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Baylin Joseph Bennett Date

Per- and Polyfluoroalkyl Substances and Cortisol Biomarkers in Wild Bottlenose Dolphins (Tursiops truncatus) from Charleston, South Carolina and Indian River Lagoon, Florida

By

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Master of Public Health

Global Environmental Health

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Matthew O. Gribble, PhD DABT

Committee Chair

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By

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B.S.
George Fox University
2014

Thesis Committee Chair: Matthew O. Gribble, PhD DABT

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Abstract

Per- and Polyfluoroalkyl Substances and Cortisol Biomarkers in Wild Bottlenose Dolphins (Tursiops truncatus) from Charleston, South Carolina and Indian River Lagoon, Florida

By Baylin Joseph Bennett

Objective: Identify associations between the presence of blood per- and polyfluoroalkyl substances (PFAS) levels and blood cortisol levels in wild bottlenose dolphins from off the coast of Charleston, South Carolina and Indian River Lagoon, Florida.

Methods: Wild bottlenose dolphin PFAS and cortisol levels were previously obtained. Associations were assessed using linear regression, Tobit regression, and parametric quantile regression controlling for bottlenose dolphin age and sex and year and location at time of sample collection. Further, results were stratified by bottlenose dolphin sex and age.

Results: Free cortisol showed statistically significant negative associations with PFOS, PFOA, and PFHxS and positive associations with PFTriA. Bound cortisol showed statistically significant positive associations with PFOS, PFDA, PFDoDA, PFDS, PFTA, PFTriA, and PFUA. Total cortisol showed negative associations with PFOA and PFHxS and positive associations with PFDA, PFDoDA, PFTA, and PFTriA.

Conclusions: Using Tobit regression to account for detection limits and parametric quantile regression as a biomarker tool are both novel approaches to assessing the presence of associations between wild bottlenose dolphin blood PFAS and blood cortisol. Taken together, the results indicate a relationship between PFAS levels and cortisol levels. Blood cortisol is a biomarker for stress. Stress has been proposed as playing a potential role in consequent autoimmune disease. Given that the wild bottlenose dolphins off the coast of Charleston, South Carolina share a crucial dietary fish source with the Gullah/Geechee population, who have a profound disparity in lupus prevalence, further research should be conducted to define the role PFAS through dietary consumption might have in developing autoimmune diseases.

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Introduction

Per- and polyfluoroalkyl substances (PFAS) are manmade chemicals that are manufactured and used because of their many useful properties, including hydrophobicity and oleophobicity. These properties have caused PFAS to be used widely, such as non-stick cookware, weather-resistant coatings for apparel, and fire suppressing foams.[1] The PFAS family comprises over 8,000 chemicals.[2] The key identifier of PFAS are carbon-fluorine bonds, which are considered the strongest bond in organic chemistry.[3] The family is commonly grouped, either "short-chain" or "long-chain", based on the length of the carbon-chain backbone.[4] In 2008, two commonly used long-chain PFAS, PFOA and PFOS, were recognized as being associated with negative human health outcomes.[5] They were quickly replaced with short-chain PFAS, because it was believed that short-chain PFAS were considered "safer". Recent findings have proven contrary.[6] Certain PFAS have shown an ability to both bioaccumulate and biomagnify in the environment.[7,8] In a study using NHANES data, 4 separate PFAS were measured in over 98% of human serum samples.[10] Further, PFAS have been measured in the blood serum of workers who use PFAS as well as in residents who live around those manufacturing plants.[10] Finally, PFAS chemicals have also been measured in the environment and wildlife.12[11,12,13]

Bottlenose dolphins (*Tursiops truncatus*) are recognized as apex predators, keystone species, and sentinel species, which makes them perfect candidates to assess environmental PFAS contamination.[14] Of particular importance to this paper, sentinel species are organisms that are used as indicators "to gain early warnings about current or potential negative impacts on individual- and population-level animal health."[15] PFAS chemicals have been measured in bottlenose dolphin blood plasma.[16] Cortisol is a common biological tool to assess wildlife stress.[17,18] Further, free cortisol, as opposed to bound cortisol or total cortisol, is the only cortisol type with any biological activity.[19] Dietary consumption of fish and shellfish have been associated with physiological PFAS levels.[20] Further, the dolphins in the Charleston, South Carolina harbor from this study are consuming the same fish population as an African American population currently being assessed for disproportional lupus disparities.[21]

The Gullah/Geechee people of South Carolina are a population of African American fishers who heavily rely on fish and other seafood as a crucial dietary source.[22] Further, it has been established this population has a profound disparity in lupus prevalence among their population.[23] A proposed link for these lupus disparities is the dietary reliance on fish consumption.[24] Further, stress has recently been suggested to play a role in "subsequent autoimmune disease", such as lupus.[25] This study aims to further the current broader one health research being done in Charleston, South Carolina on the African American disparities in lupus among the Gullah/Geechee population using veterinary epidemiology. To the best of the authors' knowledge, an association between any PFAS and bottlenose dolphin blood plasma has never been assessed.

Materials and Methods

Sample Collection

Blood samples were collected, prepared, and analyzed as previously described from two cohorts located either off the coast of Charleston, South Carolina or the Indian River Lagoon, Florida.[26]

PFOS Tertiles	Cortisol Type	Mean	25th Percentile	Median	75th Percentile	IQR
1	Free	3.031	1.996	2.887	3.920	1.925
	Bound	4.469	1.825	3.637	6.233	4.408
	Total	7.021	4.441	6.593	8.828	4.386
2	Free	2.864	1.508	2.638	4.007	2.500
	Bound	4.018	1.976	3.805	5.612	3.636
	Total	6.484	4.414	6.428	8.317	3.903
3	Free	2.555	1.663	2.576	3.300	1.638
	Bound	3.515	2.134	3.633	4.704	2.570
	Total	5.703	4.276	5.628	7.228	2.952
Total	Free	2.819	1.698	2.637	3.703	2.005
	Bound	4.003	2.003	3.726	5.501	3.498
	Total	6.406	4.386	6.069	8.110	3.724

Table 1. Values for mean, 25th percentile, median, 75th percentile, and IQR for each cortisol type within each PFOS tertile.

Statistical Analyses

Stata 16.1® software was used to perform all statistical analyses.

Linear Regression

One of three types of blood cortisol concentrations (10nM) – total, bound, or free - was used as the dependent variable. For the independent variable, each PFAS was grouped into tertiles separately. Bottlenose dolphin age and sex as well as the year and location at time of sample collection were all considered due to their confounding potential. Finally, the standard error (SE) estimates were considered robust. The linear regression model used was:

$$Y = \beta_1 X_1 + \beta_2 Z_1 + \beta_3 Z_2 + \beta_4 Z_3 + \beta_5 Z_4 + \epsilon_t$$

where Y is the specific cortisol type, X_1 is the tertiles for the specific PFAS concentration, Z_1 is the potential confounding term representing the year the dolphin was captured and sampled, Z_2 is the potential confounding term representing the sex of the dolphin, Z_3 is the potential confounding term representing the location the dolphin was captured and sampled, and Z_4 is the potential confounding term representing the age of the dolphin at capture and sampling. The ϵ_t represents the normally distributed error term. Additionally, the lowest tertile for each PFAS contained all values that were below limit of quantitation (LOQ). Results were stratified by sex and age. Age stratification categories were juveniles (females <7 years old and males <10 years old) and adults (females ≥7 years old and males ≥10 years old). Finally, all p-values reported for linear regression model results are from T-tests.

Tobit Regression

The log of the concentrations for an individual PFAS was used as the dependent variable. For the independent variable, blood cortisol (total, bound, or free) was used. Bottlenose dolphin age and sex as well as the year and location at time of sample collection were all considered due to their confounding potential. Finally, for the left-censoring variable limit for each model, the log of each LOQ for each year for each PFAS was used. The Tobit regression model used was:

$$Y_t^* = \beta_0 + \beta_1 X_1 + \beta_2 Z_1 + \beta_3 Z_2 + \beta_4 Z_3 + \beta_5 Z_4 + \mu_t$$

where Y_t^* is the PFAS concentration, X_1 is the concentration of the specific cortisol (10nM), Z_1 is the potential confounding term representing the year the dolphin was captured and sampled, Z_2 is the potential confounding term representing the sex of the dolphin, Z_3 is the potential confounding term representing the location the dolphin was captured and sampled, and Z_4 is the potential confounding term representing the age of the dolphin at capture and sampling. The μ_t represents the normally distributed error term. PFAS concentration is left-censored based on the LOQ (LOQ), which is specific to the PFAS and the year the samples were measured. Additionally, results were stratified by sex and age. Age stratification categories were juveniles (females <7 years old and males <10 years old) and adults (females ≥7 years old and males ≥10 years old). Finally, all p-values reported for Tobit regression model results are from T-tests.

Parametric Quantile Regression

For the time dependent variable, blood cortisol (total, bound, or free) was used. Tertiles of PFAS, bottlenose dolphin age and sex as well as the year and location at time of sample collection were all considered independent variables. Distribution for each model was considered separately. Most models had either a lognormal or Weibull distribution except for bound cortisol set with tertiles of PFHpA, which had a generalized gamma distribution. Finally, the standard error (SE) estimates were considered robust. The quantile regression model used was:

$$Q_\tau(Y_\tau) = \beta_0(\tau) + \beta_1(\tau) X_1 + \beta_2(\tau) Z_1 + \beta_3(\tau) Z_2 + \beta_4(\tau) Z_3 + \beta_5(\tau) Z_4 + \mu_t$$

where Y_τ is the specific cortisol type, τ is the specific quantile, X_1 is the tertiles for the specific PFAS concentration, Z_1 is the potential confounding term representing the year the dolphin was captured and sampled, Z_2 is the potential confounding term representing the sex of the dolphin, Z_3 is the potential confounding term representing the location the dolphin was captured and sampled, Z_4 is the potential confounding term representing the age of the dolphin at capture and sampling, and μ_t represents the normally

distributed error term. Also, the lowest tertile for each PFAS contained all values that were below LOQ. Finally, lognormal and Weibull distributions are special cases of the 3-parameter generalized gamma distribution (i.e., lognormal is the 3-parameter gamma with lambda = 0 and Weibull is the 3-parameter gamma with lambda =1).[27] We fit proportionate percentiles parametric quantile regression models assuming a generalized gamma distribution and evaluated if the maximum likelihood estimate for lambda was significantly different from 0 or from 1. If the maximum likelihood generalized gamma was consistent with either lognormal or Weibull distribution, we fitted a simplified model assuming lognormal or Weibull. If both distributions fit, we used Weibull. More specifically, Weibull distribution was assumed for all PFAS in the models containing total cortisol. Additionally, Weibull distribution was assumed for all PFAS in the models containing free cortisol, except for PFHpA and PFDS in which lognormal was assumed for both models. For all PFAS in the models containing bound cortisol, lognormal distribution was assumed. Results were stratified by sex and age. Age stratification categories were juveniles (females <7 years old and males <10 years old) and adults (females ≥7 years old and males ≥10 years old). Finally, all p-values reported for linear regression model results are from Z-tests.

Results

Linear Regression (see Figure 1)

When assessing the PFAS cortisol association with free cortisol while controlling for the year and dolphins' age, sex, and location at sample collection, only one PFAS showed a positive association and three PFAS showed a negative association with free cortisol. Dolphins in the middle tertile of PFTriA exposure had a mean cortisol 2.00 (10^-8 M) higher than in the lowest-exposure reference group (0.56,3.44; p=0.007). Dolphins in the highest tertile of PFTriA exposure had a mean cortisol 3.43 (10^-8 M) higher than in the lowest-exposure reference group (1.10,5.77; p=0.005). Dolphins in the highest tertile of PFOS exposure had a mean cortisol 1.45 (10^-8 M) lower than in the lowest-exposure reference group (-2.64,-0.026; p=0.017). Dolphins in the middle tertile of PFOA exposure had a mean cortisol 1.65 (10^-8 M) lower than in the lowest-exposure reference group (-2.61,-0.16; p=0.001). Dolphins in the highest tertile of PFOA exposure

had a mean cortisol 1.40 (10^-8 M) lower than in the lowest-exposure reference group (-2.65,-0.16; p=0.027). Finally, dolphins in the highest tertile of PFHxS exposure had a mean cortisol 1.36 (10^-8 M) lower than in the lowest-exposure reference group (-2.53,-0.19; p=0.023).

When assessing the PFAS cortisol association with bound cortisol while controlling for the year and dolphins' age, sex, and location at sample collection, six PFAS showed a positive association, and there were zero PFAS that showed a negative association with bound cortisol. Dolphins in the middle tertile of PFOS exposure had a mean cortisol 0.50 (10^-8 M) higher than in the lowest-exposure reference group (0.01,0.99; p=0.045). Dolphins in the middle tertile of PFDA exposure had a mean cortisol 0.55 (10^-8 M) higher than in the lowest-exposure reference group (0.09,1.02; p=0.02). Dolphins in the middle tertile of PFDoDA exposure had a mean cortisol 0.65 (10^-8 M) higher than in the lowest-exposure reference group (0.27,1.03; p=0.001). Dolphins in the highest tertile of PFDoDA exposure had a mean cortisol 0.59 (10^-8 M) higher than in the lowest-exposure reference group (0.11,1.06; p=0.015). Dolphins in the highest tertile of PFDS exposure had a mean cortisol 0.59 (10^-8 M) higher than in the lowest-exposure reference group (0.33,2.36; p=0.01). Dolphins in the middle tertile of PFTA exposure had a mean cortisol 1.08 (10^-8 M) higher than in the lowest-exposure reference group (0.30;1.86; p=0.007). Dolphins in the highest tertile of PFTA exposure had a mean cortisol 0.71 (10^-8 M) higher than in the lowest-exposure reference group (0.21,1.21; p=0.006). Finally, dolphins in the middle tertile of PFUA exposure had a mean cortisol 0.50 (10^-8 M) higher than in the lowest-exposure reference group (0.04, 0.96; p=0.035).

When assessing the PFAS cortisol association with total cortisol while controlling for the year and dolphins' age, sex, and location at sample collection, three PFAS revealed a positive association relative to tertile one of the represented PFAS and two showed a negative association. Dolphins in the highest tertile of PFDA exposure had a mean cortisol 2.02 (10^-8 M) higher than in the lowest-exposure reference group (0.30,3.74; p=0.022). Dolphins in the middle tertile of PFDoDA exposure had a mean cortisol 1.26 (10^-8 M) higher than in the lowest-exposure reference group (0.14,2.38; p=0.028). Dolphins in the middle tertile of PFTriA exposure had a mean cortisol 1.97 (10^-8 M) higher than in the lowest-exposure reference group (0.39,3.55; p=0.016). Dolphins in the highest tertile of PFTriA exposure had a mean cortisol 3.79 (10^-8 M)

higher than in the lowest-exposure reference group (1.45,6.113; p=0.002). Dolphins in the middle tertile of PFOA exposure had a mean cortisol 1.68 (10^-8 M) lower than in the lowest-exposure reference group (-2.80,-0.56; p=0.003). Dolphins in the highest tertile of PFTriA exposure had a mean cortisol 1.72 (10^-8 M) lower than in the lowest-exposure reference group (-3.06,-0.37; p=0.013). Dolphins in the highest tertile of PFHxS exposure had a mean cortisol 1.41 (10^-8 M) lower than in the lowest-exposure reference group (-2.70,-0.12; p=0.032).

Results for all of the unadjusted models, sex-stratified models, and age-stratified models are listed as Supplemental Figures 1-5 in the Appendix. When the results were stratified by sex, the sample sizes of the female dolphins were noticeably small, which yielded wider confidence intervals compared to those of the male dolphins. Comparing the statistically significant adjusted non-stratified free cortisol results, dolphins in the middle tertile of PFOA exposure held their negative association with the stronger effect being seen in female dolphins, which a mean cortisol of -3.15 (10^-8 M) lower than in the lowest-exposure reference group (-4.94,-1.35; p=0.001), compared to male dolphins, which had a mean cortisol of -1.21 (10^-8 M) lower than in the lowest-exposure reference group (-2.33,-0.90; p=0.034). Additionally, the statistically significant results of both tertiles of PFTriA exposure in the adjusted non-stratified model, held for male dolphins, where male dolphins in the middle tertile of PFTriA exposure had a mean of 2.44 (10^-8 M) higher than in the lowest-exposure reference group (0.76,4.11; p=0.005), and male dolphins in the highest tertile of PFTriA exposure had a mean of 4.42 (10^-8 M) higher than in the lowest-exposure reference group (1.96,6.88; p=0.001); but the results were not seen in female dolphins. The statistically significant results seen in dolphins in the highest tertile of PFOA exposure and dolphins in the highest tertile of PFHxS exposure compared to the lowest-exposure reference group were not seen in the sex stratified results. Finally, several new statistically significant results were revealed by sex stratification. Rather than dolphins in the highest tertile of PFOS showing statistically significantly lower results than the lowest-exposure reference groups for free cortisol, male dolphins in the middle tertile of PFOS had a mean of -1.29 (10^-8 M) lower than in the lowest-exposure reference group (-2.54,-0.04; p=0.043); neither tertile of PFOA exposure yielded statistically significant results for female dolphins. Female dolphins in the middle tertile of PFDS exposure had a mean of 1.93 (10^-8 M) higher than in the lowest-exposure reference group (0.88,2.97; p=0.002).

