analysis, we conducted the first analysis to evaluate different methods for estimating the association between multi-pollutant mixtures and absolute counts of MCHII+ cells in 82 dolphins from Charleston, South Carolina and the Indian River Lagoon, Florida using single pollutant regression, principle component regression, Bayesian Kernel Machine Regression with individual pollutants and variable selection, and Bayesian Kernel Machine Regression with principle components. This was done while simultaneously evaluating

the impact of missing data by comparing analyses conducted imputing zero concentration for missing data, accounting for missing values with random forest imputation, and accounting for values with multiple imputation.

Single pollutant regression analysis showed statistically significant associations between absolute counts of MHCII+ and ∑PFCs, ∑PFSAs, and ∑PCBs. Similarly, principle component regression showed a statistically significant association between components 1 (which had the highest weights for ∑PFCs, ∑PFSAs, and ∑PFCAs) and 2 (which had the highest weights for ∑ Pesticides and ∑PCBs. Alternatively, Bayesian Kernel Machine Regression with hierarchical variable selection identified PFCAs as most influential. Principle components 1 and 2 were still found to be significant in Bayesian Kernel Machine Regression analyses with principle components as predictors. Of the methods presented, Bayesian Kernel Machine Regression with hierarchical variable selection yielded the most straightforward results by identifying a single predictor with the most influence.

Analyses conducted with different methods for handling missing data yielded similar results across all three methods with one exception - analyses conducted with multiple imputation resulted in only slightly larger standard errors than the other methods. Of the two imputation approaches implemented, this is the most conservative.

One limitation of this study is the reduction in sample size from 125 to 82 dolphins due to missing outcome observations. Though we were able to impute missing congener values, we did not impute outcome observations. Additionally, the methods that we did implement make up only a small percentage of the available methods for investigating the impacts of multi-pollutant mixtures. Future studies can be conducted to compare these methods with those implemented in this study.

5. TABLES AND FIGURES

Figure 1. Breakdown of pollutants congeners discarded and categorized.

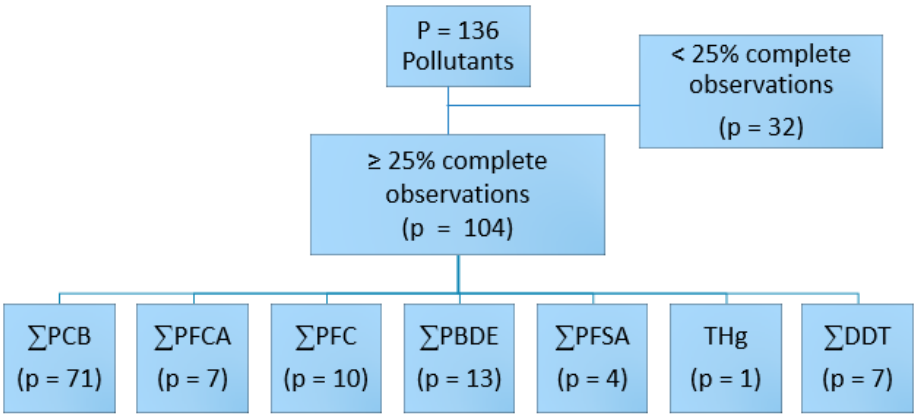


Table 1. Summary statistics of dolphin characteristics by site. Mean (± SD) are reported for continuous variables and frequency (%) are reported for categorical.

		Site	
	All [**N** = 125]	**Charleston Harbor, SC** [**n** = 60 (48%)]	**Indian River Lagoon, FL** [**n** = 65 (52%)]
Age (years)	13.02 (± 6.91)	14.63 (± 8.19)	11.55 (± 5.10)
Length[*](cm)	230.45 (± 22.21)	229.20 (± 24.41)	231.63 (± 20.02)
Gender			
Male	82 (66%)	35 (58%)	47 (72%)
Female	43 (34%)	25 (42%)	18 (28%)
Weight[**] (pounds)	321.43 (± 83.48)	330.12 (± 93.40)	314.29 (± 74.36)
Year			
2003	54 (43.2%)	33 (55%)	21 (32.31%)
2004	34 (27.2%)	10 (16.67%)	24 (36.92%)
2005	32 (25.6%)	17 (28.33%)	15 (23.08%)
2007	2 (1.6%)	0 (0%)	2 (3.08%)
2010	1 (0.8%)	0 (0%)	1 (1.54%)
2011	2 (1.6%)	0 (0%)	2 (3.08%)

[*]2 missing values; [**]12 missing values

Table 2. Comparison of polluant concentration summary statistics across methods for handling missing data.

