Perfluorinated Compounds, Thyroid Function, and Neuropsychological Status in Older Residents of Upper Hudson River Communities

by

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Abstract

Perfluorinated compounds (PFCs) are widespread in the environment. Minimal data exist regarding the neurotoxicity and thyroid disruptive property of PFCs in aging populations and the possible mediating effects of thyroid hormones (THs). In this study, we assessed associations among PFCs, thyroid function, and neuropsychological status, and determined if the neurotoxic effects of PFCs are mediated by changes in THs in an aging population. We measured perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), thyroid stimulating hormone (TSH), total thyroxine (T4), free T4 (fT4), and total triiodothyronine (T3) in serum and performed neuropsychological tests in men and women aged 55-74 years and living in upper Hudson River communities. Multivariable linear and quantile regressions were conducted. Mediation analyses were performed to obtain thyroid-mediated, non-thyroid, and total effects of a PFC on a neuropsychological test score.

Geometric means (standard deviations) of serum PFOS and PFOA were 34.20 (1.80) ng/mL and 8.10 (1.72) ng/mL respectively, higher than the levels in the general U.S. population. Serum PFOS was positively associated with fT4 and T4 in the overall study sample; one interquartile range increase in PFOS was associated with 5% and 9% increases in fT4 and T4 respectively. The results also suggested statistical interaction between PFOA and age for the effects on fT4 and T4.

Associations between THs and neuropsychological function were domain-specific. Higher T4 and fT4 were associated with improved visuospatial function, as measured by Block Design Subtest (BDT) total scores, in the overall study sample. We detected statistical interactions between age and THs for effects in tasks of memory and

learning and executive function. Concurrent increases in age and THs were associated with deficits in memory and learning and executive function.

We detected protective effects of PFCs in tasks of memory and learning and executive function. Total thyroxine partially mediated the protective effect of PFOS on BDT total scores (proportion mediated = 51%). However, the protective effects of PFCs on memory, learning and executive function were mostly mediated via pathways other than those involving alterations in THs. These findings provide insight regarding the impact of PFCs on thyroid and neuropsychological function and the role of THs.

Chapter 1: Introduction to Perfluorinated Compounds, Thyroid function, and Neuropsychological Function

1.1 Introduction

Perfluorinated compounds (PFCs) are a class of anthropogenic compounds used in the variety of consumer products and industrial applications; PFCs have been used as liquid repellants in carpets, textiles, paper coatings, leather, and fire-fighting foams, and as processing aids in the manufacture of fluoropolymers (Agency for Toxic Substances and Disease Registry (ATSDR) 2009; Lau et al. 2007; Prevedouros et al. 2006). PFCs are very stable and resistant to environmental degradation and metabolic biotransformation due to strong carbon-fluorine bonds; they also bioaccumulate at various trophic levels (Kannan et al. 2005; Lau et al. 2007). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two most predominantly manufactured PFCs in the U.S.A, and are the ones to be commonly detected in the environment (ATSDR 2009; Giesy and Kannan 2001; Kato et al. 2011).

Human exposure to PFCs can occur via ingestion of contaminated food, dust, and drinking water (ATSDR 2009). They are well-absorbed from gastrointestinal tract but poorly eliminated by the human body (Lau et al. 2007). The half-life of PFOS in humans has been estimated to be 5.4 years (Olsen et al. 2007), and that of PFOA has been estimated to range from 2.3 to 8.5 years (Bartell et al. 2010; Olsen et al. 2007; Seals et al. 2011). PFCs are structurally similar to free fatty acids, by the virtue of which they may compete for serum protein binding sites (Han et al. 2003; Jones et al. 2003). They exist in a bound form, primarily with albumin, and are concentrated in organs including liver and

kidneys that are rich in proteins (Lau et al. 2007). PFCs have been detected at greater concentrations in occupationally exposed populations and those residing in close proximity to industrial sources (Olsen and Zobel 2007; Steenland et al. 2009a), and at lower concentrations in the general U.S. populations (Kato et al. 2011).

Due to their potential link with adverse health outcomes, phase-out efforts of long-chained PFCs (i.e., PFCs with more than 7 carbon atoms), including PFOS and PFOA, were initiated in early 2000s (ATSDR 2009; Steenland et al. 2010a; U.S. Environmental Protection Agency (EPA) 2009). For example, in 2002, the 3M Company, a major manufacturer of PFCs, discontinued production of PFOS. In 2006, the EPA and the eight major fluoropolymer manufacturers launched the PFOA Stewardship Program to eliminate the emissions and product content of PFOA by 2015 (U.S. EPA 2009). In 2009, the EPA developed the Action Plan to further reduce long-chained PFCs, based on their body burden, persistence, bioaccumulative and toxic characteristics, amount of production and use in consumer products. The data from the National Health and Nutritional Examination Survey (NHANES), a nationally representative survey of the general U.S. population, indicates a gradual decrease of PFOS over time since the initiation of the phase-out efforts (Kato et al. 2011); however, PFOA levels have remained similar. PFCs were still detectable in 99% of the serum samples collected in the NHANES 2007-2008, and are therefore a potential health concern.

Potential health effects of PFCs in human include metabolic disorders, endocrine disruptions, and neurotoxicity. PFCs have been linked with dyslipidemia (Nelson et al. 2010; Steenland et al. 2009b), thyroid hormone (TH) imbalance (Knox et al. 2011; Wen et al. 2013), uric acid (Steenland et al. 2010b), fecundity (Fei et al. 2009), fetal growth

(Apelberg et al. 2007; Fei et al. 2007), and attention deficit/hyperactive disorder (ADHD) (Hoffman et al. 2010). Associations between many of such adverse health effects and PFCs are not clear because there are limited numbers of epidemiologic studies, and the results of the existing studies are not consistent. In the following review, I will focus on the effects of PFOS and PFOA on thyroid and neuropsychological function.

1.2 Thyroid Endocrinology

Normal thyroid function is essential for development of human body and brain as well as their optimal functioning throughout life. THs control metabolic functions, and are also important for proper cardiovascular system and neuropsychological well-being (Bauer et al. 2008; Klein and Ojamaa 2001; Yen and Brent 2012). Thyroid homeostasis is regulated by the hypothalamus-pituitary-thyroid axis (Santisteban 2012). Thyroid stimulating hormone (thyrotropin, TSH), a secretion of anterior pituitary gland, regulates the production and release of THs (i.e., thyroxine and triiodothyronine) from the thyroid gland, which itself is controlled by thyrotropin releasing hormone (TRH) secreted by hypothalamus. Imbalance of circulating THs in turn exerts negative feedback action on hypothalamus to inhibit or increase the secretion of TRH. Around 80% of TH secreted by the thyroid gland is thyroxine which is deiodinated to its active form triiodothyronine in peripheral tissues by thyroid deiodinases. Around 80% of triiodothyronine produced in human body is a result of extrathyroidal deiodination of thyroxine. THs are transported to target tissues in serum bound to transport proteins including thyroxine binding globulin (TBG), albumin, transthyretin, and other minor carriers (Benvenga 2012). TBG is the primary TH- carrier and transports around 64% of thyroxine and 80% of triiodothyronine; however, transthyretin is the major TH transport protein in the cerebrospinal fluid

(Herbert et al. 1986). Only 0.03% of total thyroxine (T4) and 0.3% of total triiodothyronine (T3) exist in free forms which are biologically active forms of THs available for cellular uptake.