Comparing the statistically significant adjusted non-stratified bound cortisol results, the statistically significant results for both tertiles of PFTA exposure in the adjusted non-stratified model held for male dolphins, where male dolphins in the middle tertile of PFTA exposure had a mean of 1.00 (10^-8 M) higher than in the lowest-exposure reference group (0.24,1.76; p=0.011), and male dolphins in the highest tertile of PFTA exposure had a mean of 0.92 (10^-8 M) higher than in the lowest-exposure reference group (0.26,1.58; p=0.007); but the results were not seen in female dolphins. Additionally, the statistically significant results of highest tertile of PFDS exposure in the adjusted non-stratified model, held for male dolphins, where male dolphins in the highest tertile of PFDS exposure had a mean of 1.57 (10^-8 M) higher than in the lowest-exposure reference group (0.48,2.66; p=0.005), whereas female dolphins in the highest tertile of PFDS exposure did not have results that were statistically significant, but female dolphins in the middle tertile of PFDS had a mean of 1.81 (10^-8 M) higher than in the lowest-exposure reference group (0.68,2.94; p=0.004). The statistically significant results seen in dolphins in the middle tertile of PFOS exposure, dolphins in the highest tertile of PFDoDA exposure, and dolphins in the middle tertile of PFUA exposure compared to the lowest-exposure reference group were not seen in the sex stratified results. Finally, several new statistically significant results were revealed by sex stratification. Rather than dolphins in the middle tertile of PFDA exposure showing statistically significantly lower results than the lowest-exposure reference groups for bound cortisol, male dolphins in the highest tertile of PFDA exposure had a mean of 0.92 (10^-8 M) higher than in the lowest-exposure reference group (0.15,1.70; p=0.02); neither tertile of PFDA exposure yielded statistically significant results for female dolphins. Male dolphins in the middle tertile of PFOA exposure mean of -0.84 (10^-8 M) lower than in the lowest-exposure reference group (-1.55,-0.13; p=0.02); neither tertile of PFOA exposure yielded statistically significant results for female dolphins.

Comparing the statistically significant adjusted non-stratified total cortisol results, the statistically significant results for both tertiles of PFOA exposure in the adjusted non-stratified model held for female dolphins, where female dolphins in the middle tertile of PFOA exposure had a mean of -3.04 (10^-8 M) lower than in the lowest-exposure reference group (-4.93,-1.15; p=0.002), and female dolphins in the highest tertile of PFOA exposure had a mean of -2.37 (10^-8 M) lower than in the lowest-exposure reference group (-4.60,-0.14; p=0.038); whereas the results were not seen in male dolphins in the highest tertile of PFOA exposure, but were seen in the middle tertile of PFOA exposure where male dolphins had a mean of -1.34 (10^-8 M) lower than in the lowest-exposure reference group (-2.67,-0.01; p=0.049). Additionally, the

statistically significant results of highest tertile of PFDA exposure in the adjusted non-stratified model, held for male dolphins, where male dolphins in the highest tertile of PFDA exposure had a mean of 3.19 (10^-8 M) higher than in the lowest-exposure reference group (1.21,5.17; p=0.002), whereas female dolphins in the highest tertile of PFDA exposure did not have results that were statistically significant. The statistically significant results of middle tertile of PFDoDA exposure in the adjusted non-stratified model, held for male dolphins, where male dolphins in the middle tertile of PFDoDA exposure had a mean of 1.41 (10^-8 M) higher than in the lowest-exposure reference group (0.11,2.71; p=0.034), whereas female dolphins in the middle tertile of PFDoDA exposure did not have results that were statistically significant. Additionally, the statistically significant results of highest tertile of PFHxS exposure in the adjusted non-stratified model, held for male dolphins, where male dolphins in the highest tertile of PFHxS exposure had a mean of -1.70 (10^-8 M) lower than in the lowest-exposure reference group (-3.34,-0.06; p=0.042), whereas female dolphins in the highest tertile of PFHxS exposure did not have results that were statistically significant. Both tertiles of PFTriA exposure in the adjusted non-stratified model held for male dolphins, where male dolphins in the middle tertile of PFTriA exposure had a mean of 2.58 (10^-8 M) higher than in the lowest-exposure reference group (0.48,4.67; p=0.017), and male dolphins in the highest tertile of PFTriA exposure had a mean of 4.60 (10^-8 M) higher than in the lowest-exposure reference group (2.03,7.18; p=0.001); but the results were not seen in female dolphins. Finally, several new statistically significant results were revealed by sex stratification. Male dolphins in the middle tertile of PFDA exposure had a mean of 1.88 (10^-8 M) higher than in the lowest-exposure reference group (0.38,3.39; p=0.014); neither tertile of PFDA exposure yielded statistically significant results for female dolphins. Female dolphins in the middle tertile of PFDS exposure had a mean of 3.67 (10^-8 M) higher than in the lowest-exposure reference group (2.64,4.72; p<0.001); neither tertile of PFDS exposure yielded statistically significant results for male dolphins. Female dolphins in the middle tertile of PFTA exposure had a mean of 3.85 (10^-8 M) higher than in the lowest-exposure reference group (0.02,7.69; p=0.049); neither tertile of PFTA exposure yielded statistically significant results for male dolphins.

When age stratifying the results, juvenile dolphins had much smaller sample sizes, which yield wider confidence intervals than adult dolphins. When the results were stratified by age, the sample sizes of the juvenile dolphins were noticeably small, which yielded wider confidence intervals compared to those of adult dolphins. Comparing the statistically significant adjusted non-stratified free cortisol results, the statistically

significant results for dolphins in the highest tertile of PFOS exposure in the adjusted non-stratified model held for juvenile dolphins, where juvenile dolphins in the highest tertile of PFOS exposure had a mean of -2.54 (10^-8 M) lower than in the lowest-exposure reference group (-4.96,-0.12; p=0.04); whereas the results were not seen in adult dolphins in either tertiles of PFOS exposure. Additionally, the statistically significant results for both tertiles of PFOA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the middle tertile of PFOA exposure had a mean of -1.18 (10^-8 M) lower than in the lowest-exposure reference group (-2.15,-0.20; p=0.018), and adult dolphins in the highest tertile of PFOA exposure had a mean of -1.55 (10^-8 M) lower than in the lowest-exposure reference group (-2.83,-0.27; p=0.018); whereas the results were not seen in juvenile dolphins in the highest tertile of PFOA exposure, but were seen in the middle tertile of PFOA exposure where juvenile dolphins had a mean of -2.28 (10^-8 M) lower than in the lowest-exposure reference group (-4.53,-0.03; p=0.047). The statistically significant results for dolphins in the highest tertile of PFHxS exposure in the adjusted non-stratified model held for juvenile dolphins, where juvenile dolphins in the highest tertile of PFHxS exposure had a mean of -3.81 (10^-8 M) lower than in the lowest-exposure reference group (-6.26,-1.37; p=0.003); whereas the results were not seen in adult dolphins in either tertiles of PFHxS exposure. The statistically significant results for dolphins in the highest tertile of PFTriA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the highest tertile of PFTriA exposure had a mean of 3.45 (10^-8 M) higher than in the lowest-exposure reference group (0.89,6.01; p=0.009); whereas the results were not seen in juvenile dolphins in either tertiles of PFTriA exposure. The statistically significant results seen in dolphins in the middle tertile of PFTriA exposure compared to the lowest-exposure reference group were not seen in the age stratified results. Finally, several new statistically significant results were revealed by sex stratification. Adult dolphins in the middle tertile of PFHpA exposure had a mean of 1.51 (10^-8 M) higher than in the lowest-exposure reference group (0.02,3.00; p=0.047); whereas juvenile dolphins in the middle tertile of PFHpA exposure had a mean of -2.61 (10^-8 M) lower than in the lowest-exposure reference group (-3.98,-1.24; p=0.047). Additionally, adult dolphins in the middle tertile of PFTA exposure had a mean of 1.41 (10^-8 M) higher than in the lowest-

exposure reference group (0.03,2.79; p=0.046); whereas the results were not seen in juvenile dolphins in either tertiles of PFTA exposure.

Comparing the statistically significant adjusted non-stratified bound cortisol results, the statistically significant results for dolphins in the middle tertile of PFOS exposure in the adjusted non-stratified model held for juvenile dolphins, where juvenile dolphins in the middle tertile of PFOS exposure had a mean of 1.12 (10^-8 M) higher than in the lowest-exposure reference group (0.27,1.97; p=0.011); whereas the results were not seen in adult dolphins in either tertiles of PFOS exposure. The statistically significant results for dolphins in the middle tertile of PFDA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the middle tertile of PFDA exposure had a mean of 0.57 (10^-8 M) higher than in the lowest-exposure reference group (0.04,1.10; p=0.036); whereas the results were not seen in juvenile dolphins in either tertiles of PFDA exposure. Additionally, the statistically significant results for both tertiles of PFDoDA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the middle tertile of PFDoDA exposure had a mean of 0.94 (10^-8 M) higher than in the lowest-exposure reference group (0.53,1.35; p<0.001), and adult dolphins in the highest tertile of PFDoDA exposure had a mean of 0.82 (10^-8 M) higher than in the lowest-exposure reference group 0.35,1.30; p=0.001); whereas the results were not seen in juvenile dolphins in either tertile of PFDoDA exposure. The statistically significant results for dolphins in the highest tertile of PFDS exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the highest tertile of PFDS exposure had a mean of 1.36 (10^-8 M) higher than in the lowest-exposure reference group (0.42,2.30; p=0.005); whereas the results were not seen in juvenile dolphins in either tertiles of PFDS exposure. The statistically significant results seen in dolphins in both tertiles of PFTA exposure and the middle tertile of PFUA exposure compared to the lowest-exposure reference group were not seen in the age stratified results. Finally, several new statistically significant results were revealed by sex stratification. Adult dolphins in the highest tertile of PFDA exposure had a mean of 0.85 (10^-8 M) higher than in the lowest-exposure reference group (0.08,1.62; p=0.031); whereas the results were not seen in juvenile dolphins in either tertiles of PFDA exposure. Additionally, adult dolphins in the middle tertile of PFDS exposure had a mean of 0.76 (10^-8 M) higher than in the lowest-exposure reference group

(0.003,1.51; p=0.049); whereas the results were not seen in juvenile dolphins in either tertiles of PFDS exposure.

Comparing the statistically significant adjusted non-stratified total cortisol results, the statistically significant results for dolphins in the middle tertile of PFOA exposure in the adjusted non-stratified model held for juvenile dolphins, where juvenile dolphins in the middle tertile of PFOA exposure had a mean of -2.66 (10^-8 M) lower than in the lowest-exposure reference group (-4.94,-0.37; p=0.023); whereas the results were not seen in adult dolphins in either tertiles of PFOA exposure. The statistically significant results for dolphins in the highest tertile of PFDA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the highest tertile of PFDA exposure had a mean of 2.04 (10^-8 M) higher than in the lowest-exposure reference group (0.14,3.93; p=0.035); whereas the results were not seen in juvenile dolphins in either tertiles of PFDA exposure. Additionally, the statistically significant results for the middle tertile of PFDoDA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the middle tertile of PFDoDA exposure had a mean of 1.35 (10^-8 M) higher than in the lowest-exposure reference group (0.23,2.47; p=0.019), whereas the results were not seen in juvenile dolphins in either tertile of PFDoDA exposure. The statistically significant results for dolphins in the highest tertile of PFHxS exposure in the adjusted non-stratified model held for juvenile dolphins, where juvenile dolphins in the highest tertile of PFHxS exposure had a mean of -3.89 (10^-8 M) lower than in the lowest-exposure reference group (-6.19,-1.59; p=0.001); whereas the results were not seen in juvenile dolphins in either tertiles of PFHxS exposure. The statistically significant results seen in dolphins in the highest tertile of PFOA exposure and both tertiles of PFTriA exposure compared to the lowest-exposure reference group were not seen in the age stratified results. Finally, several new statistically significant results were revealed by sex stratification. Adult dolphins in the middle tertile of PFDA exposure had a mean of 1.77 (10^-8 M) higher than in the lowest-exposure reference group (0.30,3.23; p=0.019); whereas the results were not seen in juvenile dolphins in either tertiles of PFDA exposure. Additionally, adult dolphins in the middle tertile of PFHpA exposure had a mean of 1.97 (10^-8 M) higher than in the lowest-exposure reference group (0.26,3.69; p=0.025); whereas juvenile dolphins in the middle tertile of PFHpA exposure had a mean of -4.01

(10^-8 M) lower than in the lowest-exposure reference group (-5.54,-2.48; $p<0.001$). Finally, adult dolphins in the middle tertile of PFTA exposure had a mean of 2.33 (10^-8 M) higher than in the lowest-exposure reference group (0.40,4.26; $p=0.019$); whereas juvenile dolphins in the middle tertile of PFTA exposure had a mean of -19.50 (10^-8 M) lower than in the lowest-exposure reference group (-38.95,-0.04; $p<0.05$).

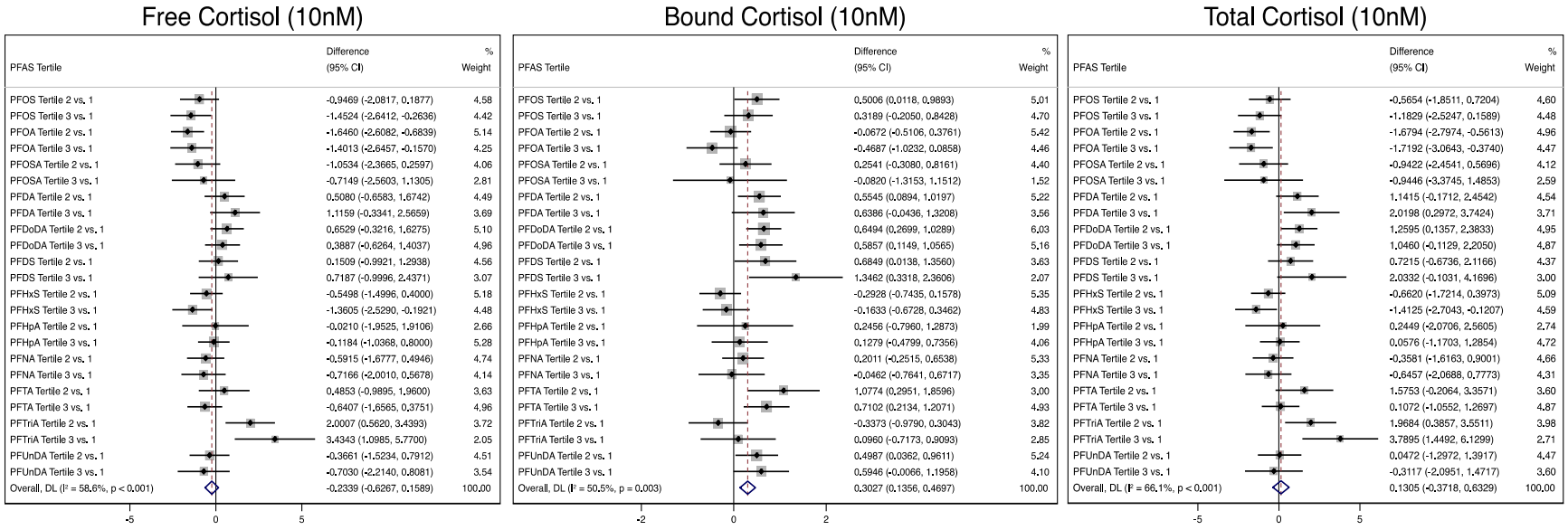

Figure 1. Results for adjusted linear regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: For free and total, PFOS (n=195), PFOA (n=214), PFOSA (n=187), PFDA (n=214), PFDoDA (n=214), PFDS (n=88), PFHxS (n=214), PFHpA (n=85), PFNA (n=214), PFTA (n=102), PFTriA (n=77), and PFUA (n=214); for Bound PFOS (n=200), PFOA (n=219), PFOSA (n=191), PFDA (n=219), PFDoDA (n=219), PFDS (n=92), PFHxS (n=219), PFHpA (n=89), PFNA (n=219), PFTA (n=104), PFTriA (n=77), and PFUA (n=219).

Tobit Regression (see Figure 2)

When assessing the PFAS cortisol association with free cortisol while controlling for the year and dolphins' age, sex, and location at sample collection under censoring conditions, one PFAS showed a positive association with free cortisol levels and two showed a negative association. The geometric mean PFTriA per 10nM free cortisol was 1.18 (1.05,1.32; p=0.004). The geometric mean PFOA per 10nM free cortisol was 0.94 (0.90,0.99; p=0.008). Finally, the geometric mean PFHxS per 10nM free cortisol was 0.93 (0.88,0.98; p=0.009).

When assessing the PFAS cortisol association with bound cortisol while controlling for the year and dolphins' age, sex, and location at sample collection under censoring conditions, two PFAS showed a positive association with bound cortisol levels and zero showed a negative association. The geometric mean PFDoDA per 10nM bound cortisol was 1.12 (1.01,1.23; p=0.029). Finally, the geometric mean PFTA per 10nM bound cortisol was 1.37 (1.14,1.64; p=0.001).

When assessing the PFAS cortisol association with total cortisol while controlling for the year and dolphins' age, sex, and location at sample collection under censoring conditions, one PFAS showed a positive association with bound cortisol levels and one showed a negative association. The geometric mean PFTriA per 10nM total cortisol was 1.19 (1.07,1.33; p=0.001). Finally, geometric mean PFOA per 10nM total cortisol was 0.95 (0.92,0.99; p=0.007).