	Without Imputation			**Random Forest Imputation**			**Average MICE Imputation**		
	Mean (SD)	**Min**	**Max**	**Mean (SD)**	**Min**	**Max**	**Mean (SD)**	**Min**	**Max**
∑ PFC	1,499.2 (1,290.49)	112	8,640.4	1,514.9 (1,289.76)	117.2	8,640.4	1,561.16 (1,276.72)	143.64	8,640.4
∑ PFCA	228.39 (304.33)	10.87	2,005.91	228.92 (304.29)	10.87	2,005.91	228.99 (304.3)	10.87	2,005.1
∑ PFSA	1,270.8 (1,077.96)	101.1	7,059.0	1,286.0 (1,076.6)	106.4	7,059.0	1,332.2 (1,064.28)	127.86	7059.0
∑ Pesticides	6,435.2 (4,376.95)	153.9	21,199.8	6,435.2 (4,376.95)	153.9	21,199.8	6,435.2 (4,376.95)	153.9	21,199.8
∑ PCB	23,472.7 (14,957.01)	428.7	75,957.9	23,920.7 (14,851.65)	428.7	75,957.9	24,144.12 (14,755.55)	428.7	75,957.9
∑ PBDE	1,159.21 (1,234.24)	98.19	8,881.72	1,159.3 (1,234.2)	101.8	8,881.7	1,159.21 (1,234.22)	100.44	8,881.71
Blood THg	0.40 (0.45)	0.03	2.75	0.40 (0.45)	0.03	2.75	0.40 (0.45)	0.03	2.75

Table 3. Derived principal components and the weights of the pollutants that make up each component across methods for handling missing data.

	Without Imputation			**Random Forest Imputation**			**Average MICE Imputation**		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
∑ PFC	-0.55	-0.18	-0.24	-0.54	-0.20	-0.23	-0.54	-0.22	-0.23
∑ PFCA	-0.51	-0.06	-0.01	-0.50	-0.08	0.00	-0.50	-0.09	0.02
∑ PFSA	-0.52	-0.20	-0.28	-0.51	-0.22	-0.28	-0.51	-0.23	-0.28
∑ Pesticides	-0.10	0.61	-0.17	-0.12	0.61	-0.16	-0.13	0.61	-0.16
∑ PCB	0.00	0.58	-0.39	-0.03	0.58	-0.39	-0.06	0.58	-0.40
∑ PBDE	-0.29	0.42	0.22	-0.30	0.41	0.23	-0.31	0.40	0.24
Blood THg	0.29	-0.17	-0.80	0.29	-0.16	-0.80	0.29	-0.16	-0.79

Figure 2. Univariate relationship between each pollutant and absolute counts of MCHII+ cells where all other exposures are fixed to their 50th percentile with **A**. variable selection not accounting for missing data, **B.** principle components as predictors not accounting for missing data, **C.** principle components as predictors with random forest imputation, and **D.** principle components as predictors with multiple imputation.