1.3 Perfluorinated Compounds and Thyroid Function

A growing literature indicates that PFCs may have thyroid disruptive effects. Animal studies have reported decreases in THs following exposures to PFOS and PFOA (Lau et al. 2003; Seacat et al. 2002; Thibodeaux et al. 2003). In humans, associations between PFCs and serum THs have been reported in occupationally exposed groups (Olsen et al. 2003; Olsen and Zobel 2007) and in adults and children exposed to high levels of PFOA and background levels of PFOS and PFOA (Knox et al. 2011; Lopez-Espinosa et al. 2012b; Wen et al. 2013). However, the findings from these studies do not concur. As with TSH, most of the studies have reported no associations (Bloom et al. 2010; Lin et al. 2013; Lopez-Espinosa et al. 2012b; Olsen and Zobel 2007; Wen et al. 2013); few have reported positive or negative associations (Dallaire et al. 2009; Jain 2013). Studies examining associations between PFCs (i.e., PFOS and PFOA) and thyroid function markers in selected populations are described briefly in Table 1. Inconsistent findings across studies, particularly for THs, limit current understandings of PFCs' thyroid disruptive properties and the associated public health impact, which warrant further research.

Mechanisms by which PFCs may influence THs are not well understood. PFCs may compete for serum TH transport proteins and increase free forms of THs by displacing them from the protein-bound forms (Han et al. 2012; Jones et al. 2003; Weiss et al. 2009). However, the potential causal link between PFOS and THs had been

questioned. Reduction in free thyroxine (fT4) observed in prior animal studies following PFOS exposure had been suggested to be due to negative bias induced by analog hormone assay techniques in the presence of compounds that interfere with binding to serum protein analog including PFOS (Chang et al. 2007). Studies have demonstrated reduction in T4 and fT4 when measured by analog method but no significant difference in fT4 levels between exposed rats and controls when measured by the gold-standard equilibrium dialysis radioimmunoassay (Chang et al. 2008; Luebker et al. 2005). Negative bias was not observed in fT4 measurement due to the presence of PFOA and PFOS in a human population with serum PFOS ranges similar to that of general U.S. population but with higher PFOA levels (Lopez-Espinosa et al. 2012a).

1.4 Thyroid Function and Neuropsychological Function

Evidence indicates that overt thyroid conditions affect neuropsychological functions, including mood and neurocognition, in adults (Bauer et al. 2008; Whybrow and Bauer 2005a, b). THs have been suggested to affect mood and behavior by interacting with neurotransmitters, including norepinephrine, serotonin, and dopamine. Brain regions including the limbic system structures that have high densities of TH receptors have also been linked with mood disorders and dementia (Bauer et al. 2008; de Jong et al. 2009). Since aging populations carry substantial burdens of both neuropsychological impairments, and thyroid conditions (Peeters 2008; Plassman et al. 2007; Plassman et al. 2008), even very small changes in TSH and TH levels may be important for neurocognitive function in the elderly (Davis et al. 2003; Osterweil et al. 1992; Williams et al. 2009). However, despite a large body of literature on thyroid and neuropsychological functions (Roberts et al. 2006; St John et al. 2009; van Boxtel et al.

2004; Volpato et al. 2002; Wahlin et al. 1998; Wahlin et al. 2005; Wijsman et al. 2013), there is no sufficiently definitive information that elucidates how subclinical or subtle changes in TSH and THs affect neuropsychological status in aging populations (Joffe et al. 2013). For example, subclinical hyperthyroidism (defined as normal range of serum fT4 and free triiodothyronine (fT3)) concentrations in the presence of low serum TSH concentration (Cooper and Biondi 2012)) have been linked with better as well as worse cognitive function (Ceresini et al. 2009; Wijsman et al. 2013). Likewise, increasing levels of THs have been associated with improved neurocognition as well as with deficits (Hogervorst et al. 2008; Prinz et al. 1999; Volpato et al. 2002). In addition, as the major limitations, the previous studies have evaluated very limited domains of neuropsychological function, mainly using global measures of cognitive function such as the Mini Mental State Examination, and utilized few measures of thyroid function markers. The results of selected studies conducted in aging populations are summarized in Table 2. So, comprehensive evaluations of neuropsychological function using wide-range of biomarkers of thyroid function may add knowledge to the existing evidence.

1.5 Perfluorinated Compounds and Neuropsychological Function

PFCs' neurotoxic effect in human is one of the most understudied health outcomes. *In-vitro* models and animal studies suggest that PFCs may induce developmental neurotoxicity (Johansson et al. 2008; Pinkas et al. 2010; Slotkin et al. 2008; Zhang et al. 2011). Deficits in cognitive and motor functions in rodents have been demonstrated following prenatal and neonatal exposures to the chemicals (Butenhoff et al. 2009; Johansson et al. 2008; Johansson et al. 2009; Liu et al. 2010; Onishchenko et al. 2011). Impairment in memory retention has been shown following the exposure to PFOS

in rats during adulthood (Fuentes et al. 2007). Only a handful of studies, however, have investigated PFCs' neurotoxic effects in humans, and the majority of those have focused on children; the overall results suggest mixed associations (Fei and Olsen 2010; Hoffman et al. 2010; Stein and Savitz 2011; Stein et al. 2013); Table 3 summarizes the findings of the studies that examined associations of PFOS and PFOA so far. Only two studies have examined the associations in adults (Gallo et al. 2013; Power et al. 2013), and their findings suggest PFCs may be neuroprotective. So the data regarding the neurobehavioral effects of PFCs in human, particularly in adults, is very minimal.

1.6 Perfluorinated Compounds, Thyroid Function, and Neuropsychological Status

The literature has suggested several mechanisms, including alterations in calcium related signaling pathway, neural proteins, and cholinergic system, for PFCs' neurotoxic effects (Johansson et al. 2008; Johansson et al. 2009; Mariussen 2012), and peroxisome proliferator activated receptor (PPAR) activation for PFCs' neuroprotective effects (Gallo et al. 2013). In addition, associations of PFCs with alterations in THs, and associations of THs with neuropsychological function hint the possibility that PFCs may alter neuropsychological function via disruption of thyroid homeostasis, one of the putative mechanisms by which other persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) cause neurotoxicity (Kodavanti 2005). A recent study in children suggests that exposure to PCBs may decrease the resin T3 reuptake, a measure of the binding capacity of TBG, which may in turn affect neurodevelopment (Julvez et al. 2011). This may be true to other POPs and in

adults as well. To date, no studies have formally established if neurotoxic effects of any POPs including PFCs are mediated by alterations in thyroid function.

1.7 Research in Aging Population

The majority of the research regarding the effects of PFCs on thyroid and neuropsychological function have been focused on children or younger adults; there is a paucity of research in aging populations. For example, only few studies have included older individuals in their study of PFCs and thyroid function (Ji et al. 2012; Wen et al. 2013), and only one has elaborated the associations specifically for elderly (Knox et al. 2011). Likewise, only two studies have reported association between PFCs and neurocognitive function in adults/ aging populations (Gallo et al. 2013; Power et al. 2013).

Aging individuals are vulnerable to increased health risks owing to compromised biological capacities (Geller and Zenick 2005). For example, they may experience greater health risks from toxicants due to diminished ability to cope with toxic effects, and metabolize and/or eliminate them. Furthermore, body burdens of neurotoxicants may increase with age. Older people are at greater risk of both subclinical thyroid conditions and overt thyroid disease (Peeters 2008) and also carry substantial burdens of neuropsychological impairments, including poor cognition and mood disorders (Bauer et al. 2008; Whybrow and Bauer 2005a, b). Exposures to such neurotoxicants have been suggested to exacerbate deficits in central and peripheral nervous system function associated with aging process (Seegal 2001). Further studies that help elucidate the role of aging on the associations of PFCs with health effects are necessary.