Results for all of the unadjusted models, sex-stratified models, and age-stratified models are listed as Supplemental Figures 6-10 in the Appendix. When the results were stratified by sex, the sample sizes of the female dolphins were noticeably small, which yielded wider confidence intervals compared to those of the male dolphins. Comparing the statistically significant adjusted non-stratified free cortisol results, the geometric mean PFOA per 10nM free cortisol in female dolphins held a negative association where the geometric mean PFOA per 10nM free cortisol was 0.90 (0.81,0.996; p=0.042); whereas male dolphins did not show this result. Additionally, the geometric mean PFTriA per 10nM free cortisol in male dolphins held a positive association where the geometric mean PFTriA per 10nM free cortisol was 1.24 (1.04,1.48; p=0.016); whereas female dolphins did not show this result. The statistically significant geometric mean PFHxS per

10nM free cortisol result seen in the adjusted non-stratified result was not seen in the sex stratified results. Finally, there were zero new statistically significant results from sex stratification.

Comparing the statistically significant adjusted non-stratified bound cortisol results, the geometric mean PFDoDA per 10nM bound cortisol in male dolphins held a positive association where the geometric mean PFDoDA per 10nM bound cortisol was 1.13 (1.02,1.26; p=0.025); whereas female dolphins did not show this result. Additionally, the geometric mean PFTA per 10nM bound cortisol in male dolphins held a positive association where the geometric mean PFTA per 10nM bound cortisol was 1.48 (1.21,1.80; p<0.001); whereas female dolphins did not show this result. Finally, several new statistically significant results were revealed by sex stratification. The geometric mean PFOA per 10nM bound cortisol in male dolphins showed a negative association where the geometric mean PFOA per 10nM bound cortisol was 0.91 (0.84,0.99; p=0.026); whereas female dolphins showed a positive association where the geometric mean PFOA per 10nM bound cortisol was 1.41 (1.07,1.86; p=0.016). Additionally, the geometric mean PFDS per 10nM bound cortisol in male dolphins showed a positive association where the geometric mean PFDS per 10nM bound cortisol was 1.17 (1.02,1.35; p=0.029); whereas female dolphins did not show this result. Finally, the geometric mean PFHxS per 10nM bound cortisol in female dolphins showed a positive association where the geometric mean PFDS per 10nM bound cortisol was 1.36 (1.03,1.81; p=0.033); whereas male dolphins did not show this result.

Comparing the statistically significant adjusted non-stratified total cortisol results, the geometric mean PFOA per 10nM total cortisol in male dolphins held a negative association where the geometric mean PFOA per 10nM total cortisol was 0.98 (0.93,0.99; p=0.016); whereas female dolphins did not show this result. Additionally, the geometric mean PFTriA per 10nM total cortisol in male dolphins held a positive association where the geometric mean PFTriA per 10nM total cortisol was 1.27 (1.08,1.50; p=0.005); whereas female dolphins did not show this result. Finally, one new statistically significant result was revealed by sex stratification. The geometric mean PFDoDA per 10nM total cortisol in male dolphins showed a positive association where the geometric mean PFDoDA per 10nM total cortisol was 1.06 (1.005,1.11; p=0.032); whereas female dolphins did not show this result.

When the results were stratified by age, the sample sizes of the juvenile dolphins were noticeably small, which yielded wider confidence intervals compared to those of the adult dolphins. Comparing the statistically significant adjusted non-stratified free cortisol results, the geometric mean PFOA per 10nM free cortisol in adult dolphins held a negative association where the geometric mean PFOA per 10nM free cortisol was 0.94 (0.89,0.98; p=0.011); juvenile dolphins also held a negative association where the geometric mean PFOA per 10nM free cortisol was 0.92 (0.86,0.99; p=0.033). Additionally, the geometric mean PFHxS per 10nM free cortisol in juvenile dolphins held a negative association where the geometric mean PFHxS per 10nM free cortisol was 0.83 (0.76,0.90; p<0.001); whereas adult dolphins did not show this result. The statistically significant geometric mean PFTriA per 10nM free cortisol result seen in the adjusted non-stratified result was not seen in the age stratified results. Finally, there were several new statistically significant results from age stratification. Juvenile dolphins showed a negative association where the geometric mean PFOS per 10nM free cortisol was 0.90 (0.83,0.97; p=0.007). Additionally, juvenile dolphins showed a negative association where the geometric mean PFNA per 10nM free cortisol was 0.92 (0.86,0.98; p=0.008).

Comparing the statistically significant adjusted non-stratified bound cortisol results, the geometric mean PFDoDA per 10nM bound cortisol in adult dolphins held a positive association where the geometric mean PFDoDA per 10nM bound cortisol was 1.16 (1.05,1.28; p=0.003); whereas juvenile dolphins did not show this result. Additionally, the geometric mean PFTA per 10nM bound cortisol in juvenile dolphins held a positive association where the geometric mean PFTA per 10nM bound cortisol was 1.79 (1.25,2.56; p=0.002); whereas adult dolphins did not show this result. Finally, one new statistically significant result was revealed by age stratification. The geometric mean PFDS per 10nM bound cortisol in adult dolphins showed a positive association where the geometric mean PFDS per 10nM bound cortisol was 1.19 (1.03,1.37; p=0.017); whereas juvenile dolphins did not show this result.

Comparing the statistically significant adjusted non-stratified total cortisol results, the geometric mean PFOA per 10nM total cortisol in adult dolphins held a negative association where the geometric mean PFOA per 10nM total cortisol was 0.95 (0.91,0.99; p=0.016); juvenile dolphins also held a negative association where the geometric mean PFOA per 10nM free cortisol was 0.93 (0.87,0.999; p=0.046). The

statistically significant geometric mean PFTriA per 10nM total cortisol result seen in the adjusted non-stratified result was not seen in the age stratified results. Finally, several new statistically significant results were revealed by age stratification. The geometric mean PFOS per 10nM total cortisol in juvenile dolphins showed a negative association where the geometric mean PFOS per 10nM total cortisol was 0.92 (0.85,0.99; $p=0.023$); whereas adult dolphins did not show this result. Additionally, the geometric mean PFHxS per 10nM total cortisol in juvenile dolphins showed a negative association where the geometric mean PFHxS per 10nM total cortisol was 0.85 (0.79,0.92; $p<0.001$); whereas adult dolphins did not show this result. Finally, the geometric mean PFNA per 10nM total cortisol in juvenile dolphins showed a negative association where the geometric mean PFNA per 10nM total cortisol was 0.95 (0.90,0.999; $p<0.001$); whereas adult dolphins did not show this result.

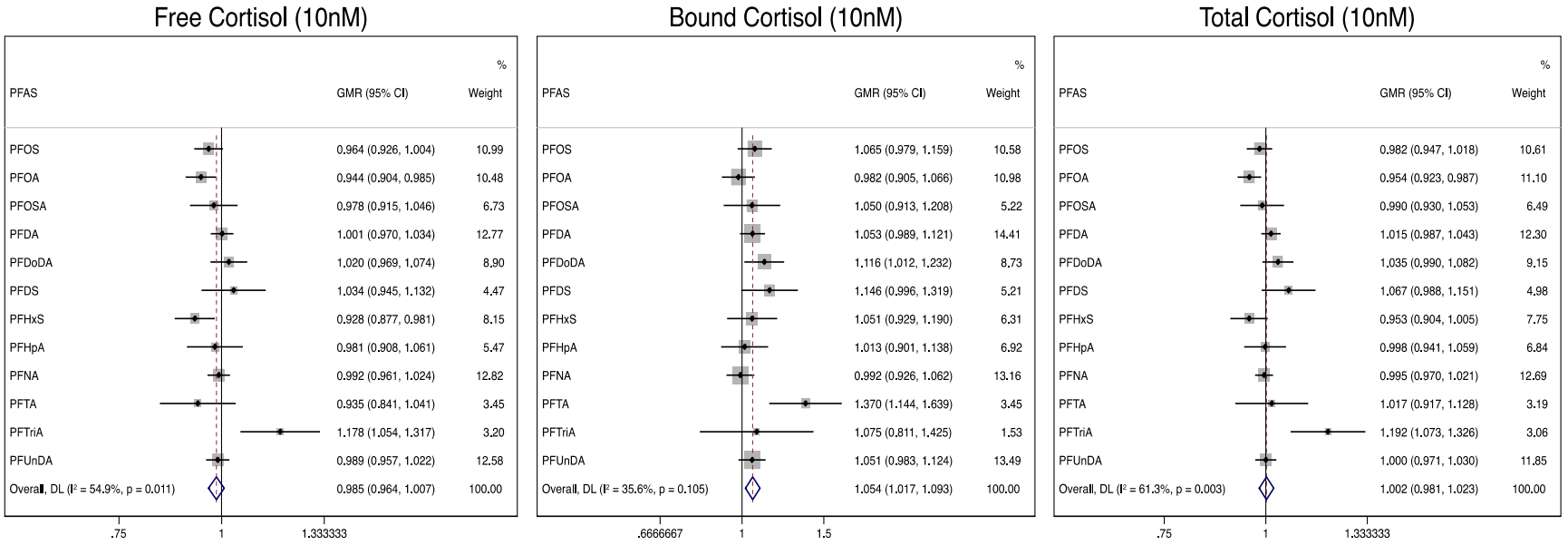

Figure 2. Results for adjusted Tobit regression models testing the GMR of PFAS per 10nM increase in cortisol. Models were adjusted for location, year, sex, and age at time of sample collection. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: For free and total, PFOS (n=195), PFOA (n=214), PFOSA (n=187), PFDA (n=214), PFDoDA (n=214), PFDS (n=88), PFHxS (n=214), PFHpA (n=85), PFNA (n=214), PFTA (n=102), PFTriA (n=77), and PFUA (n=214); for Bound PFOS (n=200), PFOA (n=219), PFOSA (n=191), PFDA (n=219), PFDoDA (n=219), PFDS (n=92), PFHxS (n=219), PFHpA (n=89), PFNA (n=219), PFTA (n=104), PFTriA (n=77), and PFUA (n=219).

Parametric Quantile Regression

When assessing the PFAS cortisol association with free cortisol while controlling for the year and dolphins' age, sex, and location at sample collection, one PFAS showed a positive association, and three showed a negative association (see Figure 3). Dolphins in the middle tertile of PFTriA exposure had cortisol levels 50% higher than in the lowest tertile (1.08,2.09; p=0.015). Dolphins in the highest tertile of PFTriA exposure had cortisol levels 90% higher than in the lowest tertile (1.31,2.76; p=0.001). Dolphins in the middle tertile of PFOS exposure had cortisol levels 26% lower than in the lowest tertile (0.57,0.97; p=0.028). Dolphins in the middle tertile of PFOA exposure had cortisol levels 32% lower than in the lowest tertile (0.55,0.85; p=0.001). Finally, dolphins in the highest tertile of PFHxS exposure had cortisol levels 22% lower than in the lowest tertile (0.61,0.99; p=0.047).

When assessing the PFAS cortisol association with bound cortisol while controlling for the year and dolphins' age, sex, and location at sample collection, there were five PFAS that showed a positive association. No PFAS showed a negative association. Dolphins in the middle tertile of PFDA exposure had cortisol levels 26% higher than in the lowest tertile (1.10,1.46; p=0.001). Dolphins in the middle tertile of PFDoDA exposure had cortisol levels 30% higher than in the lowest tertile (1.15,1.46; p<0.001). Dolphins in the middle tertile of PFDS exposure had cortisol levels 25% higher than in the lowest tertile (1.01,1.54; p=0.044). Dolphins in the highest tertile of PFDS exposure had cortisol levels 61% higher than in the lowest tertile (1.20,2.17; p=0.002). Dolphins in the middle tertile of PFTA exposure had cortisol levels 54% higher than in the lowest tertile (1.13,2.09; p=0.006). Dolphins in the highest tertile of PFTA exposure had cortisol levels 36% higher than in the lowest tertile (1.14,1.62; p=0.001). Dolphins in the highest tertile of PFUA exposure had cortisol levels 29% higher than in the lowest tertile (1.03,1.60; p=0.021).

When assessing the PFAS cortisol association with total cortisol while controlling for the year and dolphins' age, sex, and location at sample collection, four PFAS showed a positive correlation and one PFAS showed a negative association. Dolphins in the highest tertile of PFDA exposure had cortisol levels 29% higher than in the lowest tertile (1.02,1.63; p=0.033). Dolphins in the middle tertile of PFDoDA exposure had cortisol levels 24% higher than in the lowest tertile (1.07,1.44; p=0.003). Dolphins in the highest tertile of

PFDoDA exposure had cortisol levels 21% higher than in the lowest tertile (1.03,1.42; p=0.018). Dolphins in the middle tertile of PFTA exposure had cortisol levels 50% higher than in the lowest tertile (1.07,2.11; p=0.019). Dolphins in the highest tertile of PFTriA exposure had cortisol levels 59% higher than in the lowest tertile (1.24,2.06; p<0.001). Dolphins in the middle tertile of PFOA exposure had cortisol levels 20% lower than in the lowest tertile (0.68,0.93; p=0.004). Finally, dolphins in the highest tertile of PFOA exposure had cortisol levels 19% lower than in the lowest tertile (0.66,0.99; p=0.041).

Results for all of the unadjusted models, sex-stratified models, and age-stratified models are listed as Supplemental Figures 11-15 in the Appendix. When the results were stratified by sex, the sample sizes of the female dolphins were noticeably small, which yielded wider confidence intervals compared to those of the male dolphins. Comparing the statistically significant adjusted non-stratified free cortisol results, female dolphins the middle tertile of PFOS exposure held the negative association with cortisol levels 26% lower than in the lowest tertile (0.57,0.97; p=0.028); whereas this result was not seen in male dolphins. Additionally, the statistically significant results seen in the middle tertiles of PFOA exposure in the adjusted non-stratified model held for both female and male dolphins, with female dolphins in the middle tertiles of PFOA exposure had cortisol levels 46% lower than in the lowest tertile (0.39,0.77; p<0.001) and male dolphins in the middle tertiles of PFOA exposure had cortisol levels 30% lower than in the lowest tertile (0.54,0.91; p=0.020). The statistically significant results for both tertiles of PFTriA exposure in the adjusted non-stratified model held for male dolphins, where male dolphins in the middle tertile of PFTriA exposure had cortisol levels 63% higher than in the lowest tertile (1.08,2.46; p=0.019), and male dolphins in the highest tertile of PFTA exposure had cortisol levels 138% higher than in the lowest tertile (1.50,3.79; p<0.001); but the results were not seen in female dolphins. The statistically significant results seen in dolphins in the highest tertile of PFHxS exposure compared to the lowest-exposure reference group were not seen in the sex stratified results. Finally, several new statistically significant results were revealed by sex stratification. Female dolphins in the highest tertile of PFOA exposure had cortisol levels 40% lower than in the lowest tertile (0.37,0.99; p=0.044); whereas this result was not seen in male dolphins. Male dolphins in the highest tertile of PFDA exposure had cortisol levels 57% higher than in the lowest tertile (1.06,2.32; p=0.025); whereas this result was not seen in

female dolphins. Female dolphins in the middle tertile of PFDS exposure had cortisol levels 35% higher than in the lowest tertile (1.10,1.65; p=0.004); whereas this result was not seen in male dolphins. Finally, male dolphins in the highest tertile of PFTA exposure had cortisol levels 23% lower than in the lowest tertile (0.61,0.99; p=0.038); whereas female dolphins in the middle tertile of PFTA exposure had cortisol levels 224% higher than in the lowest tertile (1.51,6.96; p=0.003).

Comparing the statistically significant adjusted non-stratified bound cortisol results, male dolphins in both tertiles of PFDA exposure held the negative associations where male dolphins in the middle tertile of PFDA exposure had cortisol levels 22% higher than in the lowest tertile (1.04,1.43; p=0.013) and male dolphins in the highest tertile of PFDA exposure had cortisol levels 41% higher than in the lowest tertile (1.06,1.87; p=0.017); whereas this result was not seen in female dolphins. Additionally, the statistically significant results seen in dolphins in the middle tertile of PFDoDA exposure in the adjusted non-stratified model held for male dolphins, where male dolphins in the middle tertile of PFDoDA exposure had cortisol levels 32% higher than in the lowest tertile (1.15,1.52; p<0.001); whereas this result was not seen in female dolphins. The statistically significant results seen in dolphins in both tertiles of PFDS exposure in the adjusted non-stratified model held for male and female dolphins, where male dolphins in the highest tertile of PFDS exposure had cortisol levels 75% higher than in the lowest tertile (1.28,2.38; p<0.001) but female dolphins in the middle tertile of PFDS exposure had cortisol levels 87% higher than in the lowest tertile (1.30,2.70; p=0.001). Additionally, the statistically significant results seen in dolphins in both tertiles of PFTA exposure in the adjusted non-stratified model held for male dolphins where male dolphins in the middle tertile of PFTA exposure had cortisol levels 49% higher than in the lowest tertile (1.07,2.07; p=0.018) and male dolphins in the highest tertile of PFDA exposure had cortisol levels 43% higher than in the lowest tertile (1.17,1.76; p=0.001); whereas this result was not seen in female dolphins. The statistically significant results seen in dolphins in the middle tertile of PFUA exposure in the adjusted non-stratified model held for female dolphins, where female dolphins in the middle tertile of PFUA exposure had cortisol levels 57% higher than in the lowest tertile (1.06,2.34; p=0.025); whereas this result was not seen in male dolphins. The statistically significant results seen in dolphins in the highest tertile of PFDoDA exposure and in the highest tertile of

PFUA exposure compared to the lowest-exposure reference group were not seen in the sex stratified results. Finally, several new statistically significant results were revealed by sex stratification. Male dolphins in the highest tertile of PFOA exposure had cortisol levels 23% lower than in the lowest tertile (0.61,0.96; p=0.021); whereas this result was not seen in female dolphins. Additionally, female dolphins in the middle tertile of PFHpA had cortisol levels 54% higher than in the lowest tertile (1.02,2.32; p=0.038); whereas this result was not seen in male dolphins.