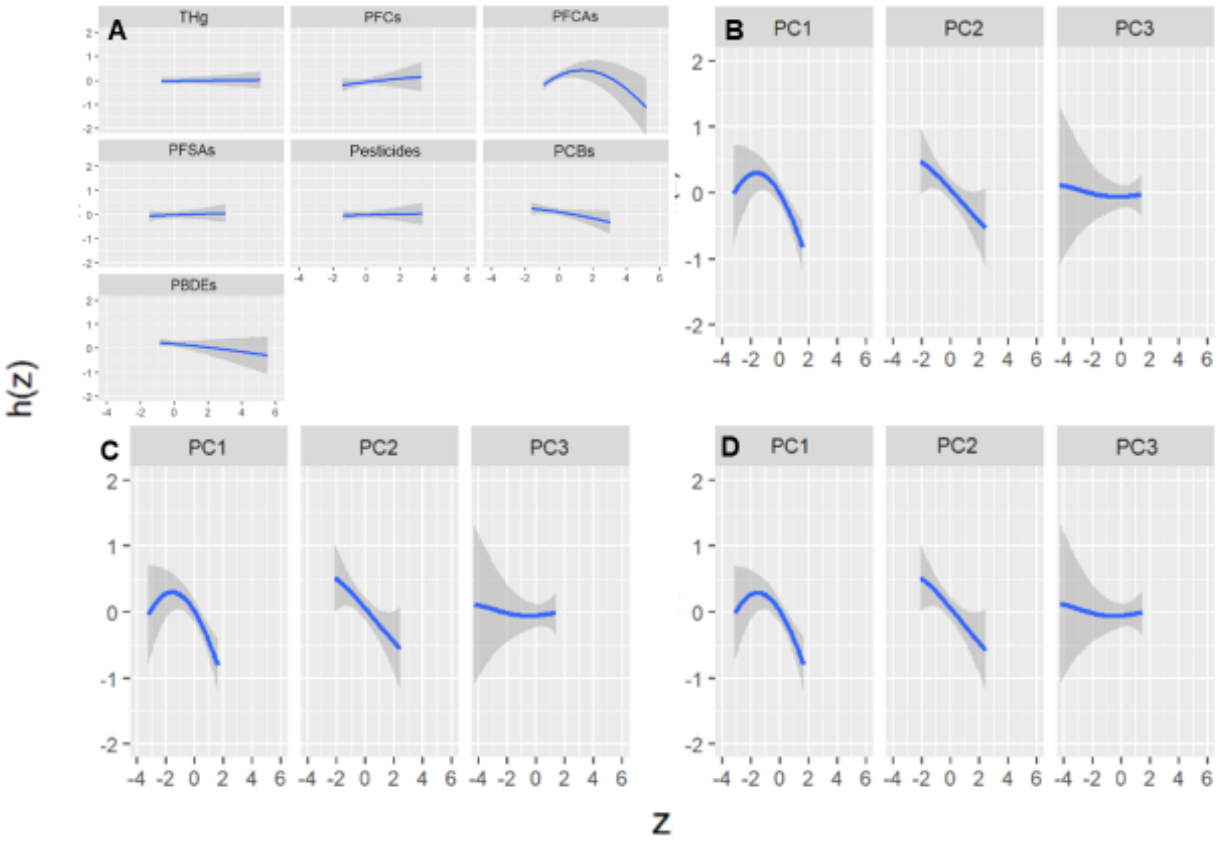


Figure 3. Comparison of the value of the exposure-response function when all predictors are at a particular percentile as compared to when all of them are at their 50th percentile with **A**. variable selection not accounting for missing data, **B.** principle components as predictors not accounting for missing data, **C.** principle components as predictors with random forest imputation, and **D.** principle components as predictors with multiple imputation.