1.8 Potential Sex Difference in the Associations of PFCs with Thyroid and Neuropsychological Function

Sex-related differences in pharmacokinetic-pharmacodynamic profiles of PFCs have been reported in a few animal species. For instance, sex-dependent renal elimination of PFOA has been observed across several animal species (Han et al. 2012; Lau et al. 2007). Laboratory studies suggest sex hormones-mediated PFOA clearance in rats (Kudo et al. 2002; Vanden Heuvel et al. 1992) may be related to involvement of organic anion transport proteins (OAT) in renal system (Kudo et al. 2002; Nakagawa et al. 2008). Greater PFC body burdens have also been reported in men than in women (Steenland et al. 2007; Knox et al. 2011; Steenland et al. 2009a), although renal clearances of PFOS and PFOA have been found to be negligible in humans, and no sex-dependent elimination was detected (Bartell et al. 2010; Harada et al. 2004; Nakagawa et al. 2009; Seals et al. 2011).

Sex-hormones, particularly estrogen, may play important roles in TH metabolism and neuroprotection. For example, sex hormones may alter thyroid function by altering clearance of TBG (Tahboub and Arafah 2009). The literature suggests estrogen is neuroprotective and neurotrophic in nature (Brann et al. 2007). Sex-specific associations between several environmental toxicants and thyroid and neuropsychological function have been observed in several instances (Bloom et al. 2013; Knox et al. 2011; Persky et al. 2001; Seegal et al. 2010; Turyk et al. 2007). It is possible that PFC may exert sex-specific effects on thyroid and neuropsychological function, owing to sexually dimorphic physiological processes; however, these are understudied areas.

1.9 Exposure to Other POPs

U.S. populations are exposed to other widespread, persistent, and bioaccumulative chemicals, including PCBs, PBDEs dichlorodiphenyl trichlorethane (DDT) and its metabolite *p,p*-dichlorodiphenyl dichloroethene (DDE) (Boas et al. 2012; Centers for Disease Control and Prevention 2005; Giesy and Kannan 2001; Johnson-Restrepo et al. 2005; Sjodin et al. 2008). Like PFCs, these chemicals are also linked with thyroid disruption and neurotoxicity (Bloom et al. 2013; Fitzgerald et al. 2008; Fitzgerald et al. 2012). Given their link with adverse health effects, the production and use of PCBs, a class of chlorinated aromatic hydrocarbons that were widely used for various commercial and industrial purposes such as in electric capacitors, lubricants, caulking etc, was discontinued in late 1970s in the U.S.A (ATSDR 2000). Similarly, PBDEs, a class of brominated aromatic hydrocarbons widely used as flame retardants in numerous consumer products, are also being phased-out (ATSDR 2004), and DDT, widely used pesticide, is no longer used (ATSDR 2002). Though the production of PCBs and DDT has been ceased, and PBDEs are being phased-out, they still remain in the environment given their persistent and bioaccumulative nature, and therefore, are a potential concern.

PFCs, PBDEs, and PCBs could potentiate each others' effect on thyroid and neuropsychological function, because as previously mentioned there is a strong likelihood that they share some mechanisms of action even though they are completely different classes of chemicals. Such interactive associations have been demonstrated in few toxicological (Eriksson et al. 2006; Wang et al. 2011) and epidemiologic studies (Bloom et al. 2013; Fitzgerald et al. 2007). For example, PBDE-associated deficits in memory and learning were reported to be greater among those individuals with higher

serum total PCB (Fitzgerald et al. 2007). In another study, co-exposures to serum PCBs and PBDEs exhibited synergistic effect on increase in T3 levels in women (Bloom et al. 2013). In the present context when the general populations are exposed to mixture of these POPs, assessment of joint effects on health effects due to co-exposure to these chemicals is important from a public health perspective.

1.10 Specific Aims

Numerous research gaps exist regarding the relationships among PFCs, thyroid function, and neuropsychological function. They include: i) insufficient information on associations of PFCs with thyroid function in aging populations and neuropsychological function in adults; ii) no data regarding whether such associations may be modified by other POPs; iii) insufficient information on associations between thyroid function and neuropsychological function among adults with euthyroid condition and/or subclinical thyroid deviation (especially aging populations); and iv) no data regarding whether any association between PFCs and neuropsychological may be mediated by THs .

The current study was performed to help address these research gaps. In this study, we investigated if PFOS and PFOA are associated with thyroid and neuropsychological function, if thyroid function is associated with neuropsychological function, and if thyroid function mediates effects of PFOA and PFOS on neuropsychological function. The specific aims of the study were:

1. *To assess associations between thyroid function, as measured by serum TSH, T4, fT4, and T3, and serum PFOS and PFOA among older men and women residing in the upper Hudson River communities.*

We also explored if such associations are age- and sex-dependent, and are modified by serum total PCB, PBDE, DDE, and DDE. The proposed hypothesis is that PFOS and PFOA will be positively associated with THs, particularly fT4.

2. *To examine associations between thyroid function, as measured by TSH, T4, fT4, and T3, and neuropsychological function.*

   The proposed hypothesis is thyroid imbalances will be associated with deficits in neuropsychological test scores. We predict that aging would accelerate deficits in neuropsychological function associated with serum TSH and THs.

3. *To examine associations between serum PFOS and PFOA and neuropsychological function, and to examine if such associations are mediated by TSH and THs.*

   The proposed hypothesis is that PFCs will be associated with deficits in neuropsychological test scores, and the effects of PFCs on neuropsychological function will be partially mediated by changes in TSH and THs.

1.11 Study Area Overview

The current study is derived from a larger project designed to assess exposure to POPs including PCBs, PBDEs, and PFCs, and their effects on neuropsychological function (Fitzgerald et al. 2008). The study population is comprised of men and women aged 55 to 74 years, who lived in three demographically similar communities near the Hudson River: Fort Edward, Hudson Falls, and Glens Falls of New York State (NYS). The study areas were chosen because General Electric plants situated in Hudson Falls and

Fort Edward used PCBs to manufacture electric capacitors from 1947 until 1977, and these facilities discharged almost one million pounds of PCBs into the upper Hudson River (U.S. EPA 2011).

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Table 1: Literature summary - PFOS and PFOA and thyroid function in selected populations

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Olsen et al (2003)	Workers involved in two perfluorooctanyl-manufacturing facilities of the 3M company in Antwerp (n = 255) and Decatur (n = 263)	PFOS: GMs (CIs) = 0.44 (0.38–0.51) ppm for Antwerp and 0.91 (0.82–1.02) ppm for Decatur; PFOA: GMs (CIs) = 0.33 (0.27–0.40) for Antwerp and 1.13 (0.99–1.30) for Decatur	TSH, T4, fT4, T3, T3 uptake, and fT4 index	Age, BMI, alcohol, cigarette, location	PFOS and PFOA positively associated with T3 in a cross-sectional assessment
Olsen et al (2007)	Workers involved in the production and the use of ammonium salt of PFOA in three different facilities of the 3M company; n = 552 (Antwerp = 206, Cottage Grove = 131, Decatur = 215)	Medians (ranges) of serum PFOS and PFOA levels were 0.72 (0.2-6.24) µg/mL and 1.10 (0.01-92.03) µg/mL respectively	TSH, T4, fT4, and T3	Age, BMI, and alcohol	PFOA negatively associated with fT4, and positively associated with T3 in all locations combined; facility-specific association detected
Dallaire et al (2009)	Inuit adult population of Nunavik, Canada (n = 623)	GM (CI) = 18.28 (17.19–19.44) ng/mL for PFOS	TSH, fT4, T3, and TBG	Age, sex, BMI, plasma lipids, cigarette, and education	PFOS was negatively associated with TSH, T3, and TBG, and positively associated with fT4.
Bloom et al (2010)	Sport fish anglers living in 16 New York State counties adjacent to Lake Erie and Ontario (n = 31, age range = 31-45 years)	GMs (CIs) = 19.57 (16.3–23.5) ng/mL and 1.33 (1.15–1.53) ng/mL for PFOS and PFOA	TSH and fT4	Age	No association