Comparing the statistically significant adjusted non-stratified total cortisol results, both female and male dolphins in the middle tertiles of PFOA exposure held the negative associations where female dolphins in the middle tertile of PFOA exposure had cortisol levels 32% lower than in the lowest tertile (0.55,0.87; p=0.013) and male dolphins in the middle tertile of PFOA exposure had cortisol levels 20% lower than in the lowest tertile (0.67,0.95; p=0.001). Additionally, the statistically significant results seen in dolphins in the highest tertile of PFDA exposure in the adjusted non-stratified model held for male dolphins, where male dolphins in the middle tertile of PFDA exposure had cortisol levels 56% higher than in the lowest tertile (1.19,2.05; p=0.001); whereas this result was not seen in female dolphins. The statistically significant results seen in dolphins in both tertiles of PFDoDA exposure in the adjusted non-stratified model held for male dolphins, where male dolphins in the middle tertile of PFDoDA exposure had cortisol levels 29% higher than in the lowest tertile (1.08,1.54; p=0.004) and male dolphins in the highest tertile of PFDoDA exposure had cortisol levels 22% higher than in the lowest tertile (1.02,1.46; p=0.034); whereas these results were not seen in female dolphins. Additionally, the statistically significant results seen in dolphins in the middle tertile of PFTA exposure in the adjusted non-stratified model held for female dolphins where female dolphins in the middle tertile of PFTA exposure had cortisol levels 144% higher than in the lowest tertile (1.38,4.32; p=0.002); whereas this result was not seen in male dolphins. The statistically significant results seen in dolphins in the highest tertile of PFTriA exposure in the adjusted non-stratified model held for male dolphins where male dolphins in the highest tertile of PFTriA exposure had cortisol levels 73% higher than in the lowest tertile (1.22,2.47; p=0.002); whereas this result was not seen in female dolphins. The statistically significant results seen in dolphins in the highest tertile of PFOA exposure compared to the lowest-exposure

reference group were not seen in the sex stratified results. Finally, several new statistically significant results were revealed by sex stratification. Male dolphins in the middle tertile of PFDA exposure had cortisol levels 26% higher than in the lowest tertile (1.04,1.53; p=0.018); whereas this result was not seen in female dolphins. Additionally, female dolphins in the middle tertile of PFDS exposure had cortisol levels 71% higher than in the lowest tertile (1.41,2.07; p<0.001); whereas this result was not seen in male dolphins. Finally, male dolphins in the highest tertile of PFHxS exposure had cortisol levels 19% lower than in the lowest tertile (0.66,0.99; p=0.044); whereas this result was not seen in female dolphins.

Similarly, when age stratifying the results, juvenile dolphins had much smaller sample sizes, which yield wider confidence intervals than adult dolphins. When the results were stratified by age, the sample sizes of the juvenile dolphins were noticeably small, which yielded wider confidence intervals compared to those of adult dolphins. Comparing the statistically significant adjusted non-stratified free cortisol results, juvenile dolphins the highest tertile of PFOS exposure held the negative association with cortisol levels 37% lower than in the lowest tertile (0.40,0.98; p=0.04); whereas this result was not seen in adult dolphins. Additionally, the statistically significant results seen in the middle tertiles of PFOA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the middle tertile of PFOA exposure had cortisol levels 26% lower than in the lowest tertile (0.60,0.91; p=0.006); whereas the results were not seen in juvenile dolphins. The statistically significant results dolphins in the highest tertile of PFHxS exposure in the adjusted non-stratified model held for juvenile dolphins, where juvenile dolphins in the highest tertile of PFHxS exposure had cortisol levels 49% lower than in the lowest tertile (0.33,0.79; p=0.003); whereas the results were not seen in adult dolphins. The statistically significant results seen in dolphins in both tertiles of PFTriA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the middle tertile of PFTriA exposure had cortisol levels 42% higher than in the lowest tertile (1.01,1.999; p=0.043) and adult dolphins in the highest tertile of PFTriA exposure had cortisol levels 84% higher than in the lowest tertile (1.31,2.59; p<0.001); whereas these results were not seen in juvenile dolphins. Finally, several new statistically significant results were revealed by age stratification. Adult dolphins in the highest tertile of PFOA exposure had cortisol levels 27% lower than in the lowest tertile (0.55,0.97; p=0.027); whereas this result was

not seen in juvenile dolphins. Juvenile dolphins in the highest tertile of PFHpA exposure had cortisol levels 78% lower than in the lowest tertile (0.07,0.70; p=0.011); whereas this result was not seen in adult dolphins. Finally, juvenile dolphins in both tertiles of PFTA exposure showed negative associations, where juvenile dolphins in the middle tertile of PFTA exposure had cortisol levels 99% lower than in the lowest tertile (0.0003,0.10; p<0.001) and juvenile dolphins in the highest tertile of PFTA exposure had cortisol levels 49% lower than in the lowest tertile (0.30,0.85; p=0.01); whereas these results were not seen in adult dolphins.

Comparing the statistically significant adjusted non-stratified bound cortisol results, adult dolphins in both tertiles of PFDA exposure held the negative associations where adult dolphins in the middle tertile of PFDA exposure had cortisol levels 32% higher than in the lowest tertile (1.09,1.61; p=0.005) and adult dolphins in the highest tertile of PFDA exposure had cortisol levels 43% higher than in the lowest tertile (1.04,1.97; p=0.027); whereas this result was not seen in juvenile dolphins. Additionally, the statistically significant results seen in adult dolphins in both tertiles of PFDoDA exposure held the negative associations where adult dolphins in the middle tertile of PFDoDA exposure had cortisol levels 46% higher than in the lowest tertile (1.26,1.70; p<0.001) and adult dolphins in the highest tertile of PFDoDA exposure had cortisol levels 38% higher than in the lowest tertile (1.14,1.68; p=0.001); whereas this result was not seen in juvenile dolphins. The statistically significant results seen in dolphins in both tertiles of PFDS exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the highest tertile of PFDS exposure had cortisol levels 58% higher than in the lowest tertile (1.21,2.07; p=0.001); whereas this result was not seen in juvenile dolphins. Additionally, the statistically significant results seen in dolphins in both tertiles of PFUA exposure in the adjusted non-stratified model held for adult dolphins but only held for juvenile dolphins in the middle tertile of PFUA exposure, where adult dolphins in the middle tertile of PFUA exposure had cortisol levels 26% higher than in the lowest tertile (1.03,1.53; p=0.024) and adult dolphins in the highest tertile of PFUA exposure had cortisol levels 38% higher than in the lowest tertile (1.03,1.85; p=0.03); and juvenile dolphins in the middle tertile of PFUA exposure had cortisol levels 32% higher than in the lowest tertile (1.02,1.70; p=0.032). The statistically significant results seen in dolphins in the middle tertile of PFDS exposure and in both tertiles of PFTA exposure compared to the lowest-exposure reference group were not seen in the sex stratified results. Finally, one new statistically significant result was revealed by age

stratification. Juvenile dolphins in the middle tertile of PFOS exposure had cortisol levels 40% higher than in the lowest tertile (1.08,1.80; p=0.01); whereas this result was not seen in adult dolphins.

Comparing the statistically significant adjusted non-stratified total cortisol results, juvenile and adult dolphins in both tertiles of PFOA exposure held the negative associations where juvenile dolphins in the middle tertile of PFOA exposure had cortisol levels 24% lower than in the lowest tertile (0.58,0.99; p=0.042) and juvenile dolphins in the highest tertile of PFOA exposure had cortisol levels 27% lower than in the lowest tertile (0.55,0.97; p=0.033); and adult dolphins in the middle tertile of PFOA exposure had cortisol levels 15% lower than in the lowest tertile (0.73,0.9996; p=0.049) and adult dolphins in the highest tertile of PFOA exposure had cortisol levels 21% lower than in the lowest tertile (0.65,0.97; p=0.024). Additionally, the statistically significant results seen in dolphins in the middle tertile of PFTA exposure in the adjusted non-stratified model held for adult dolphins but was opposite for juvenile dolphins, where adult dolphins in the middle tertile of PFTA exposure had cortisol levels 64% higher than in the lowest tertile (1.21,2.20; p=0.001) and juvenile dolphins in the middle tertile of PFTA exposure had cortisol levels 99% lower than in the lowest tertile (0.002,0.13; p<0.001). The statistically significant results seen in dolphins in the highest tertile of PFTriA exposure in the adjusted non-stratified model held for adult dolphins, where adult dolphins in the highest tertile of PFTriA exposure had cortisol levels 48% higher than in the lowest tertile (1.11,1.98; p=0.008); whereas these results were not seen in juvenile dolphins. The statistically significant results seen in dolphins in the highest tertile of PFDA exposure and the highest tertile of PFDoDA exposure compared to the lowest-exposure reference group were not seen in the age stratified results. Finally, several new statistically significant results were revealed by age stratification. Adult dolphins in the middle tertile of PFDA exposure had cortisol levels 27% higher than in the lowest tertile (1.08,1.50; p=0.005); whereas this result was not seen in juvenile dolphins. Additionally, juvenile dolphins in the highest tertile of PFHxS had cortisol levels 35% lower than in the lowest tertile (0.51,0.82; p<0.001); whereas this result was not seen in adult dolphins. Adult dolphins in the middle tertile of PFHpA exposure had cortisol levels 35% higher than in the lowest tertile (1.08,1.70; p=0.009), whereas juvenile dolphins in the middle tertile of PFHpA exposure had cortisol levels 54% lower than in the lowest tertile (0.36,0.58; p<0.001). Finally, juvenile dolphins in the highest tertile of PFTA exposure had cortisol levels 31% lower than in the lowest tertile (0.50,0.96; p=0.027).

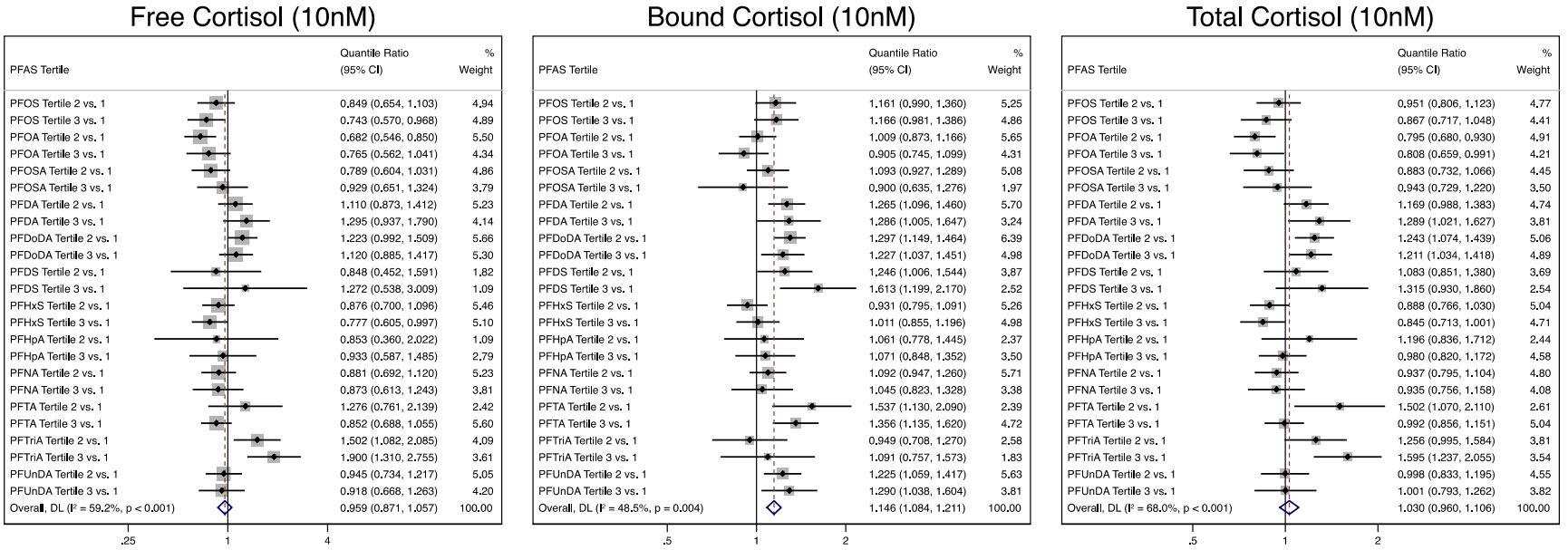

Figure 3. Results for adjusted parametric quantile regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: For free and total, PFOS (n=192), PFOA (n=211), PFOSA (n=184), PFDA (n=211), PFDoDA (n=211), PFDS (n=88), PFHxS (n=211), PFHpA (n=85), PFNA (n=211), PFTA (n=102), PFTriA (n=77), and PFUA (n=211); for Bound PFOS (n=200), PFOA (n=219), PFOSA (n=191), PFDA (n=219), PFDoDA (n=219), PFDS (n=92), PFHxS (n=219), PFHpA (n=89), PFNA (n=219), PFTA (n=104), PFTriA (n=77), and PFUA (n=219).

Discussion

In general, PFOS, PFOA, and PFHxS were negatively associated with free and total cortisol. PFTriA was positively associated with free and total cortisol. In the linear regression and parametric quantile regression models, bound cortisol was consistently positively associated with PFDA, PFDoDA, PFDS, PFTA, and PFUA. This study demonstrates the presence of associations between the different types of cortisol (free, bound, and total) and multiple individual PFAS. Further, the differences in PFAS associations with the different types of cortisol should be considered. The results from this study show that PFAS associations can also differ among the types PFAS, as well as among different sexes and ages. Taken together, this study indicates that further exploration into a possible link between lupus and dietary PFAS exposure among the Gullah/Geechee population in Charleston, South Carolina is needed.

Limitations

Due to sample size limitations, data sparsity is present. Therefore, data extrapolation is conceivable. Further, despite non-independent observations being addressed using Huber-White robust standard errors in order to address false-positive issues, selection bias within this study is still possible. Additionally, PFAS exist in mixtures within in the environment. Possible associations between PFAS mixtures and cortisol levels should be explored. Also, cortisol is a stress-response hormone that has been shown to respond in wildlife during capture and handling scenarios.[27] Consequently, other stress-response hormones should be studied to further understand the possible mechanisms of the relationship between environmental PFAS contamination and the hypothalamic-pituitary-adrenal axis in wildlife and humans.

Public Health Implications

Environmental PFAS contamination is a public health issue. Understanding the impacts of this issue is necessary in order to take proper actions regarding management and clean up. This study furthers that understanding. Further, it also adds to the growing evidence that bottlenose dolphins are useful tools in comprehending the global burden of PFAS contamination. Taken together, this study indicates that further

exploration into a possible link between lupus and dietary PFAS exposure among the Gullah/Geechee population in Charleston, South Carolina is needed.