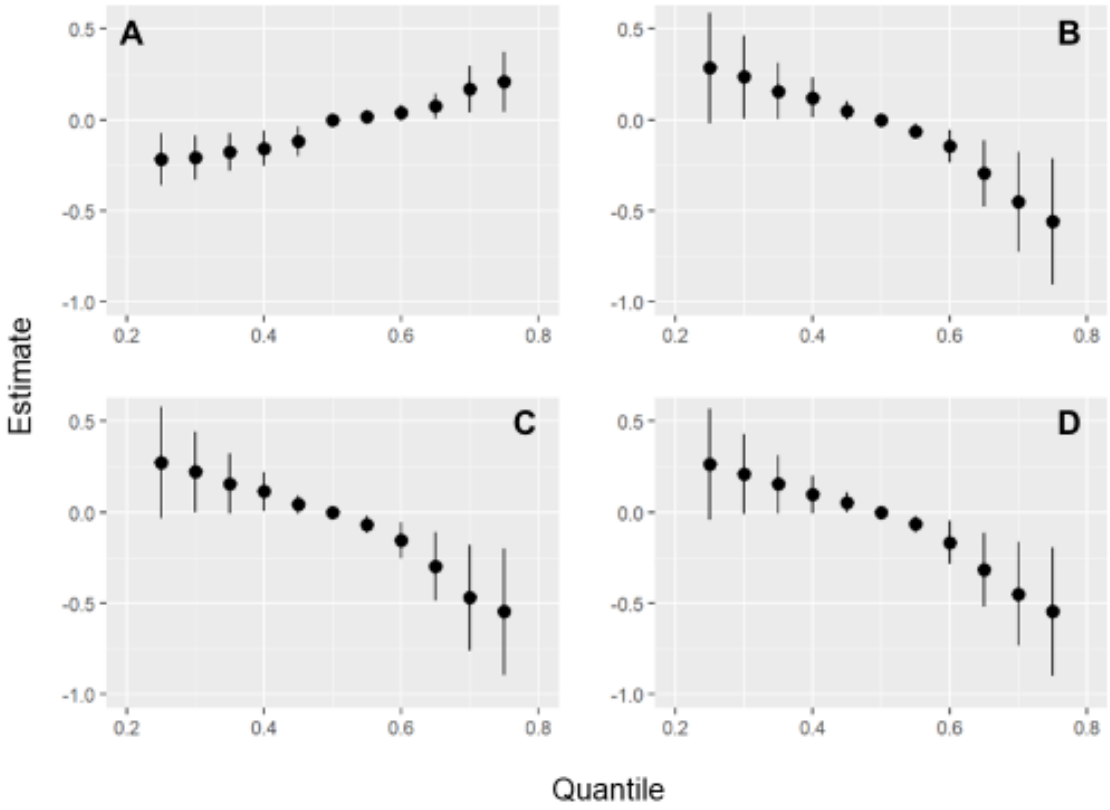
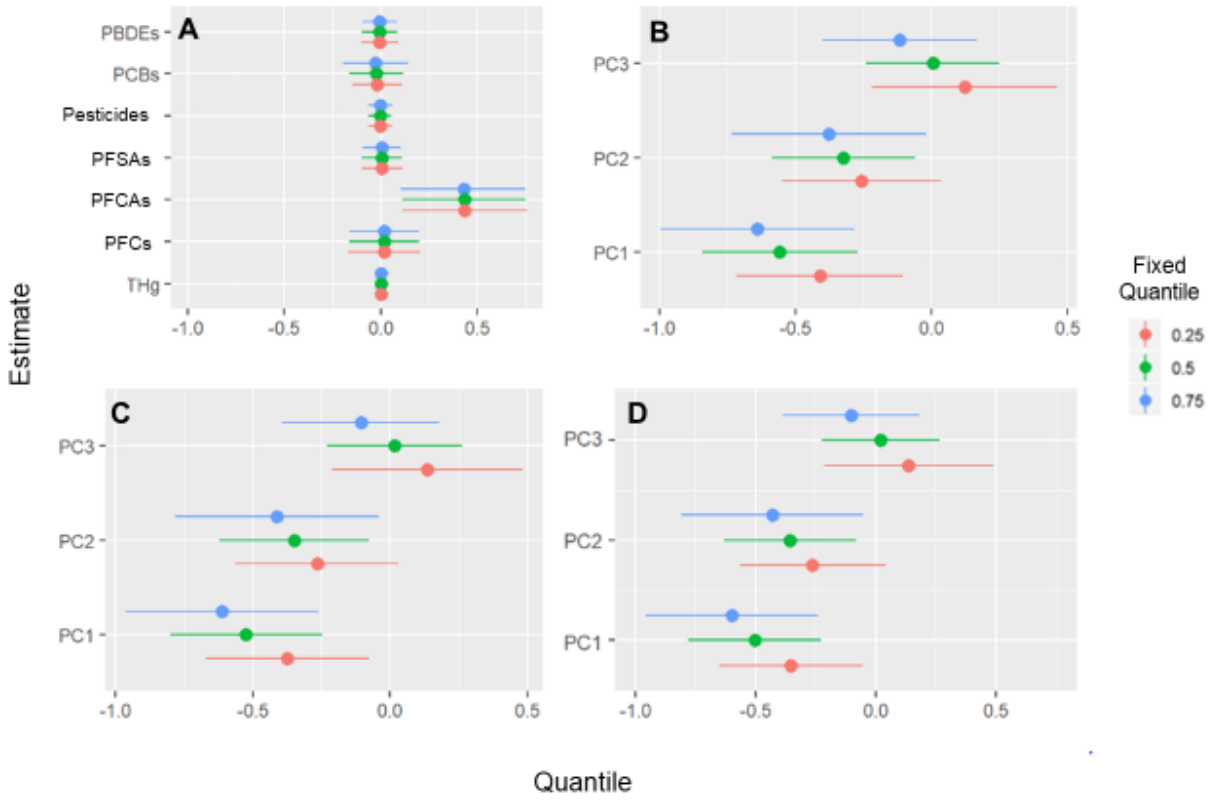


Figure 4. Value of the exposure-response function for a predictor at its 75th percentile as compared to when that predictor is at its 25th percentile while the remaining exposures are fixed to particular percentiles with **A**. variable selection not accounting for missing data, **B.** principle components as predictors not accounting for missing data, **C.** principle components as predictors with random forest imputation, and **D.** principle components as predictors with multiple imputation.