Table 1: Literature summary - PFOS and PFOA and thyroid function in selected populations

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Melzer et al (2010)	NHANES 1999-2006 (n = 3,974)	PFOA: GMs (CIs) = 4.91 (4.64–5.2) ng/mL and 3.77 (3.52–4.04) ng/mL for men and women PFOS: GMs (CIs) = 25.08 (23.63–26.62) ng/mL and 19.14 (17.8–20.58) ng/mL for men and women respectively	Thyroid disease ever and thyroid disease current with medication	Age, sex, race/ethnicity, education, BMI, smoking status, and alcohol consumption	Women with PFOA in the 4th quartile were more likely to report thyroid disease (ever/ current medication) compared to women in the 1st and 2nd quartiles; a similar but non-significant trend in men; for PFOS, men in the 4th quartile were more likely to report current treated thyroid disease as compared to men in the 1st and 2nd quartile
Lopez-espinosa et al (2012)	A cross-sectional association of 10,725 children aged 1 to 17 years, who lived in contaminated water districts near a chemical plant that used PFOA to manufacture fluoropolymers	Serum PFOS: Median (IQR) = 20 (15, 28) ng/mL Serum PFOA: Median (IQR) = 29 (13, 68) ng/mL In utero PFOA: Median (IQR) = 12 (5.4- 37) ng/mL	TSH and T4	Age, sex, and month of sampling	Increased odds of hypothyroidism associated with serum PFOA; Positive association of serum PFOS with T4

Table 1: Literature summary - PFOS and PFOA and thyroid function in selected populations

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Knox et al (2011)	A cross-sectional association of 52,296 adults, aged > 20 years, who lived in contaminated water districts near a chemical plant that used PFOA to manufacture fluoropolymers	PFOS: GMs (SDs) = 25.7 (17.5) ng/mL & 29.1 (20.6) ng/mL for women & men aged> 50 years PFOA: GMs (SDs) = 98.6 (230.2) ng/mL and 124.3 (380.8) ng/mL for women and men aged> 50 years	T4, T3 uptake, TSH, and albumin	Age, serum estradiol, and alcohol	Positive association of PFOA with T4 and inverse association with T3 uptake in women (for those aged < and > 50 years) and men (aged > 50 years); no significant main effects, but significant interaction between serum PFOA and sex for TSH; significant interaction between PFOA and sex for T3 uptake; PFOS positively associated with T4 and inversely associated with T3 uptake in all age and sex groups; significant interaction between sex and PFOS for T4 and T3 uptake; positive association of PFOA and PFOS with albumin in all age and sex groups
Ji et al (2012)	Siheung cohort near Seoul, South Korea (n = 633, 258 males and 375 females, > 12 years of age)	Medians (IQRs) = 7.96 (5.58–12.10) ng/mL and 2.74 (2.04–3.64) ng/mL for serum PFOS and PFOA	T4 and TSH	Age and BMI	No association

Table 1: Literature summary - PFOS and PFOA and thyroid function in selected populations

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Lin et al (2012)	567 participants in a population-based cohort of Taiwanese adolescents and young adults with abnormal urinalysis (age range = 12–30 years)	GMs (CIs) = 7.78 (2.42) ng/mL and 2.67 (2.96) ng/mL for serum PFOS and PFOS	fT4 and TSH	Age, gender, and lifestyle factors	No association
Wen et al (2013)	Participants of the NHANES 2007-2010 aged > 20 years (n = 1181, 673 Men and 509 Women)	GMs (CIs) = 14.2 (13.59 –14.86) ng/mL and 4.15 (4.02– 4.29) ng/mL for serum PFOS and PFOA	T4, fT4, T3, fT3, TSH, and thyroglobulin	Age, race, drinking, smoking, and natural log-urinary iodine	Positive association of serum PFOS with thyroglobulin among women ($p<0.06$); positive association between PFOA and T3 among women
Jain et al (2013)	NHANES 2007-2008 participants	NA	TSH, total and fT4, total and fT3, and thyroglobulin	NA	Positive association of PFOA with TSH and 3

Abbreviations: BMI, Body Mass Index; CI, 95% Confidence Interval; fT3, Free Triiodothyronine; fT4, Free Thyroxine; GM; Geometric Mean; IQR, Interquartile Range; NA, Not Available; NHANES, National Health and Nutritional Examination Survey; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; SD, Standard Deviation; T3, Total Triiodothyronine; T4, Total Thyroxine; TBG, Thyroid Binding Globulin; TSH, Thyroid Stimulating Hormone.

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
Jaeschke et al (1996)	A randomized double-blind placebo controlled trial among 37 sub-clinically hypothyroid patients aged 55 to 86 years	Placebo or L-T4 replacement therapy to achieve normal TSH level.	Word Fluency Test, Digit Symbol Substitution, Trial Making, Logical memory, and Word Learning Test		A subtle improvement in composite memory score in L-T4 treated patients as compared to placebo-treated patients; however, the authors question clinical importance of the difference detected in the cognitive measure between two groups
Wahlin et al (1998)	A cross-sectional study of 200 non-demented elderly aged 75 to 96 years	TSH and fT4	Episodic memory, verbal fluency, visuospatial ability (Block Design Test), short-term memory (Digit Span Test), and perceptual motor speed (Trail Making Tests A and B)	Age, education, and depressive symptoms	TSH positively associated to episodic memory performance
Prinz et al (1999)	A cross-sectional study of 44 healthy euthyroid elderly men (mean age = 72 years)	T4, T3, T3 uptake, and fT4 index	WAIS-R, Dementia Rating Scale, and Rivermead Behavioral Profile etc	Age, and education	T4 was positively associated with a general cognition factor, and performance and verbal scores of the WAIS-R; fT4 index associated with a general cognition factor.

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
Volpato et al (2002)	Assessment of associations between thyroid function markers measured at baseline and cognitive function at a baseline, and after 1, 2, and 3 years in 628 physically impaired euthyroid women aged > 65 years	TSH and T4	MMSE	Age, race, education, prevalent stroke, hypertension, diabetes, ankle-brachial index, depressive symptoms, albumin, L-T4 use, & estrogen replacement therapy.	Women in the lowest tertile of baseline T4 had increased risk of cognitive decline over a three-year period as compared to the women in the highest tertile
Gussekloo et al (2004)	An unselected population-based study of elderly in the Netherlands (= 599, age > 85 years)	TSH, fT4, and fT3	Depressive symptoms (GDS); Cognitive function (MMSE); in the participants with MMSE score > 18, cognitive function further investigated for attention (Stroop test), cognitive speed (Letter Digit Coding test), & memory (Word Learning Test)	Sex and education	Low fT3 was associated with poor global cognitive function and depressive symptoms