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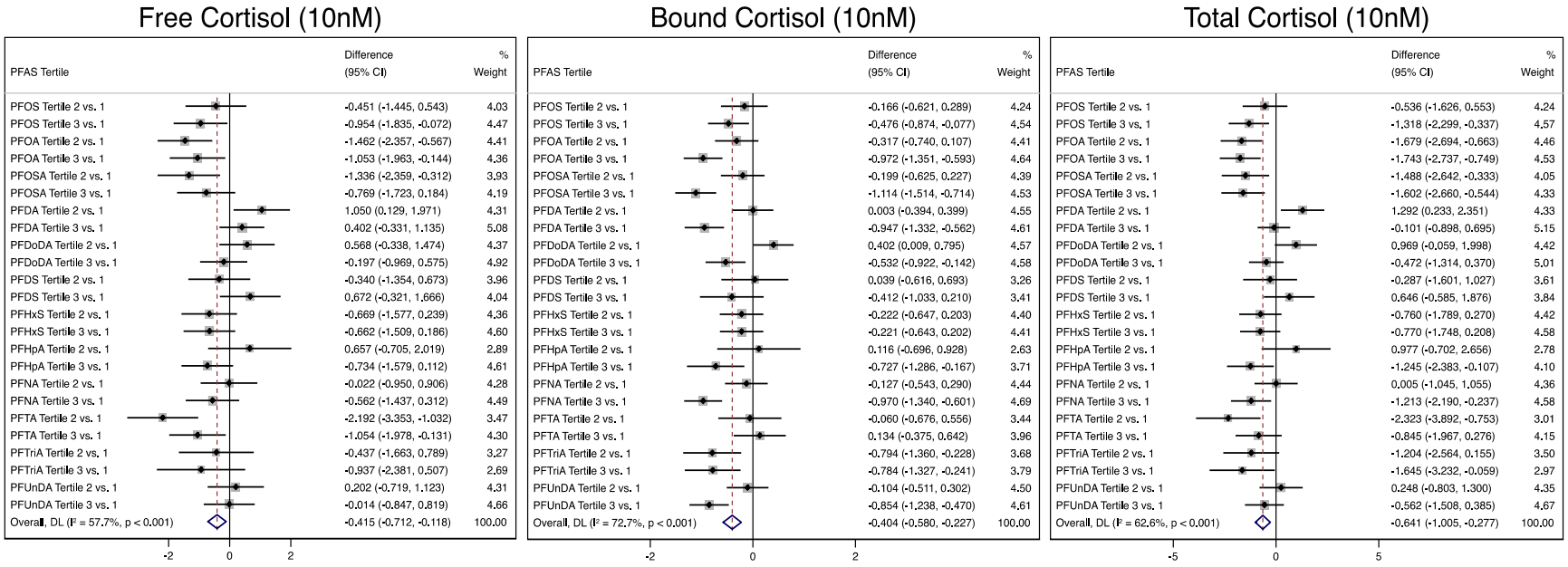
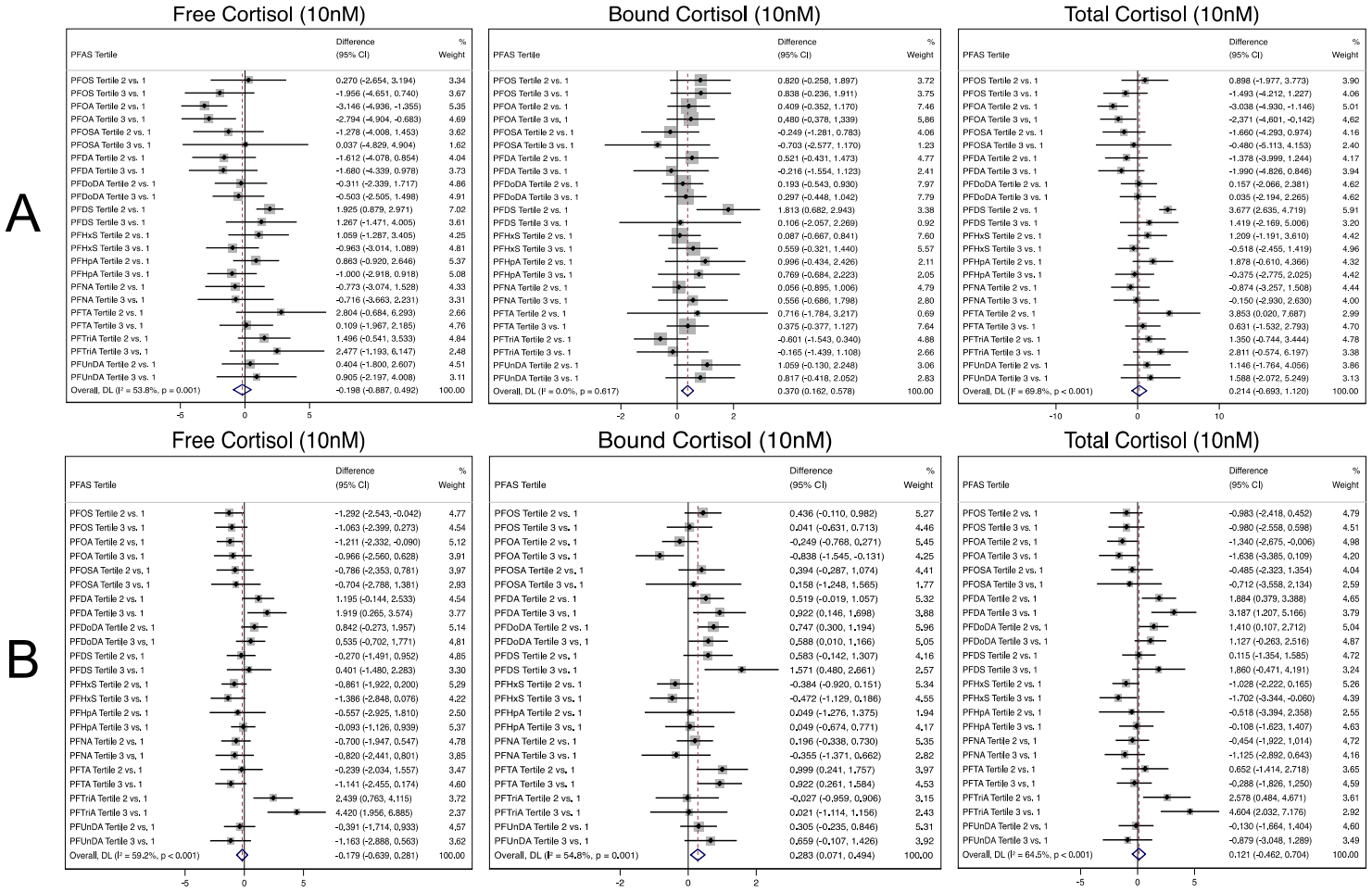
Appendix

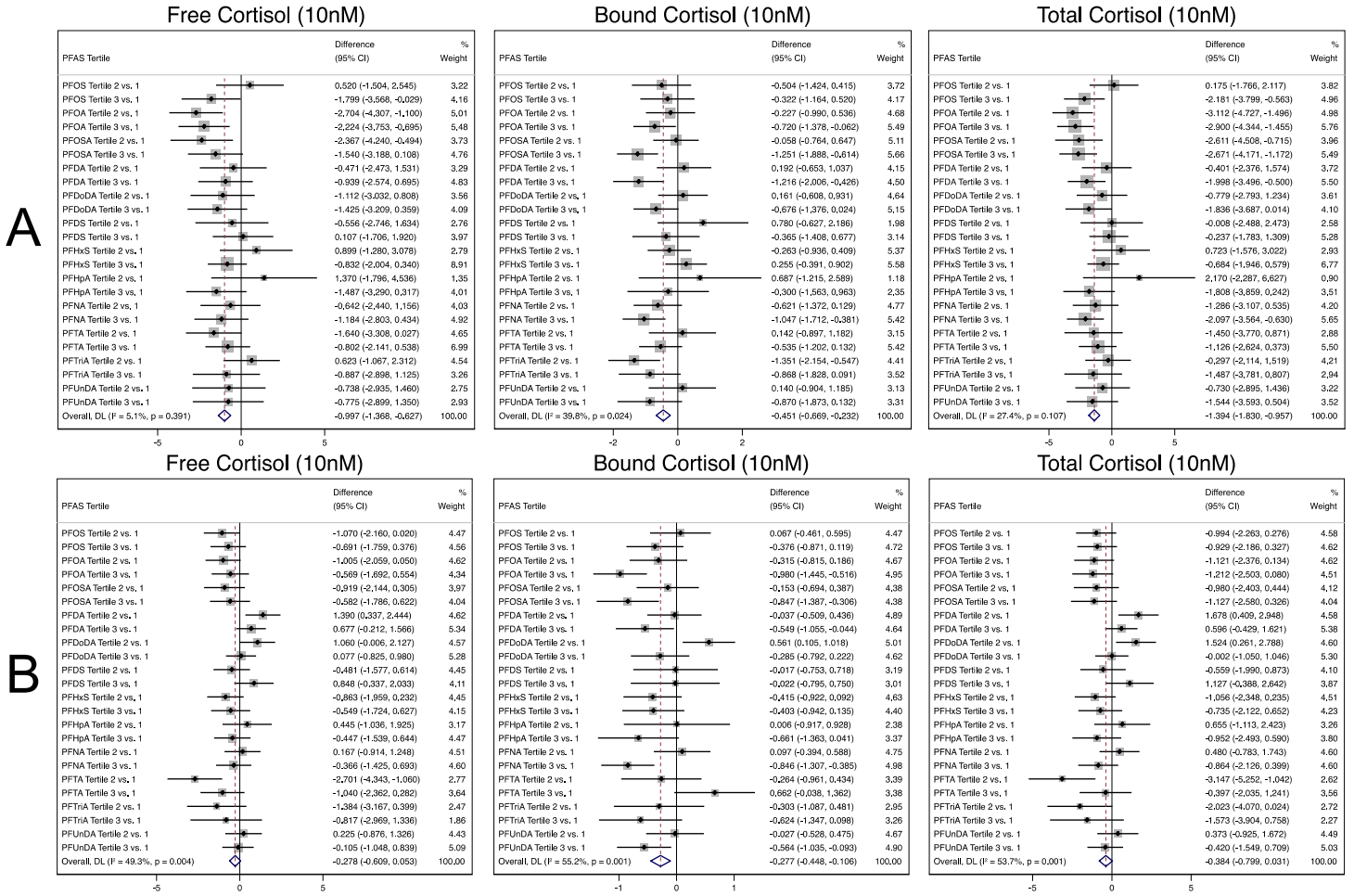
Linear Regression



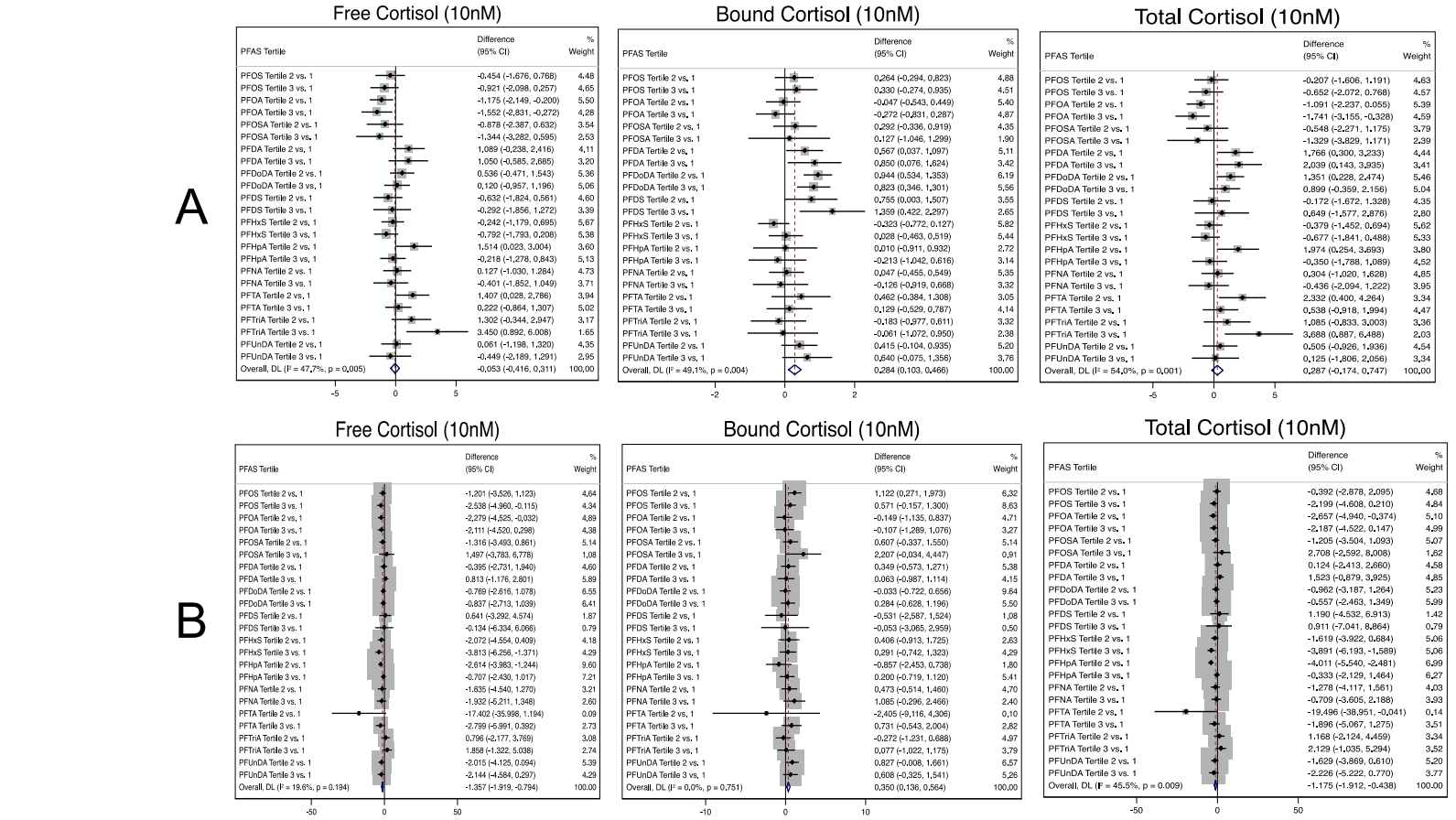
Supplemental Figure 1. Results for unadjusted linear regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau^2. % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for free and total, PFOS (n=225), PFOA (n=251), PFOSA (n=218), PFDA (n=251), PFDoDA (n=251), PFDS (n=104), PFHxS (n=251), PFHpA (n=101), PFNA (n=251), PFTA (n=120), PFTriA (n=93), and PFUA (n=251); for Bound PFOS (n=230), PFOA (n=257), PFOSA (n=223), PFDA (n=257), PFDoDA (n=257), PFDS (n=109), PFHxS (n=257), PFHpA (n=106), PFNA (n=257), PFTA (n=123), PFTriA (n=94), and PFUA (n=257).



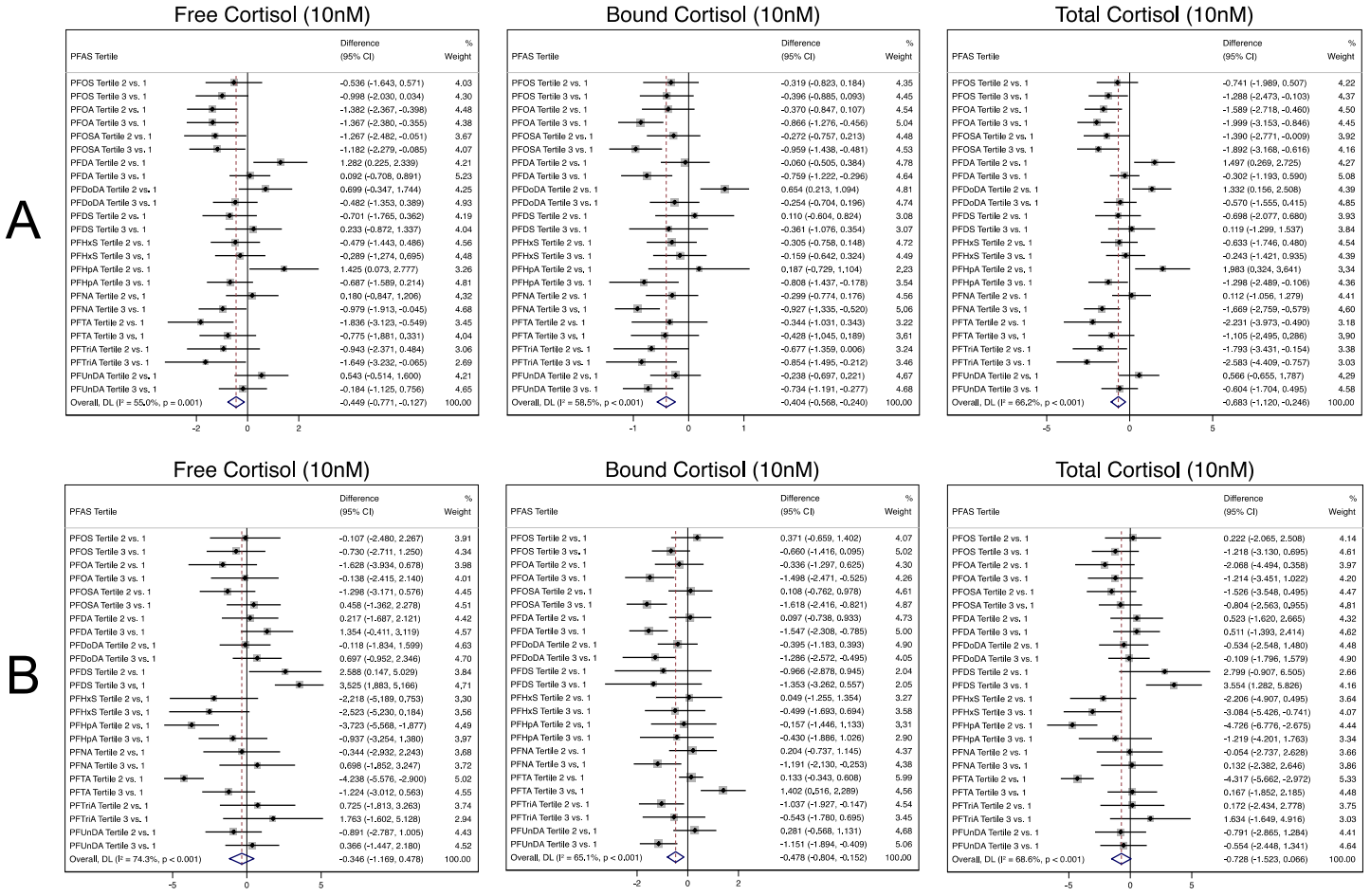
Supplemental Figure 2. Sex-stratified results, A) female and B) male, for adjusted linear regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for female free, bound, and total, PFOS (n=55), PFOA (n=61), PFOSA (n=61), PFDA (n=61), PFDoDA (n=61), PFDS (n=18), PFHxS (n=61), PFHpA (n=18), PFNA (n=61), PFTA (n=41), PFTriA (n=31), and PFUA (n=61); For male free and total, PFOS (n=140), PFOA (n=153), PFOSA (n=126), PFDA (n=153), PFDoDA (n=153), PFDS (n=70), PFHxS (n=153), PFHpA (n=67), PFNA (n=153), PFTA (n=61), PFTriA (n=46), and PFUA (n=153); for male bound PFOS (n=145), PFOA (n=158), PFOSA (n=130), PFDA (n=158), PFDoDA (n=158), PFDS (n=74), PFHxS (n=158), PFHpA (n=71), PFNA (n=158), PFTA (n=63), PFTriA (n=46), and PFUA (n=158).