Supplementary Tables

Table 1. Parameter estimates across methods for handling missing data from single-pollutant regression analyses conducted between each individual pollutant and log-transformed absolute count of MCHII+ cells, adjusted for age and sex.

	Without Imputation	**Random Forest Imputation**	**Average MICE Imputation**
	Estimate (± Standard Error)	**Estimate (± Standard Error)**	**Estimate (± Standard Error)**
∑ PFC	0.00 (± 0.00)**	0.00 (± 0.00)**	0.00 (± 0.00)**
∑ PFCA	0.00 (± 0.00)*	0.00 (± 0.00)*	0.00 (± 0.00)*
∑ PFSA	0.00 (± 0.00)**	0.00 (± 0.00)**	0.00 (± 0.00)**
∑ Pesticides	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
∑ PCB	0.00 (± 0.00)**	0.00 (± 0.00)**	0.00 (± 0.00)**
∑ PBDE	0.00 (± 0.00)	0.00 (± 0.00)	0.00 (± 0.00)
Blood THg	-0.11 (± 0.14)	-0.11 (± 0.14)	-0.11 (± 0.14)

* P-value < 0.1; ** P-value < 0.05; *** P-value < 0.01

Table 2. Results of regression analysis between principal component scores one through three and log-transformed absolute count of MCHII+ cells across methods for handling missing data, adjusted for age and sex.

Method	**PC1**	**PC2**	**PC3**
Without Imputation	-0.17 (± 0.06)***	-0.13 (± 0.06)**	-0.01 (± 0.07)
Random Forest Imputation	-0.16 (± 0.06)***	-0.14 (± 0.06)**	-0.00 (± 0.07)
Average MICE Imputation	-0.16 (± 0.06)***	-0.14 (± 0.06)**	-0.01 (± 0.07)

* P-value < 0.1; ** P-value < 0.05; *** P-value < 0.01

6. REFERENCES

Agay-Shay, K., Martinez, D., Valvi, D., Garcia-Esteban, R., Basagaña, X., Robinson, O., Casas, M., Sunyer, J., & Vrijheid, M. (2015). Exposure to endocrine-disrupting chemicals during pregnancy and weight at 7 years of age: A multi-pollutant approach. *Environmental Health Perspectives*. https://doi.org/10.1289/ehp.1409049

Anderson, T. W. (1985). An Introduction to Multivariate Statistical Analysis, 2nd Edition. *Biometrics*. https://doi.org/10.2307/2531310

Billionnet, C., Sherrill, D., & Annesi-Maesano, I. (2012). Estimating the Health Effects of Exposure to Multi-Pollutant Mixture. In *Annals of Epidemiology*. https://doi.org/10.1016/j.annepidem.2011.11.004

Bobb, J. F., Valeri, L., Claus Henn, B., Christiani, D. C., Wright, R. O., Mazumdar, M., Godleski, J. J., & Coull, B. A. (2014). Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics*. https://doi.org/10.1093/biostatistics/kxu058

Bossart, G. D. (2011). Marine mammals as sentinel species for oceans and human health. *Veterinary Pathology*. https://doi.org/10.1177/0300985810388525

Campion, W. M., & Rubin, D. B. (1989). Multiple Imputation for Nonresponse in Surveys. *Journal of Marketing Research*. https://doi.org/10.2307/3172772

Chiu, Y. H., Bellavia, A., James-Todd, T., Correia, K. F., Valeri, L., Messerlian, C., Ford, J. B., Mínguez-Alarcón, L., Calafat, A. M., Hauser, R., & Williams, P. L. (2018). Evaluating effects of prenatal exposure to phthalate mixtures on birth weight: A comparison of three statistical approaches. *Environment International*. https://doi.org/10.1016/j.envint.2018.02.005

Fair, P. A., Adams, J., Mitchum, G., Hulsey, T. C., Reif, J. S., Houde, M., Muir, D., Wirth, E., Wetzel, D., Zolman, E., McFee, W., & Bossart, G. D. (2010). Contaminant blubber burdens in Atlantic bottlenose dolphins (Tursiops truncatus) from two southeastern US estuarine areas: Concentrations and patterns of PCBs, pesticides, PBDEs, PFCs, and PAHs. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2009.12.021

Fair, P. A., Romano, T., Schaefer, A. M., Reif, J. S., Bossart, G. D., Houde, M., Muir, D., Adams, J., Rice, C., Hulsey, T. C., & Peden-Adams, M. (2013). Associations between perfluoroalkyl compounds and immune and clinical chemistry parameters in highly exposed bottlenose dolphins (Tursiops truncatus). *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.2122