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
van Boxtel et al (2004)	Netherlands-based study of adults aged between 49-71 years (n = 120)	TSH	Memory (Word Learning Task), behavioral planning and evaluation (Concept Shifting Task), and attention (Stroop Color Word Test)	Age, sex, education, & mood status	Higher level of TSH associated with poor memory performance which disappeared when those with thyroid disorder and those on TH replacement therapy were excluded, and when mood status was controlled for
Wahlin et al (2005)	Longitudinal assessment of the survivors (n = 45) who participated in a previous study investigating aging and dementia; participants were aged 75-93 years at baseline	TSH	Episodic memory (free recall, and cued recall), verbal fluency, visuospatial ability (Block Design Test) & short-term memory (Digit Span Test), and perceptual motor speed (Trail Making Tests A and B)	Age at baseline, education, gender, and incident dementia diagnosis at either after 3 years or after 6 years of follow-up, and the relevant mood score	TSH change over 6 years was positively associated with episodic memory
Jorde et al (2006)	A total of 89 subjects with SHT & 154 control subjects (with normal TSH, fT4 and T3) were	TSH, fT4, and fT3; T4 supplementation for randomized trial	Fourteen tests of cognitive function, BDI, General Health Questionnaire	Age, sex, BMI, T4, T3, and TSH	TSH positively associated with Trail Making Test A; fT4 negatively associated with Stroop Part 1 and 2; fT3 negatively associated

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
	recruited from a general health survey; 69 with SHT included in placebo-controlled trial (36 in T4 group and 34 in Placebo group); mean age ranges from 61 to 63 years for all groups				with visual recall; those with SHT had significantly more favorable scores than controls; no significant difference in test scores for those taking T4 compared to those taking placebo.
Roberts et al (2006)	A cross-sectional study of elderly recruited from 20 primary care practices in central England (5,865 patients 65 years of age or older)	TSH and fT4; SHT, subclinical hyperthyroidism, overt hypo-and hyper-thyroidism, euthyroidism	Depression and anxiety (HADS), and cognitive function (the MEAMS and the Folstein MMSE)	Age, sex, comorbid conditions, deprivation, and medications	Statistically significant associations between anxiety and TSH, and between cognition and both TSH and fT4; however, authors emphasize no clinical relevance; statistical significance disappeared but magnitude of association persisted when overt thyroid disease were excluded

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
Hogervost et al (2008)	Study of an aging population of England and Wales (age range = 64-94 years, n = 1,047) to assess whether hypothyroidism and/or subclinical hyperthyroidism at baseline preceded was associated with cognitive decline after a 2-year follow-up	TSH and fT4	MMSE, WMS-R, Mood (SAD scale)	Age, sex, education, mood and baseline MMSE, vascular risk factors and disease, and study center	High TSH level associated with lower MMSE at baseline; when cases with thyroid disorders, stroke, and those with suspected neurobehavioral impairment were excluded, high level of fT4 was associated with worse MMSE performance after 2 year follow up
Kritz-Silverstein et al (2009)	Community dwelling 447 men & 663 women (age = 42 to 99 years)	TSH	Depression (BDI), Memory assessment (Buschke-Fuld Selective Test), cognitive impairment (3MSE), visuomotor tracking and attention (Trail Making Test B)	Age, education, exercise, smoking & BDI for cognitive function	No association between TSH & scores on cognitive function tests; TSH was inversely associated with BDI in men only.
Ceresini et al (2009)	A community based cross-sectional study of 1,171 men and women aged 23 to	TSH, fT4, and fT3	MMSE (MMSE < 24 considered as cognitive impairment)	Age, sex, education, physical activity,	Participants with subclinical hyperthyroidism were more likely to have

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
	102 years			smoking, stroke, Parkinson's disease, hypertension chronic heart failure & diabetes.	cognitive impairment
St John et al. (2009)	Study conducted in the participants of B-Vitamin Atherosclerosis Intervention Trial, a randomized trial to assess effect of vitamin B supplementation on progression of early carotid artery atherosclerosis (n = 489, age range = 40 to 88 years)	TSH	14 cognitive tests to generate 4 factors: general cognition, word list learning, logical memory, and visual memory	Age, gender, race-ethnicity, education, homocysteine levels, low density lipoproteins, and smoking status.	TSH not associated with any neurocognitive measures, although those aged > 60 years with higher TSH level demonstrated poor paragraph recall ($p<0.06$); gender-stratified analyses showed that TSH positively associated with scores on word list learning for women; significant interaction of TSH with age and gender for effects on visual memory and logical memory respectively

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
Parle et al (2010)	A randomized double-blind placebo controlled trial of 94 subjects with SHT, aged > 65 years, aimed to achieve euthyroidism	T4 or placebo was given for 12 months	MMSE, MEAMS, Trail Making A and B		No significant changes in any of the measures of cognitive function over time and no between-group difference in cognitive scores at 6 and 12 months
Resta et al (2012)	337 outpatients (177 men and 160 women); mean age = 74.3 years	Overt hypothyroidism; SHT; euthyroidism; subclinical hyperthyroidism; and overt hyperthyroidism	GDS, MMSE, Prose Memory Test, and Matrix Test	Sex, smoking, GDS, hypertension, diabetes mellitus, chronic heart failure, stroke and Parkinson's disease.	Patients with SHT or increased TSH had increased risk of developing cognitive impairment.
Castellano et al (2013)	A case control study assessing the association between thyroid function markers and cognitive decline over a 3-year period in a subsample of 62 participants (31 pairs) aged 67 years and over at baseline	TSH, T4, fT4, T3, & fT3	3MSE, GDS, and SMAF performed at entry and annually thereafter; cases were participant with a 3MSE score ≤ 79 three years after baseline whereas controls corresponded to participant for whom	Education, thyroid medication use; matched by age, sex, and global cognition; additionally adjusted for GDS and SMAF	No association

Table 2: Literature summary - thyroid function and neuropsychological function in aging populations

Author (Year)	Study Description/ Population	Exposure	Outcome	Covariates Adjusted	Results
			a 3MSE score > 79 was maintained over the same period.		
Wijsman et al (2013)	A longitudinal study of participants from a multicenter, randomized placebo-controlled trial designed to study the benefits of a cholesterol lowering drug among adults, aged between 70-82 years, with pre-existing vascular diseases/ with risk factors for vascular diseases (n = 5154)	Subclinical hyperthyroidism, euthyroidism, and SHT	Global cognition (MMSE), attention (Stroop Color Word Test), cognitive speed (Letter-Digit Coding Test), memory (Picture-Word Learning Test); cognitive performance was tested at baseline, after 9, 18, 30 months and at the end of the study (36-48 months).	Age, sex, education, country, and Apo E genotype	Subclinical hyperthyroidism was associated with improved cognition, as measured by the MMSE score, as compared to euthyroid group.

Abbreviations: 3MSE, Modified Mini Mental State Examination; BDI, Beck Depression Inventory; BMI, Body Mass Index; fT3, Free Triiodothyronine; fT4, Free Thyroxine; GDS, Geriatric Depression Scale; HADS, Hospital Anxiety and Depression Scale; L-T4, Levothyroxine; MEAMS, Middlesex Elderly Assessment of Mental State; MMSE, Mini Mental State Examination; SAD, Symptoms of Anxiety and Depression; SHT, Subclinical Hypothyroidism; SMAF, Functional Autonomy Measurement System; T3, Total

Triiodothyronine; T4, Total Thyroxine; TBG, Thyroid Binding Globulin; TSH, Thyroid Stimulating Hormone; WAIS-R, Wechsler Adult Intelligence-Revised; WMS-R, Wechsler Memory Scale-Revised