Supplemental Figure 3. Sex-stratified results, A) female and B) male, for unadjusted linear regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for female free, bound, and total, PFOS (n=72), PFOA (n=81), PFOSA (n=80), PFDA (n=81), PFDoDA (n=81), PFDS (n=23), PFHxS (n=81), PFHpA (n=23), PFNA (n=81), PFTA (n=52), PFTriA (n=41), and PFUA (n=81); For male free and total, PFOS (n=153), PFOA (n=168), PFOSA (n=136), PFDA (n=168), PFDoDA (n=168), PFDS (n=79), PFHxS (n=168), PFHpA (n=76), PFNA (n=168), PFTA (n=66), PFTriA (n=50), and PFUA (n=168); for male bound PFOS (n=158), PFOA (n=173), PFOSA (n=140), PFDA (n=173), PFDoDA (n=173), PFDS (n=83), PFHxS (n=173), PFHpA (n=80), PFNA (n=173), PFTA (n=68), PFTriA (n=50), and PFUA (n=173).

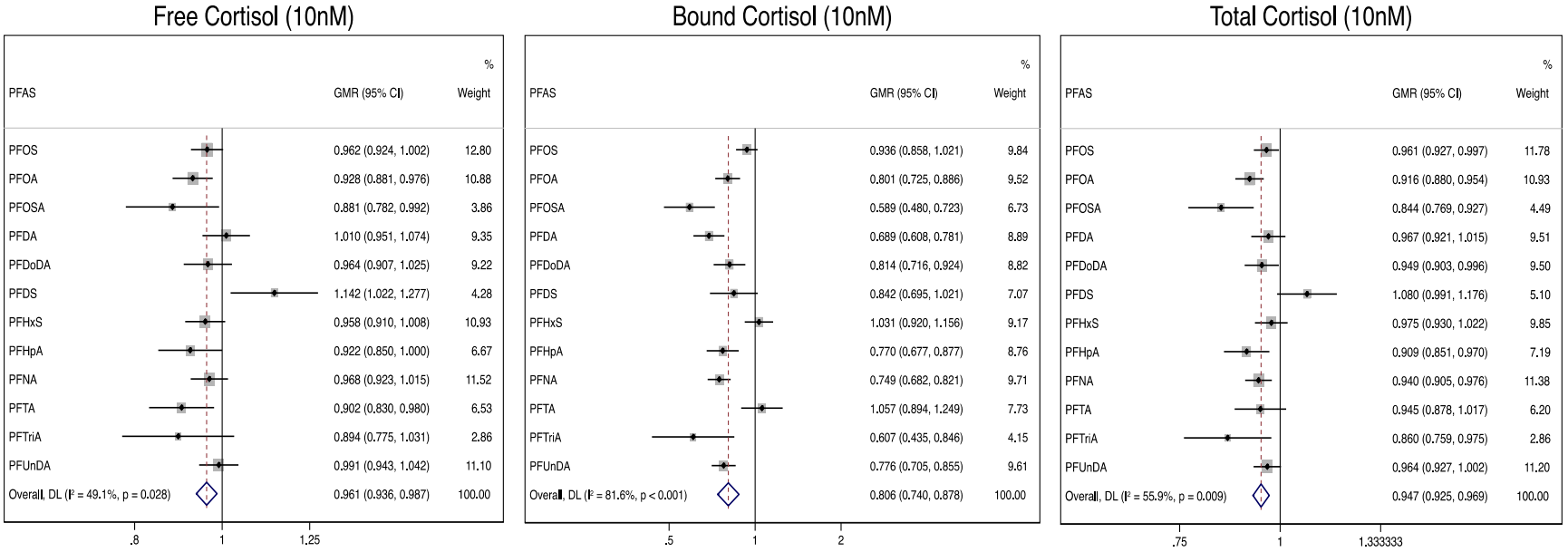


Supplemental Figure 4. Age-stratified results, A) adult (females ≥7yo and males ≥10yo) and B) juvenile females <7yo and males <10yo), for adjusted linear regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for adult free and total, PFOS (n=166), PFOA (n=189), PFOSA (n=159), PFDA (n=189), PFDoDA (n=189), PFDS (n=82), PFHxS (n=189), PFHpA (n=79), PFNA (n=189), PFTA (n=82), PFTriA (n=65), and PFUA (n=189); for adult bound PFOS (n=169), PFOA (n=192), PFOSA (n=161), PFDA (n=192), PFDoDA (n=192), PFDS (n=84), PFHxS (n=192), PFHpA (n=81), PFNA (n=192), PFTA (n=83), PFTriA (n=65), and PFUA (n=192); For juvenile free and total, PFOS (n=59), PFOA (n=60), PFOSA (n=57), PFDA (n=60), PFDoDA (n=60), PFDS (n=20), PFHxS (n=60), PFHpA (n=20), PFNA (n=60), PFTA (n=36), PFTriA (n=26), and PFUA (n=60); for juvenile bound PFOS (n=61), PFOA (n=62), PFOSA (n=59), PFDA (n=62), PFDoDA (n=62), PFDS (n=22), PFHxS (n=62), PFHpA (n=22), PFNA (n=62), PFTA (n=37), PFTriA (n=26), and PFUA (n=62).

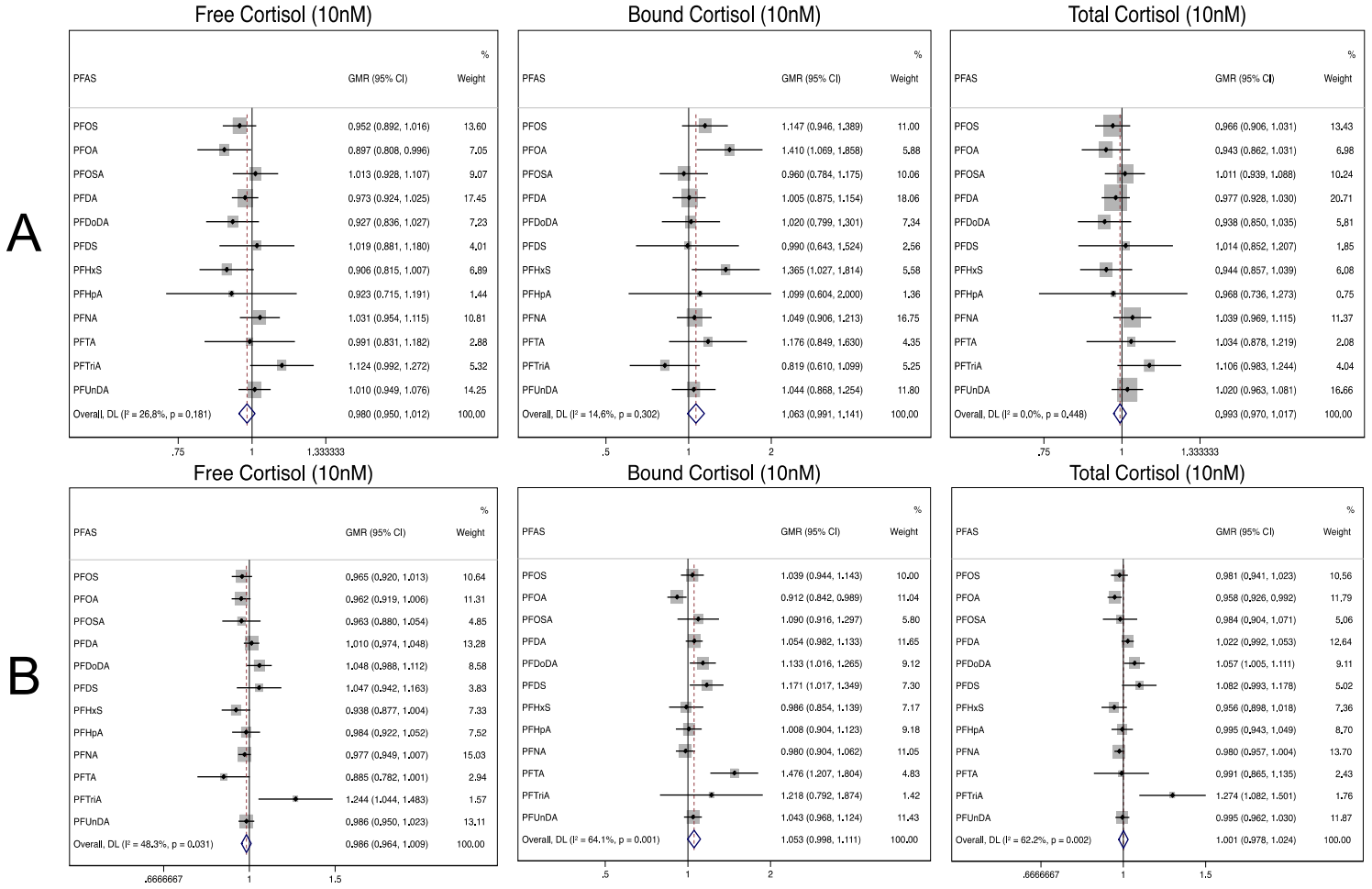


Supplemental Figure 5. Age-stratified results, A) (females ≥7yo and males ≥10yo) and B) juvenile females <7yo and males <10yo), for unadjusted linear regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for adult free and total, PFOS (n=166), PFOA (n=189), PFOSA (n=159), PFDA (n=189), PFDoDA (n=189), PFDS (n=82), PFHxS (n=189), PFHpA (n=79), PFNA (n=189), PFTA (n=82), PFTriA (n=65), and PFUA (n=189); for adult bound PFOS (n=169), PFOA (n=192), PFOSA (n=161), PFDA (n=192), PFDoDA (n=192), PFDS (n=84), PFHxS (n=192), PFHpA (n=81), PFNA (n=192), PFTA (n=83), PFTriA (n=65), and PFUA (n=192); For juvenile free and total, PFOS (n=59), PFOA (n=60), PFOSA (n=57), PFDA (n=60), PFDoDA (n=60), PFDS (n=20), PFHxS (n=60), PFHpA (n=20), PFNA (n=60), PFTA (n=36), PFTriA (n=26), and PFUA (n=60); for juvenile bound PFOS (n=61), PFOA (n=62), PFOSA (n=59), PFDA (n=62), PFDoDA (n=62), PFDS (n=22), PFHxS (n=62), PFHpA (n=22), PFNA (n=62), PFTA (n=37), PFTriA (n=26), and PFUA (n=62).

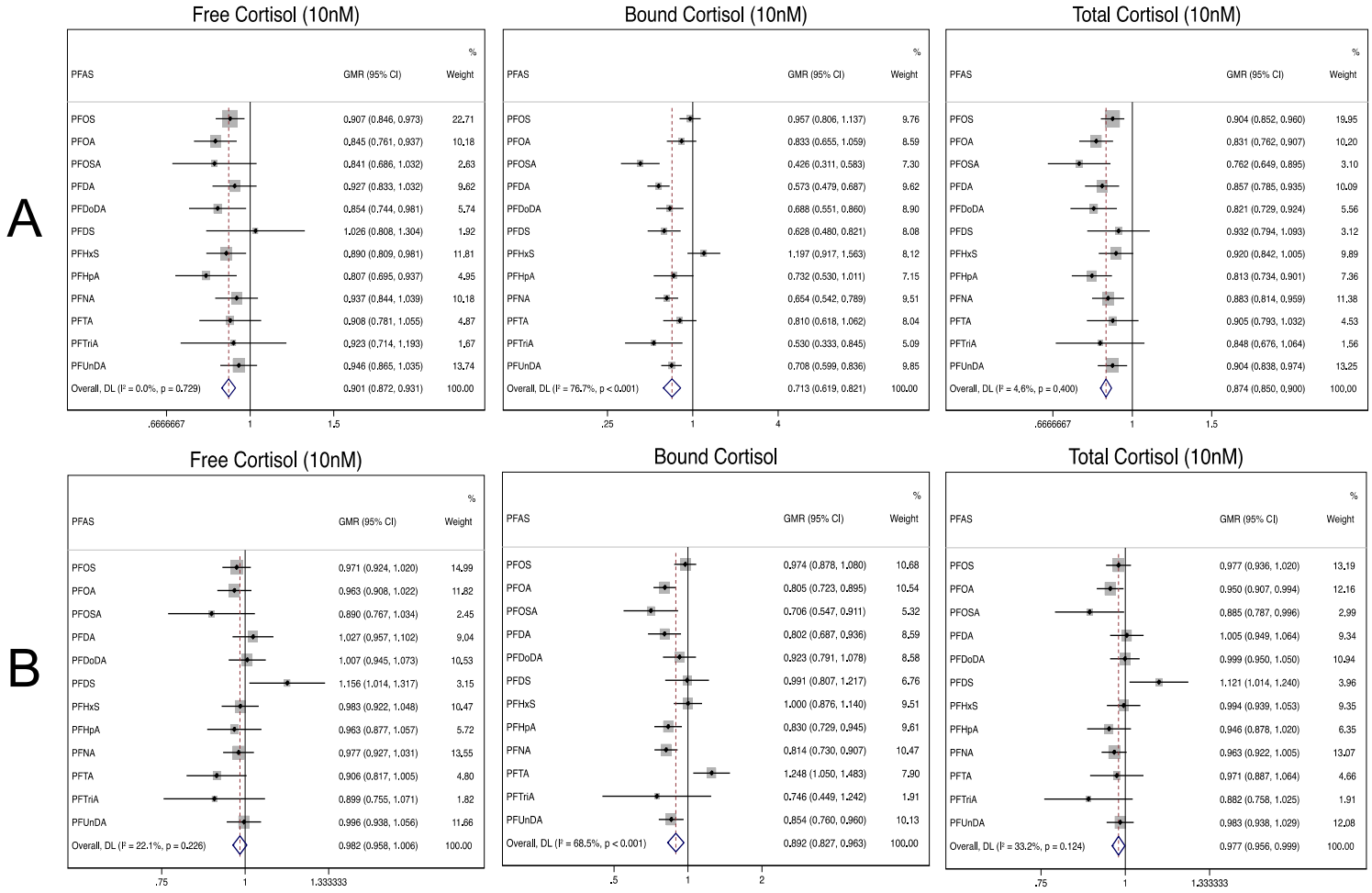
Tobit Regression



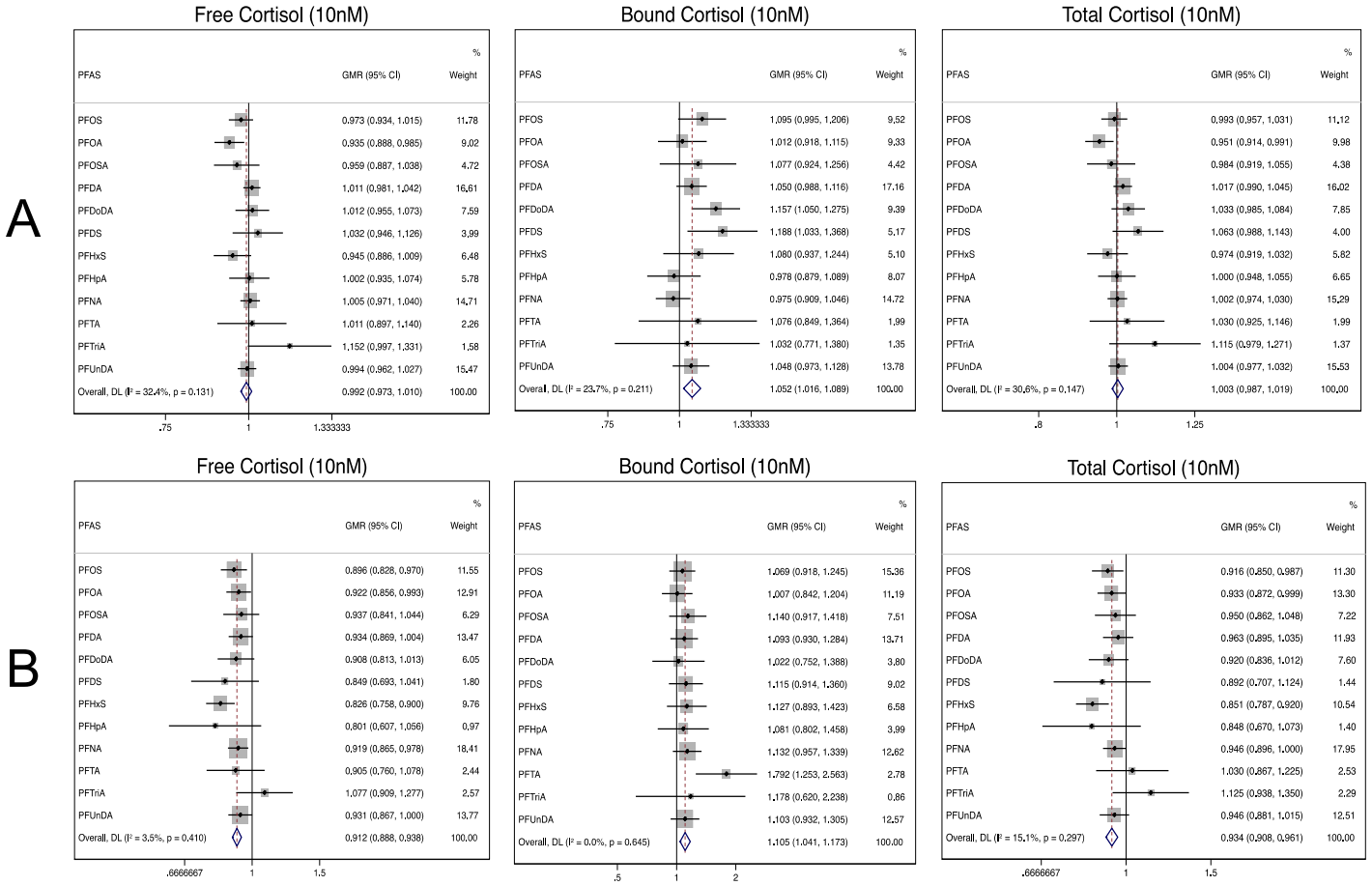
Supplemental Figure 6. Results for unadjusted Tobit regression models testing the GMR of PFAS per 10nM increase in cortisol. Models were adjusted for location, year, sex, and age at time of sample collection. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for free and total, PFOS (n=225), PFOA (n=251), PFOSA (n=218), PFDA (n=251), PFDoDA (n=251), PFDS (n=104), PFHxS (n=251), PFHpA (n=101), PFNA (n=251), PFTA (n=120), PFTriA (n=93), and PFUA (n=251); for Bound PFOS (n=230), PFOA (n=257), PFOSA (n=223), PFDA (n=257), PFDoDA (n=257), PFDS (n=109), PFHxS (n=257), PFHpA (n=106), PFNA (n=257), PFTA (n=123), PFTriA (n=94), and PFUA (n=257).