Gelman, A., & Hill, J. (2006). Data Analysis Using Regression and Multilevel/Hierarchical Models. In *Data Analysis Using Regression and Multilevel/Hierarchical Models*. https://doi.org/10.1017/cbo9780511790942

Hohn, A. A., Scott, M. D., Wells, R. S., Sweeney, J. C., & Irvine, A. B. (1989). GROWTH LAYERS IN TEETH FROM KNOWN-AGE, FREE-RANGING BOTTLENOSE DOLPHINS. *Marine Mammal Science*. https://doi.org/10.1111/j.1748-7692.1989.tb00346.x

Kim, S. S., Meeker, J. D., Keil, A. P., Aung, M. T., Bommarito, P. A., Cantonwine, D. E., McElrath, T. F., & Ferguson, K. K. (2019). Exposure to 17 trace metals in pregnancy and associations with urinary oxidative stress biomarkers. *Environmental Research*. https://doi.org/10.1016/j.envres.2019.108854

Lahvis, G. P., Wells, R. S., Kuehl, D. W., Stewart, J. L., Rhinehart, H. L., & Via, C. S. (1995). Decreased lymphocyte responses in free-ranging bottlenose dolphins (Tursiops truncatus) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environmental Health Perspectives*. https://doi.org/10.2307/3432414

Li, M. C., Mínguez-Alarcón, L., Bellavia, A., Williams, P. L., James-Todd, T., Hauser, R., Chavarro, J. E., & Chiu, Y. H. (2019). Serum beta-carotene modifies the association between phthalate mixtures and insulin resistance: The National Health and Nutrition Examination Survey 2003–2006. *Environmental Research*. https://doi.org/10.1016/j.envres.2019.108729

Murdoch, M. E., Varela, R., Hansen, L., Townsend, F., Bryan, C., Christopher, S., Pugh, R., & Bossart, G. D. (2006). Protocols for Conducting Dolphin Capture-Release Health Assessment Studies. *NOAA Technical Memorandum NOS NCCOS*.

Reif, J. S., Schaefer, A. M., & Bossart, G. D. (2015). Atlantic bottlenose dolphins (Tursiops truncatus) as a sentinel for exposure to mercury in humans: Closing the loop. In *Veterinary Sciences*. https://doi.org/10.3390/vetsci2040407

Reif, J. S., Schaefer, A. M., Bossart, G. D., & Fair, P. A. (2017). Health and environmental risk assessment project for bottlenose dolphins Tursiops truncatus from the southeastern USA. II. Environmental aspects. In *Diseases of Aquatic Organisms*. https://doi.org/10.3354/dao03143

Richards, S., Paterson, E., Withers, P. J. A., & Stutter, M. (2016). Septic tank discharges as multi-pollutant hotspots in catchments. *Science of the Total Environment*. https://doi.org/10.1016/j.scitotenv.2015.10.160

Schaefer, A. M., Stavros, H. C. W., Bossart, G. D., Fair, P. A., Goldstein, J. D., & Reif, J. S. (2011). Associations between mercury and hepatic, renal, endocrine, and hematological parameters in atlantic bottlenose dolphins (tursiops truncatus) along the eastern coast of Florida and South Carolina. *Archives of Environmental Contamination and Toxicology*. https://doi.org/10.1007/s00244-011-9651-5

Stafoggia, M., Breitner, S., Hampel, R., & Basagaña, X. (2017). Statistical Approaches to Address Multi-Pollutant Mixtures and Multiple Exposures: the State of the Science. In *Current environmental health reports*. https://doi.org/10.1007/s40572-017-0162-z

Stekhoven, D. J., & Bühlmann, P. (2012). Missforest-Non-parametric missing value imputation for mixed-type data. *Bioinformatics*. https://doi.org/10.1093/bioinformatics/btr597

Valeri, L., Mazumdar, M. M., Bobb, J. F., Henn, B. C., Rodrigues, E., Sharif, O. I. A., Kile, M. L., Quamruzzaman, Q., Afroz, S., Golam, M., Amarasiriwardena, C., Bellinger, D. C., Christiani, D. C., Coull, B. A., & Wright, R. O. (2017). The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20–40 months of age: Evidence from rural Bangladesh. *Environmental Health Perspectives*. https://doi.org/10.1289/EHP614

van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*. https://doi.org/10.18637/jss.v045.i03