Table 3: Literature summary - PFOS and PFOA and neuropsychological function

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Hoffman et al (2010)	A cross-sectional study of 1999-2000 and 2003-2004 NHANES participants aged 12-15 years (n = 571)	Medians (ranges) = 22.6 (2.1-87.2) ng/mL and 4.4 (0.4-21.7) ng/mL for serum PFOS and PFOA	Parental report of previous ADHD	Age, sex, race, environmental tobacco smoke, maternal smoking during pregnancy, and NHANES sample cycle	Increased odds of ADHD associated with both PFOS and PFOA
Fei et al (2010)	Assessment of association between prenatal exposure to PFOS and PFOS, as measured in maternal blood levels drawn around 8 weeks of gestation, and behavioral health and motor coordination of the offspring at the age of 7 years; used the data from the Danish National Birth Cohort, which enrolled mothers in early pregnancy (n = 787)	Medians (ranges)= 34.4 (7.3-106.7) ng/mL and 5.4 (0.5-21.9) ng/mL for PFOS and PFOA; PFCs measured in maternal blood	SDQ (n = 787), and DCDQ (n = 526) completed by mothers	Parity, maternal age, prepregnancy BMI, smoking and alcohol use during pregnancy, SES, sex of child, breast-feeding, birth year, home density, gestational age at blood drawing, and parental behavioral problem scores during their childhood	Women in the 2nd quartile of PFOA had decreased odds of having a child with higher scores in emotional symptoms and hyperactivity, and women in the 3rd quartile of PFOA had decreased odds of having a child with higher scores in total difficulties and hyperactivity as compared with women in the 1st quartile; no significant associations with PFOS

Table 3: Literature summary - PFOS and PFOA and neuropsychological function

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Stein et al (2011)	A cross-sectional study of Non-Hispanic White children aged 5-18 years who lived in a Mid-Ohio Valley community highly exposed to PFOA through contaminated drinking water (n = 10,546)	Means (SDs) = 22.9 (12.5) ng/mL and 66.3 (106.1) ng/mL for serum PFOS and PFOA	ADHD	Age and sex	Children in the 4th quartile of PFOA associated with decreased odds of ADHD prevalence & children in the 2nd, 3rd, and 4th quartiles of PFOS associated with decreased odds of learning problem as compared with children in the 1st quartile
Stein et al (2011)	Assessment of association between PFOA (estimated *in utero* PFOA exposure and childhood serum PFOA) and performance on neuropsychological tests (assessed 3-4 years later at ages 6-12 years after PFOA measurement) among children who have lived in a Mid-Ohio Valley community highly exposed to PFOA through contaminated	Median (range) = 43.7 (4.5-649.2) ng/mL for estimated *in utero* PFOA; median (range)= 35.0 (0.7-838.6) ng/mL for childhood PFOA in serum	IQ, academic skills, neuropsychological function and attention/impulsivity	Age, sex, home environment, test examiner, maternal IQ, and child BMI	Children in the 3rd and 4th quartiles as compared with lowest quartile ofestimated *in utero* PFOA had increases in Numerical Operation Standard Score and Full Scale IQ respectively; children in the 3rd and 4th quartiles of estimated *in utero* PFOA had increases in attention as measured by the CCI; PFOA measured in childhood also associated with increase in attention

Table 3: Literature summary - PFOS and PFOA and neuropsychological function

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
	drinking water (n = 320)				as measured by the CCI
Power et al (2012)	A cross-sectional study of 1999-2000 and 2003-2008 NHANES participants aged 60-85 years (n = 1,766)	GMs(SDs) = 22.63 (2.13) ng/mL and 4.08 (1.97) ng/mL for serum PFOS and PFOA	Self-reported limitation due to difficulty remembering or period of confusion; self-reported difficulty with ADL due to senility; performance on the Digit Symbol Substitution Task (n = 275)	Age, race/ethnicity, gender, education, NHANES cycle, education, poverty income-ratio, food security, health insurance status, social support, physical activity, alcohol consumption, and smoking status	Protective, non-significant associations between PFOA and PFOS and self-reported cognitive limitations (due to difficulty remembering or periods of confusion; difficulty with ADL due to senility); when stratified by diabetic status, significant, protective association of PFOS with self-reported limitation due to difficulty remembering or period of confusion detected only among diabetics and diabetics with no medication.

Table 3: Literature summary - PFOS and PFOA and neuropsychological function

Author (Year)	Study Description/Population	Exposure	Outcome	Covariates Adjusted	Results
Gallo et al (2013)	A cross-sectional study of 21,024 adults aged > 50 years who lived in contaminated water districts near a chemical plant that used PFOA in the manufacture of fluoropolymers	GMs (CIs) = 42.6 (41.8, 43.3) ng/mL for serum PFOS and 22.4 (22.2, 22.6) ng/mL for PFOS	Self-reported short term memory impairment	Age, ethnicity, gender, school level, household income, alcohol consumption, and cigarette smoking	PFOS and PFOA were associated with reduced odds of a self-reported short term memory impairment; when stratified by diabetic status, the association did not persist among diabetics and did not differ by antidiabetic treatment

Abbreviations: ADHD, Attention Deficit/Hyperactivity Disorder; ADL, Activities of Daily Living; BMI, Body Mass Index; CCI, Clinical Confidence Index; DCDQ, Developmental Coordination Disorder Questionnaire; GM; Geometric Mean; IQ, Intelligence Quotient; NHANES, National Health and Nutritional Examination Survey; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; SD, Standard Deviation; SDQ, Strengths and Difficulties Questionnaire; SES, Socioeconomic Status

Chapter 2: Perfluorinated Compounds and Thyroid Function in Older Adults

2.1 Abstract

Current understandings of the thyroid disruptive properties of perfluorinated compounds (PFCs), particularly in aging populations, are limited. The objectives of this study were to (i) assess associations between thyroid function, as measured by serum thyroid stimulating hormone (TSH), total thyroxine (T4), free T4 (fT4), and total triiodothyronine (T3), and serum perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in an aging population, and (ii) explore if other persistent organic pollutants with thyroid disruptive properties, including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), modified such associations. We conducted a cross-sectional study among 87 men and women, aged 55 to 74 years, without clinically diagnosed thyroid disease, and residing in the upper Hudson River communities in New York. Geometric means (standard deviations) of serum PFOS and PFOA were 31.60 (1.70) ng/mL and 9.17 (1.72) ng/mL, respectively. Multivariable linear regression analyses indicated that serum PFOS was positively associated with fT4 and T4 in the overall study sample. The results suggested statistical interactions between PFOA and age for the effects on fT4 and T4. We detected statistical interactions between PFOS and total PCB for T3 and between PFOA and total PBDE for TSH. The results suggest that PFCs are associated with subtle alterations in levels of thyroid hormones in this population, and that age, PCBs, and PBDEs may interact to alter thyroid hormone levels.

2.2 Introduction

Perfluorinated compounds (PFCs) have been extensively used over the last five decades, mainly due to their surfactant properties in a variety of consumer products and industrial applications, including textiles, fire-fighting foams, and fluoropolymers (Agency for Toxic Substances and Disease Registry (ATSDR) 2009; Prevedouros et al. 2006). PFCs are very stable and resistant to environmental degradation and metabolic biotransformation due to strong carbon-fluorine bonds. Due to their persistence and ability to bioaccumulate, they are widespread in the environment at present (ATSDR 2009; Giesy and Kannan 2001; Kato et al. 2011). Given their ubiquity and potential link with health effects, efforts to phase out production and use of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the two most predominant PFCs, were initiated in early 2000s in the U.S.A (ATSDR 2009). They are well-absorbed from gastrointestinal tract but poorly eliminated by the human body; the half-life of PFOS has been estimated to be 5.4 years (Olsen et al. 2007) and that of PFOA has been estimated to range from 2.3 to 8.5 years (Bartell et al. 2010; Olsen et al. 2007; Seals et al. 2011).

Thyroid hormones (THs) are important for proper cardiovascular system and neuropsychological function (Bauer et al. 2008; Klein and Ojamaa 2001; Yen and Brent 2012). Thyroid homeostasis is regulated by the hypothalamus-pituitary-thyroid (HPT) negative feedback system (Davis et al. 2003). THs circulate bound to serum TH transport proteins (TTPs), with only small fractions of the hormones circulating in free forms. Rather than directly interfering with the HPT axis, PFCs may compete for TTPs, including albumin and transthyretin (Chang et al. 2008; Han et al. 2012; Jones et al.