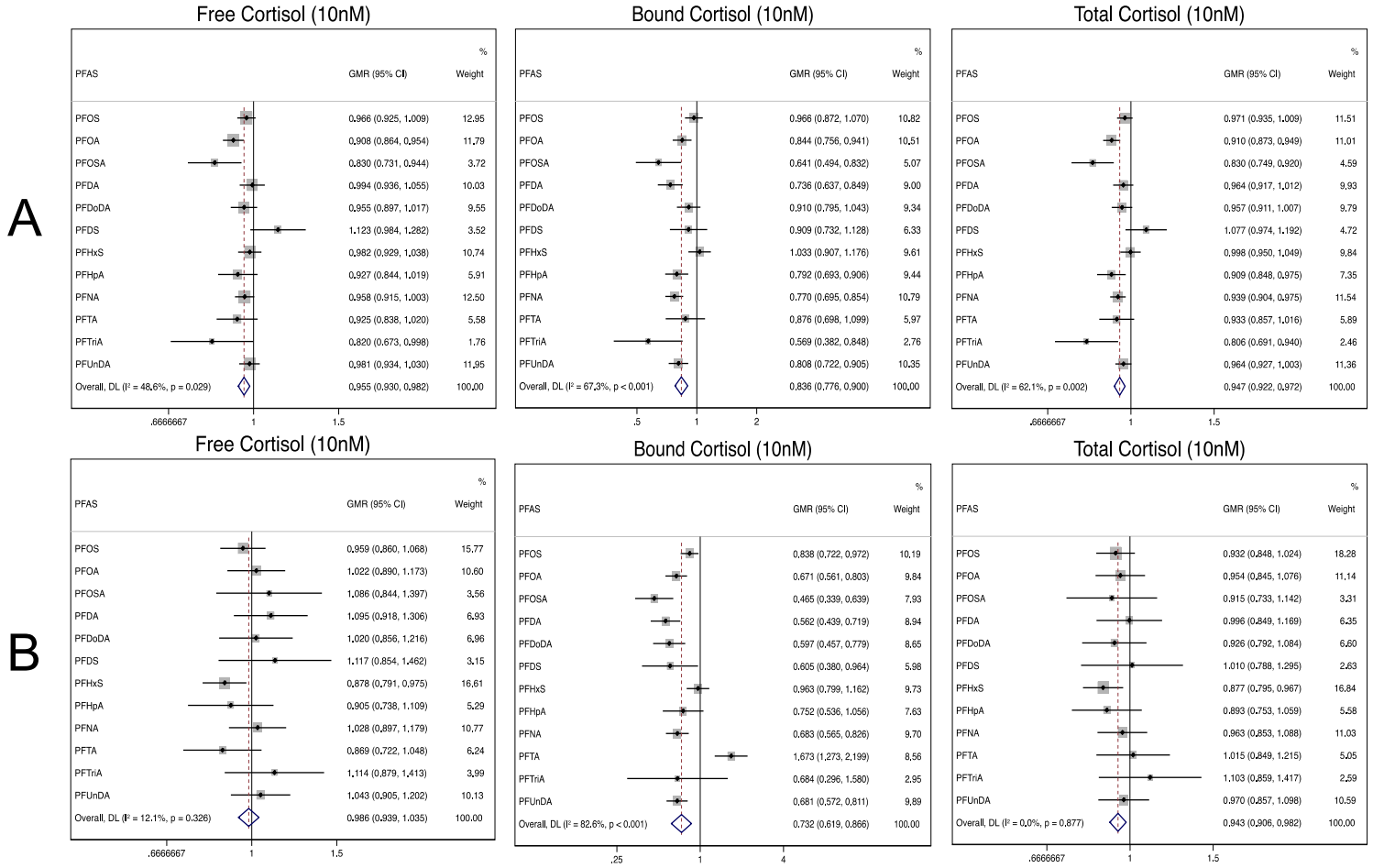
Supplemental Figure 7. Sex-stratified results, A) female and B) male, for adjusted Tobit regression models testing the GMR of PFAS per 10nM increase in cortisol. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for female free, bound, and total, PFOS (n=55), PFOA (n=61), PFOSA (n=61), PFDA (n=61), PFDoDA (n=61), PFDS (n=18), PFHxS (n=61), PFHpA (n=18), PFNA (n=61), PFTA (n=41), PFTriA (n=31), and PFUA (n=61); For male free and total, PFOS (n=140), PFOA (n=153), PFOSA (n=126), PFDA (n=153), PFDoDA (n=153), PFDS (n=70), PFHxS (n=153), PFHpA (n=67), PFNA (n=153), PFTA (n=61), PFTriA (n=46), and PFUA (n=153); for male bound PFOS (n=145), PFOA (n=158), PFOSA (n=130), PFDA (n=158), PFDoDA (n=158), PFDS (n=74), PFHxS (n=158), PFHpA (n=71), PFNA (n=158), PFTA (n=63), PFTriA (n=46), and PFUA (n=158).



Supplemental Figure 8. Sex-stratified results, A) female and B) male, for unadjusted Tobit regression models testing the GMR of PFAS per 10nM increase in cortisol. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for female free, bound, and total, PFOS (n=72), PFOA (n=81), PFOSA (n=80), PFDA (n=81), PFDoDA (n=81), PFDS (n=23), PFHxS (n=81), PFHpA (n=23), PFNA (n=81), PFTA (n=52), PFTriA (n=41), and PFUA (n=81); For male free and total, PFOS (n=153), PFOA (n=168), PFOSA (n=136), PFDA (n=168), PFDoDA (n=168), PFDS (n=79), PFHxS (n=168), PFHpA (n=76), PFNA (n=168), PFTA (n=66), PFTriA (n=50), and PFUA (n=168); for male bound PFOS (n=158), PFOA (n=173), PFOSA (n=140), PFDA (n=173), PFDoDA (n=173), PFDS (n=83), PFHxS (n=173), PFHpA (n=80), PFNA (n=173), PFTA (n=68), PFTriA (n=50), and PFUA (n=173).

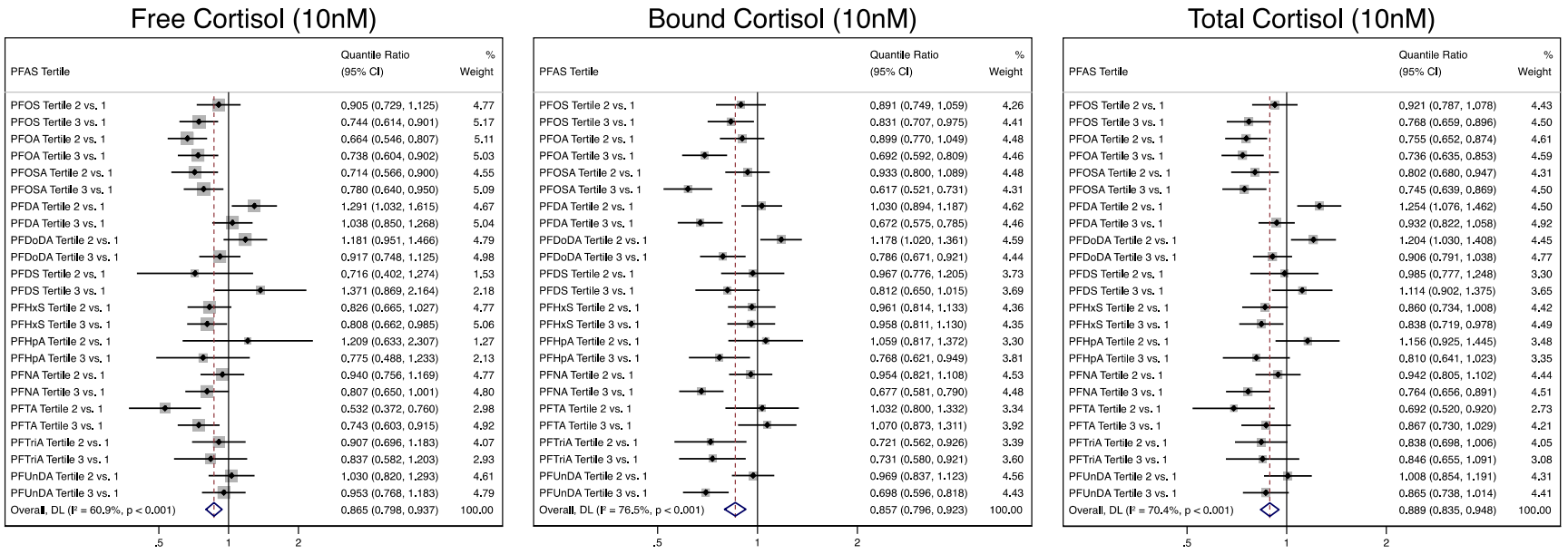


Supplemental Figure 9. Age-stratified results, A) adult (females ≥7yo and males ≥10yo) and B) juvenile females <7yo and males <10yo), for adjusted Tobit regression models testing the GMR of PFAS per 10nM increase in cortisol. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for adult free and total, PFOS (n=166), PFOA (n=189), PFOSA (n=159), PFDA (n=189), PFDoDA (n=189), PFDS (n=82), PFHxS (n=189), PFHpA (n=79), PFNA (n=189), PFTA (n=82), PFTriA (n=65), and PFUA (n=189); for adult bound PFOS (n=169), PFOA (n=192), PFOSA (n=161), PFDA (n=192), PFDoDA (n=192), PFDS (n=84), PFHxS (n=192), PFHpA (n=81), PFNA (n=192), PFTA (n=83), PFTriA (n=65), and PFUA (n=192); For juvenile free and total, PFOS (n=59), PFOA (n=60), PFOSA (n=57), PFDA (n=60), PFDoDA (n=60), PFDS (n=20), PFHxS (n=60), PFHpA (n=20), PFNA (n=60), PFTA (n=36), PFTriA (n=26), and PFUA (n=60); for juvenile bound PFOS (n=61), PFOA (n=62), PFOSA (n=59), PFDA (n=62), PFDoDA (n=62), PFDS (n=22), PFHxS (n=62), PFHpA (n=22), PFNA (n=62), PFTA (n=37), PFTriA (n=26), and PFUA (n=62).

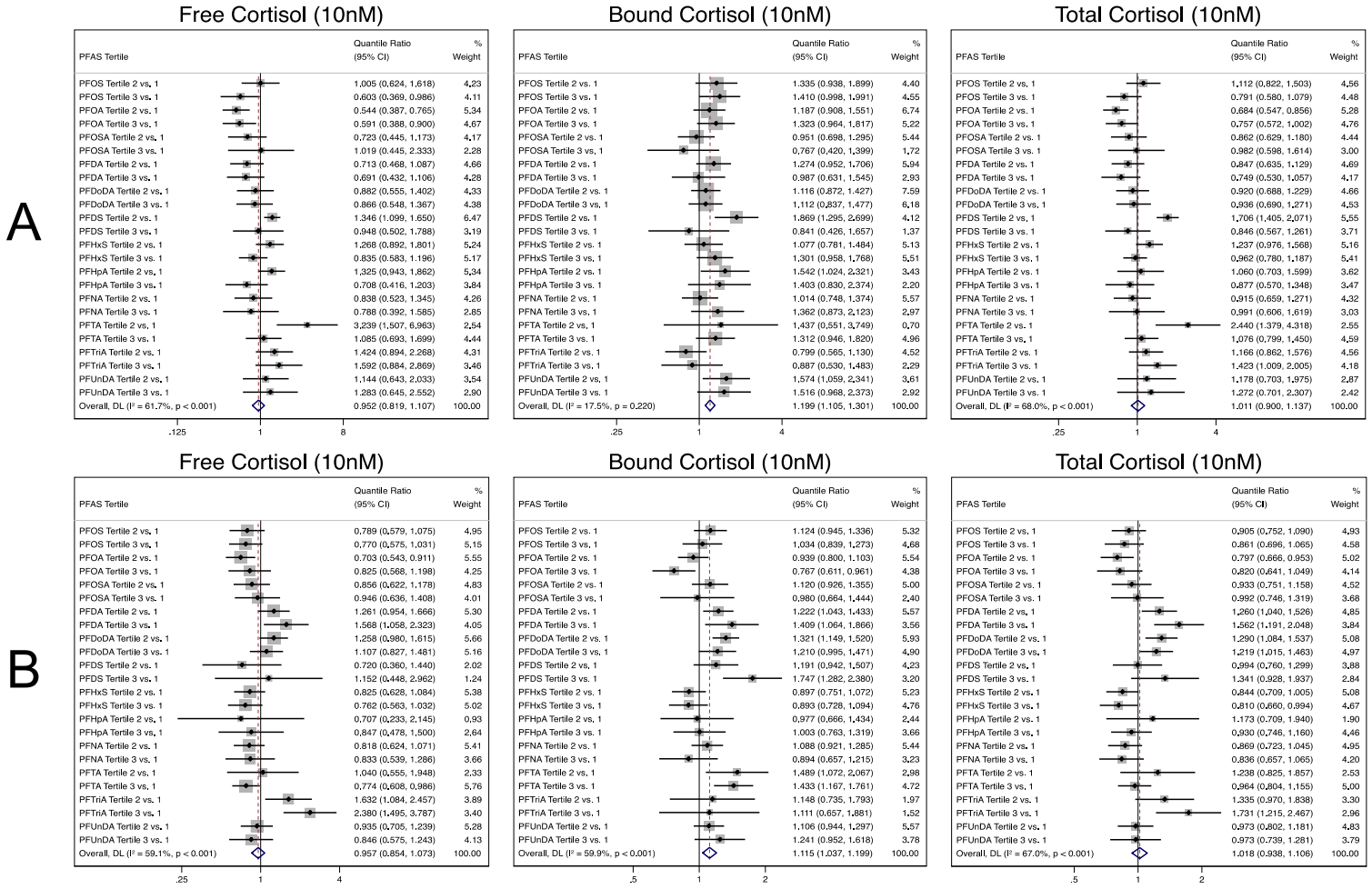


Supplemental Figure 10. Age-stratified results, A) adult (females ≥7yo and males ≥10yo) and B) juvenile females <7yo and males <10yo), for unadjusted Tobit regression models testing the GMR of PFAS per 10nM increase in cortisol. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for adult free and total, PFOS (n=166), PFOA (n=189), PFOSA (n=159), PFDA (n=189), PFDoDA (n=189), PFDS (n=82), PFHxS (n=189), PFHpA (n=79), PFNA (n=189), PFTA (n=82), PFTriA (n=65), and PFUA (n=189); for adult bound PFOS (n=169), PFOA (n=192), PFOSA (n=161), PFDA (n=192), PFDoDA (n=192), PFDS (n=84), PFHxS (n=192), PFHpA (n=81), PFNA (n=192), PFTA (n=83), PFTriA (n=65), and PFUA (n=192); For juvenile free and total, PFOS (n=59), PFOA (n=60), PFOSA (n=57), PFDA (n=60), PFDoDA (n=60), PFDS (n=20), PFHxS (n=60), PFHpA (n=20), PFNA (n=60), PFTA (n=36), PFTriA (n=26), and PFUA (n=60); for juvenile bound PFOS (n=61), PFOA (n=62), PFOSA (n=59), PFDA (n=62), PFDoDA (n=62), PFDS (n=22), PFHxS (n=62), PFHpA (n=22), PFNA (n=62), PFTA (n=37), PFTriA (n=26), and PFUA (n=62).

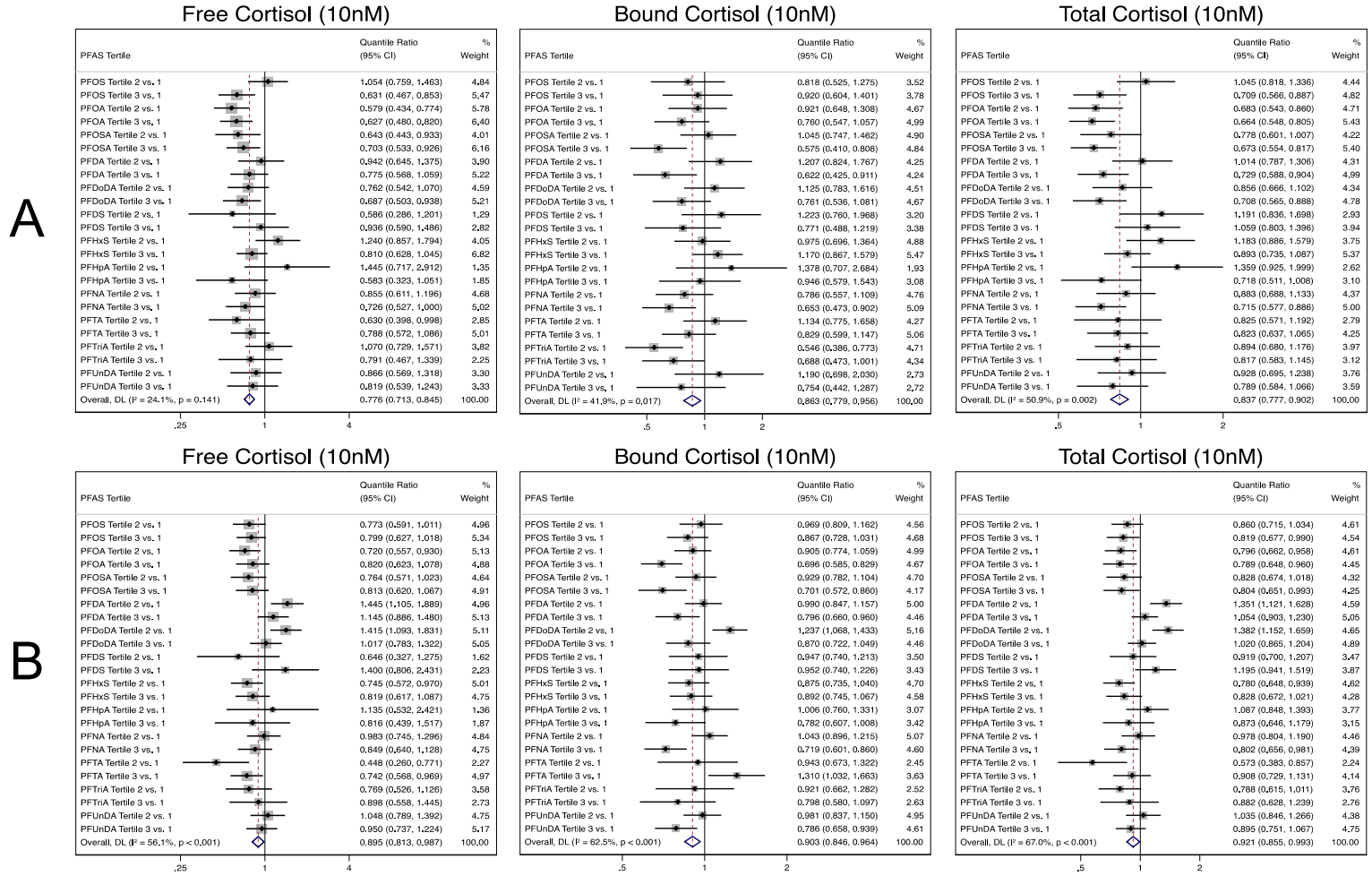
Parametric Quantile Regression



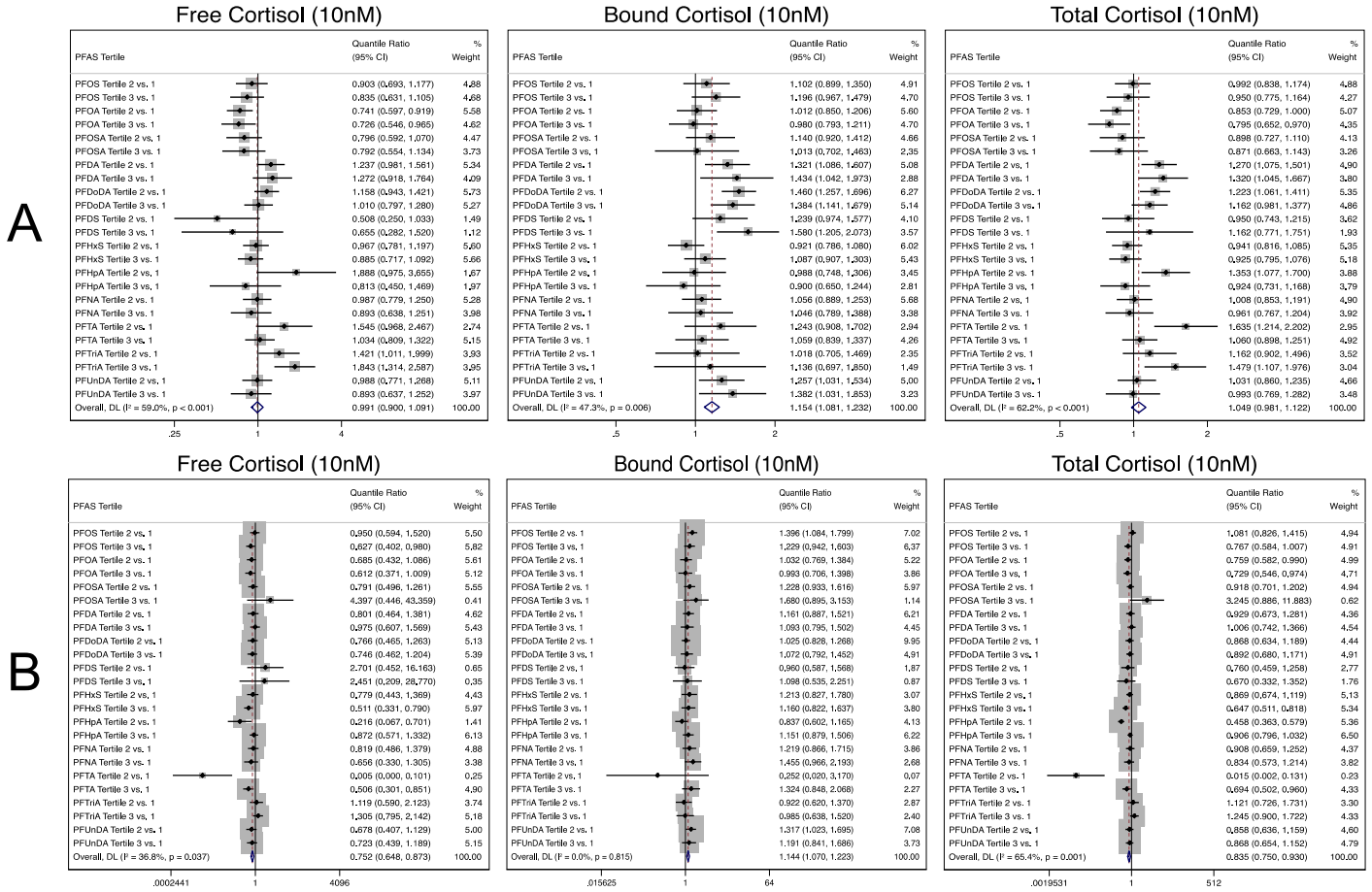
Supplemental Figure 11. Results for unadjusted parametric quantile regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau^2. % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for free and total, PFOS (n=222), PFOA (n=247), PFOSA (n=214), PFDA (n=247), PFDoDA (n=247), PFDS (n=103), PFHxS (n=247), PFHpA (n=100), PFNA (n=247), PFTA (n=119), PFTriA (n=92), and PFUA (n=247); for bound PFOS (n=230), PFOA (n=257), PFOSA (n=223), PFDA (n=257), PFDoDA (n=257), PFDS (n=109), PFHxS (n=257), PFHpA (n=106), PFNA (n=257), PFTA (n=123), PFTriA (n=94), and PFUA (n=257).



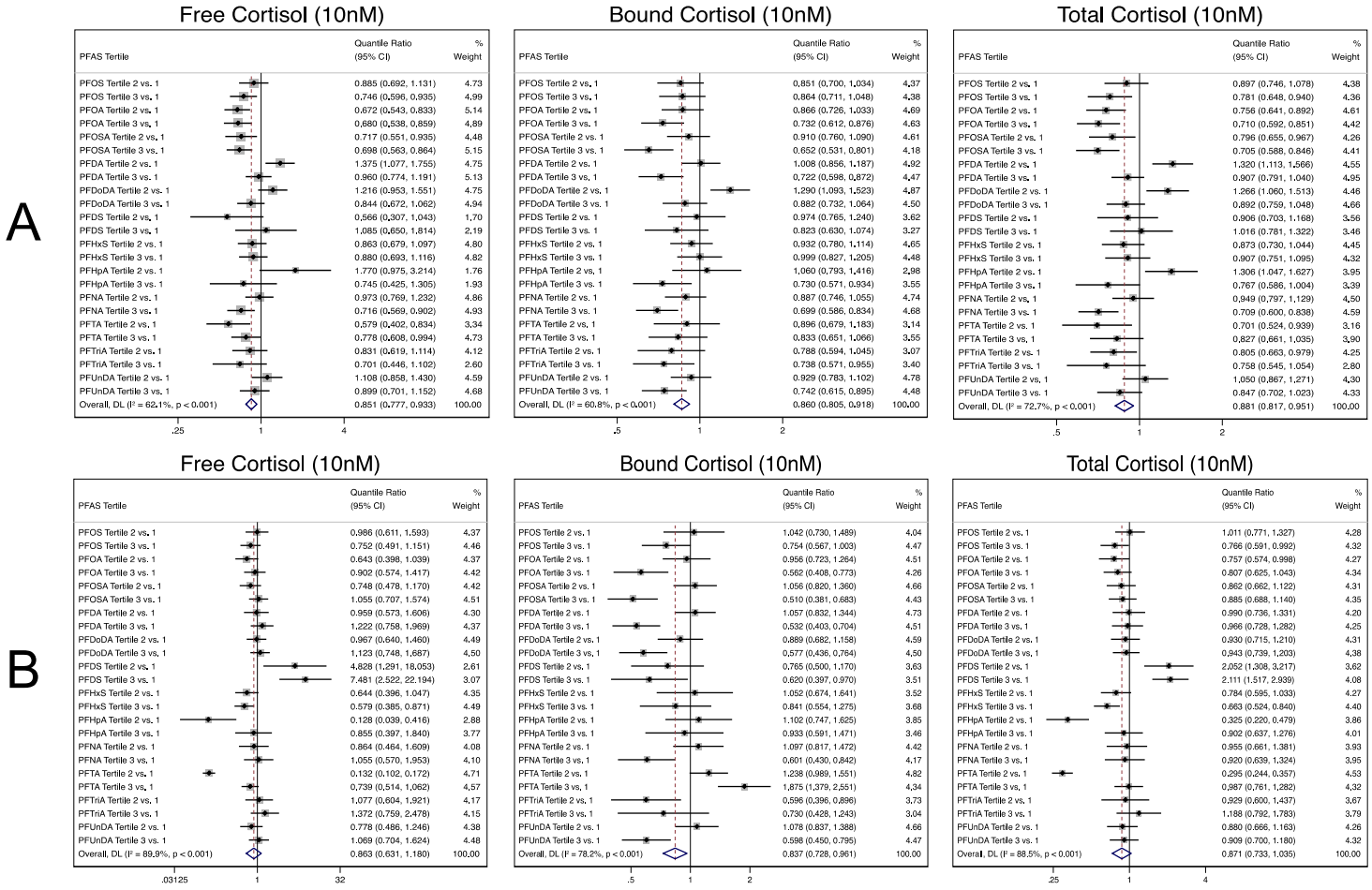
Supplemental Figure 12. Sex-stratified results, A) female and B) male, for adjusted parametric quantile regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for female free, bound, and total, PFOS (n=55), PFOA (n=61), PFOSA (n=61), PFDA (n=61), PFDoDA (n=61), PFDS (n=18), PFHxS (n=61), PFHpA (n=18), PFNA (n=61), PFTA (n=41), PFTriA (n=31), and PFUA (n=61); For male free and total, PFOS (n=137), PFOA (n=150), PFOSA (n=123), PFDA (n=150), PFDoDA (n=150), PFDS (n=70), PFHxS (n=150), PFHpA (n=67), PFNA (n=150), PFTA (n=61), PFTriA (n=46), and PFUA (n=150); for male bound PFOS (n=145), PFOA (n=158), PFOSA (n=130), PFDA (n=158), PFDoDA (n=158), PFDS (n=74), PFHxS (n=158), PFHpA (n=71), PFNA (n=158), PFTA (n=63), PFTriA (n=46), and PFUA (n=158).



Supplemental Figure 13. Sex-stratified results, A) female and B) male, for unadjusted parametric quantile regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for female free, bound, and total, PFOS (n=72), PFOA (n=81), PFOSA (n=80), PFDA (n=81), PFDoDA (n=81), PFDS (n=23), PFHxS (n=81), PFHpA (n=23), PFNA (n=81), PFTA (n=52), PFTriA (n=41), and PFUA (n=81); For male free and total, PFOS (n=150), PFOA (n=164), PFOSA (n=132), PFDA (n=164), PFDoDA (n=164), PFDS (n=78), PFHxS (n=164), PFHpA (n=75), PFNA (n=164), PFTA (n=65), PFTriA (n=49), and PFUA (n=164); for male bound PFOS (n=158), PFOA (n=173), PFOSA (n=140), PFDA (n=173), PFDoDA (n=173), PFDS (n=83), PFHxS (n=173), PFHpA (n=80), PFNA (n=173), PFTA (n=68), PFTriA (n=50), and PFUA (n=173).



Supplemental Figure 14. Age-stratified results, A) adult (females ≥7yo and males ≥10yo) and B) juvenile females <7yo and males <10yo), for adjusted parametric quantile regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Models were adjusted for location, year, sex, and age at time of sample collection. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for adult free and total, PFOS (n=164), PFOA (n=186), PFOSA (n=156), PFDA (n=186), PFDoDA (n=186), PFDS (n=81), PFHxS (n=186), PFHpA (n=78), PFNA (n=186), PFTA (n=81), PFTriA (n=64), and PFUA (n=186); for adult bound PFOS (n=169), PFOA (n=192), PFOSA (n=161), PFDA (n=192), PFDoDA (n=192), PFDS (n=84), PFHxS (n=192), PFHpA (n=81), PFNA (n=192), PFTA (n=83), PFTriA (n=65), and PFUA (n=192); For juvenile free and total, PFOS (n=58), PFOA (n=59), PFOSA (n=56), PFDA (n=59), PFDoDA (n=59), PFDS (n=20), PFHxS (n=59), PFHpA (n=20), PFNA (n=59), PFTA (n=36), PFTriA (n=26), and PFUA (n=59); for juvenile bound PFOS (n=61), PFOA (n=62), PFOSA (n=59), PFDA (n=62), PFDoDA (n=62), PFDS (n=22), PFHxS (n=62), PFHpA (n=22), PFNA (n=62), PFTA (n=37), PFTriA (n=26), and PFUA (n=62).



Supplemental Figure 15. Age-stratified results, A) adult (females ≥7yo and males ≥10yo) and B) juvenile females <7yo and males <10yo), for unadjusted parametric quantile regression models testing for differences in mean cortisol (10nM) across PFAS tertiles. Regression coefficients are relative to the first tertile of each exposure. Meta-analyses use the random-effects inverse-variance model with DerSimonian-Laird estimate of tau². % weight indicates the weighted average using inverse variance. The grey boxes are a graphical representation of the % weight. I^2 indicates proportion of variance explained between congener as a measure of heterogeneity. Sample sizes varied amongst PFAS and cortisol: for adult free and total, PFOS (n=164), PFOA (n=186), PFOSA (n=156), PFDA (n=186), PFDoDA (n=186), PFDS (n=81), PFHxS (n=186), PFHpA (n=78), PFNA (n=186), PFTA (n=81), PFTriA (n=64), and PFUA (n=186); for adult bound PFOS (n=169), PFOA (n=192), PFOSA (n=161), PFDA (n=192), PFDoDA (n=192), PFDS (n=84), PFHxS (n=192), PFHpA (n=81), PFNA (n=192), PFTA (n=83), PFTriA (n=65), and PFUA (n=192); For juvenile free and total, PFOS (n=58), PFOA (n=59), PFOSA (n=56), PFDA (n=59), PFDoDA (n=59), PFDS (n=20), PFHxS (n=59), PFHpA (n=20), PFNA (n=59), PFTA (n=36), PFTriA (n=26), and PFUA (n=59); for juvenile bound PFOS (n=61), PFOA (n=62), PFOSA (n=59), PFDA (n=62), PFDoDA (n=62), PFDS (n=22), PFHxS (n=62), PFHpA (n=22), PFNA (n=62), PFTA (n=37), PFTriA (n=26), and PFUA (n=62).