2003; Weiss et al. 2009), and increase free forms of THs by displacing them from the protein-bound forms, although the mechanisms are not well-understood.

Alterations in serum THs have been demonstrated in occupational groups exposed to PFCs (Olsen et al. 2003; Olsen and Zobel 2007), and adults and children exposed to high levels of PFOA and background levels of PFOS (Knox et al. 2011; Lopez-Espinosa et al. 2012; Wen et al. 2013) while other studies have shown no associations (Bloom et al. 2010). As for TSH, most of the studies have reported no associations (Bloom et al. 2010; Lopez-Espinosa et al. 2012; Olsen and Zobel 2007; Wen et al. 2013). Inconsistent findings across studies, particularly for THs, limit current understandings of PFCs' thyroid disruptive properties and the associated public health impact, which warrant further research. Additionally, general aging populations are underrepresented in prior research (Dallaire et al. 2009; Wen et al. 2013), with only one study illustrating associations in aging individuals (Knox et al. 2011). Aging individuals may experience increased health risks owing to compromised biological capacities and higher body burdens of the chemicals (Geller and Zenick 2005). Therefore, characterization of the associated risks in such populations is important but understudied areas.

Besides PFCs, U.S. populations are also exposed to other persistent organic pollutants (POPs) with thyroid disruptive properties, including polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) (Boas et al. 2012; Centers for Disease Control Prevention 2009). In our previous research, we reported that such POPs may interact to affect thyroid function and neuropsychological function (Bloom et al. 2013; Fitzgerald et al. 2012). Likewise, given a strong likelihood for shared biological mechanisms, including interference with serum TTPs (Boas et al. 2012), PBDEs, PCBs,

and other POPs may modify the effects of PFCs on thyroid function. However, this has not been well-addressed in previous studies.

This study intended to address the insufficient information on associations of PFCs with thyroid function in aging populations and potential modifying effects of the other POPs on such associations. We assessed associations between serum PFOS and PFOA and thyroid function among older men and women residing in upper Hudson River communities. We predict that PFOS and PFOA will be positively associated with THs. Additionally, we examined if serum total PCB, PBDE, and dichlorodiphenyl trichlorethane (DDT) and its metabolite *p,p*-dichlorodiphenyl dichloroethene (DDE) modified such associations.

2.3 Methods

2.3.1 Sample Selection

The study population consisted of men and women, aged 55 to 74 years, who lived in three demographically similar communities near the Hudson River: Fort Edward, Hudson Falls, and Glens Falls of New York State (NYS), for 25 years or more (Figure 1). The study participants were originally recruited to investigate associations between environmental PCB exposure and neuropsychological function given the proximity to General Electric plants in Hudson Falls and Fort Edward that used PCBs to manufacture electric capacitors from 1947 until 1977, and discharged almost one million pounds of PCBs into the upper Hudson River (U.S. EPA 2011).

The procedures for participant recruitment have been described in detail elsewhere (Fitzgerald et al. 2007; Fitzgerald et al. 2008). Briefly, the source population was identified using an online telephone directory search engine and a digital database

(InfoUSA). A total of 2704 men and women aged 55 to 74 years and residing in one of the three target communities were contacted by telephone to determine eligibility for the study. Those who had not lived in their respective areas for at least 25 years, who had been involved in PCB-related job for ≥ 1 year, or who had certain medical conditions, including a history of stroke, severe head injury, Parkinson's disease, Alzheimer's disease, or severe cognitive impairment were ineligible for the study. Among those invited to participate, only 40% agreed.

The final cohort consisted of 253 participants from all three communities. During the years 2000-2002, structured in-person interviews were conducted to obtain information on socio-demographics, and histories on residence, fish consumption, occupation and medication use. Serum samples were also collected during 2000-2002 to measure levels of several pertinent biomarkers, including serum PCBs.

A flowchart outlining the sample selection process is displayed in Figure 2. Of the 253 participants from the original PCB study, 181 had sufficient serum remaining (≥ 1.0 mL), and 144 agreed for TH and PBDE determinations in 2005. In 2010, 166 participants who had adequate archived serum samples (volume ≥ 0.2 mL) were asked for consent to analyze serum PFCs and 157 individuals agreed. A total of 109 participants had information on both PFCs and thyroid biomarkers. Participants with clinical thyroid disease, who were taking any thyroid-related medications (n = 9), or who were sex hormone therapy (n = 13) were excluded (Tahboub and Arafah 2009). The final analysis was restricted to 87 participants.

2.3.2 Serum Chemical Analysis

A fasting sample of 25 mL of venous blood was drawn and centrifuged to obtain serum which was pipetted into a glass bottle. Serum PCBs, organochlorine pesticides, including DDE and DDT, cholesterol, and triglycerides were analyzed in the Wadsworth Center of the NYS Department of Health (NYSDOH) during 2000-2002 (Fitzgerald et al. 2007; Fitzgerald et al. 2008). Leftover sera were archived at -20ºC, which were later used for PBDE and PFC analysis in 2005 and 2010, respectively. The analytical procedures for serum PBDE analysis can be found elsewhere (Fitzgerald et al. 2010). The 30 PCB congeners were summed to get serum total PCB, and the 9 commonly detected PBDE congeners were summed to get serum total PBDE. Concentrations of serum DDE and DDT were also summed to get DDE+DDT.

The analytical procedure for the analysis of PFOS and PFOA is described in detail elsewhere (Kannan et al. 2004). The methods utilized initial extraction of the chemicals from serum using an ion-pairing method with subsequent subjection to a high performance liquid chromatograph-tandem mass spectrometer (HPLC-MS/MS). The limit of quantitation (LOQ) ranged from 0.5 to 1 ng/mL, which was determined based on the linear range of the calibration curve prepared at a concentration range of 0.5 ng/mL to 100 ng/mL. There was one observation below the LOQ for PFOA, for which the machine-read value was assigned.

2.3.3 Thyroid Function Biomarkers

Levels of TSH, free thyroxine (fT4), total thyroxine (T4), and total triiodothyronine (T3) in serum were determined using an immunoelectrochemiluminometric assay (Roche Elecsys 1010 system, Roche

Diagnostics, U.S.A) in 2005 by the Clinical Laboratory, Wadsworth Center, NYSDOH. The laboratory is CLIA-88 accredited. The assays make use of a competition test principle using antibodies that are specific for each analyte. Endogenous TH in the sample competes with exogenous analog in the test, which has been labeled with a ruthenium complex for binding sites on the biotinylated antibody. The reaction mixture is exposed to a voltage to induce the chemiluminescent emission which is measured by a photomultiplier. The average inter-run coefficients of variation for TSH, fT4, T4, and T3 were 2.5% (5.1% at concentrations < 0.2 μIU/mL), 2.2%, 4.5%, and 5.9%, respectively. The laboratory reference intervals were 0.3-4.2 μIU/mL for TSH, 5.1-14.1 μg/dL for T4, 0.9-1.7 ng/dL for fT4, and 80-200 ng/dL for T3.

2.3.4 Statistical Analysis

Serum total lipids (2.27 × cholesterol + triglycerides + 0.623) were estimated, and serum total PCB, total PBDE, and DDE+DDT were expressed on lipid basis, i.e. ng/g of serum total lipids (Fitzgerald et al. 2008; Fitzgerald et al. 2010; Phillips et al. 1989). Serum PFCs, total PCB, total PBDE, DDE+DDT, and TSH were log transformed to base 'e' to achieve normality. Student t-test, analysis of variance, Pearson correlation coefficients (r) and non-parametric tests, such as Wilcoxon's two sample test and Kruskal-Wallis test, were employed to assess bivariate associations between PFCs and THs, and with covariates.

To assess the associations of serum PFOS and PFOA with THs and TSH adjusting for potential confounders, multivariable linear regression models were performed. Directed acyclic graphs (DAGs), delineating hypothesized causal pathways between serum PFCs and THs, and with covariates based on literature, were used to

identify covariates for confounding adjustment (Greenland et al. 1999). The regression models were adjusted for age, sex, years of education, and serum total PCBs (lipid basis) (Bloom et al. 2013; Davis et al. 2003; Kato et al. 2011; Tahboub and Arafah 2009). Linear regression assumptions, including linearity, homoscedasticity, normality, and independence of errors, were assessed. We evaluated influential observations in the regression models using statistics, including Cook's D, dfbetas, and dffits (Kleinbaum et al. 1998). Parameter estimates reported in the results section indicate the change in TH level per interquartile range (IQR) increase in ln PFC for all regression models.

To check potential interactions of the PFCs with covariates including age, total PCB, total PBDE, & DDE+DDT, we performed regression analyses with two-way product terms between PFCs and theses covariates. In a linear regression, a product term assesses departure from additivity (i.e., if the joint effect differs from the sum of the individual effects). In a linear regression with a log-transformed outcome, a product term assesses departure from multiplicativity (i.e., if the joint effect differs from the product of the individual effects). For the models with p-value (p) for a product term < 0.10, we have reported an individual effect of PFC (i.e., change in TH level per IQR increase in ln PFC among individuals in the first quartile of a covariate), individual effect of a covariate (i.e., change in TH level per IQR increase in a covariate among individuals in the first quartile of ln PFC), and joint effect of ln PFC and a covariate (i.e., change in TH level per concurrent IQR increases in ln PFC and a covariate) (Knol et al. 2009).

As lipid standardization of PCBs (Phillips et al. 1989) have been suggested to produce biased estimates (Schisterman et al. 2005), we repeated the analyses using a PCB on a wet-weight basis while adjusting for total lipids as a covariate. All the statistical tests

were two-tailed, and are considered statistically significant at $p < 0.05$ for main effect and at $p < 0.10$ for product term. All the analyses were performed using SAS version 9.3 (SAS Institute, Inc. Cary, NC).

2.4 Results

Table 1 presents the background characteristics for the study participants. Mean age (standard deviation (SD)) of the participants was 63.6 (6.1) years; 58.6% (n = 51) were men. Median number of packs of cigarettes in the last year among smokers was 274. Table 2 presents the descriptive statistics of PFCs and thyroid biomarkers. Geometric means (GMs (SDs)) of serum PFOS and PFOA were 31.6 (1.7) ng/mL and 9.2 (1.7) ng/mL respectively. GMs (SDs) of serum TSH, T4, fT4, and T3 were 2.25 (1.72) µIU/mL, 8.6 (1.2) µg/dL, 1.2 (1.2) ng/dL, and 124.7 (1.14) ng/dL, respectively. GMs (SDs) of serum total PCB, total PBDE, and DDE+DDT were 458.12 (1.57) ng/g, 30.52 (3.38) ng/g, and 506.45 (3.12) ng/g, respectively.

PFOS and PFOA were positively correlated ($r = 0.52$, $p < 0.001$). PFOS was positively associated with T4 ($r = 0.39$, $p = <0.001$) and fT4 ($r = 0.23$, $p = 0.03$) and PFOA was positively associated with T4 ($r = 0.27$, $p = 0.01$) and T3 ($r = 0.23$, $p = 0.03$). PFOS was negatively associated with education ($r = -0.21$, $p = 0.05$) and income ($r = -0.25$, $p = 0.03$), and positively associated with serum total PCB ($r = 0.30$, $p = 0.01$). Similarly, serum PFOA was positively associated with cigarette consumption ($r = 0.28$, $p = 0.01$). Mean fT4 was significantly higher in men than in women ($p = 0.02$). Both T4 and T3 were associated with income ($r = -0.26$, $p = 0.02$ for T4 and $r = -0.22$, $p = 0.05$ for T3), and cigarette smoking ($r = 0.27$, $p = 0.012$ for T4 and $r = 0.22$, $p = 0.04$ for T3); T4 was negatively associated with alcohol consumption ($r = -0.22$, $p = 0.04$).

Table 3 presents the results of our multivariable analyses adjusted for age, sex, years of education, and serum total PCBs (lipid basis). Serum PFOS was positively associated with fT4 (β = 0.054, 95% Confidence Interval (CI) = 0.002, 0.106) and T4 (β = 0.766, CI = 0.327, 1.205). A positive association was suggested between serum PFOS and TSH, although not of statistical significance (β = 0.129, CI = -0.023, 0.281; p = 0.094). A similar association was suggested for serum PFOA and T4 (p = 0.097). When regression analysis was run with both PFOS and PFOA in the single model, statistical significance was detected only for the association between PFOS and T4 (Appendix A).

We detected departure from additivity between age and PFOA for effects on fT4 and T4 (Table 4). For T4, individual effect of PFOA was non-significant and very small, and that of age was negative and non-significant. However, joint increases in age and PFOA was associated with around 0.6 μg/dL or 7% increase in T4 while decrease in T4 was expected. Effects were very subtle for fT4. Figure 3 presents the association between PFOA and T4 stratified by median value of age (i.e., 63 years), which shows increasing slope for older group (i.e., age > 63 years) and no effect in younger group (i.e., age ≤ 63 years).

We also detected departure from additivity between the effects of PFOS and total PCB on T3 (β = 4.300, CI = -1.100, 9.701 for individual PFOS effect; β = 1.981, CI = -3.019, 6.981 for individual serum total PCB effect; β = -0.084, CI = -5.598, 5.430 for joint effect; p for a product term = 0.03). Here individual effects of PFOS and total PCB correspond to 3.4% and 1.6% increases in T3; however, joint effect indicated negligible change in T3 (i.e., -0.06%). We also detected statistical interaction between PFOA and total PBDE for the effect on TSH (β = 0.192, CI = 0.010, 0.375 for individual PFOA

effect; β = 0.035, CI = -0.124, 0.194 for individual serum total PBDE effect; β = 0.046, CI= -0.146, 0.239 for joint effect; p for a product term = 0.07). Joint effect of 0.04% change was greater than the expected change of 0.006% (i.e., 0.192 times 0.035). We did not observe any evidence of statistical interaction between either PFCs, and with sex and serum DDE+DDT.

We repeated the analyses with total lipids and serum total PCB (wet basis) as the covariates; the results were similar to those with PCBs expressed on lipid basis (Appendix A) except that p for interactions between PFOS and PCB (p = 0.047) and between PFOA and PBDE (p = 0.091) were slightly inflated. We repeated the analyses including 13 women taking sex hormones (n =100). Substantive difference was observed for the association between PFOS and T3; positive significant association was detected (β = 5.154, CI = 0.349, 9.960). In addition, no interactions with total PCB and total PBDE were detected.

2.5 Discussion

In the current study of older residents of the upper Hudson River communities, we detected positive associations between serum PFOS and fT4 and T4 as we predicted. Our results suggest statistical interactions between serum PFOA and age for effects on fT4 and T4; joint exposure accounted for small increases in fT4 and T4. We also detected statistical interactions between PFOS and total PCB on T3, and between PFOA and total PBDE on TSH.

This is one of the few studies to focus the associations in aging population. Although prior studies have included aging individuals in their analyses, only one study has elaborated associations in aging group (Knox et al. 2011), and our results

complement its findings. In the study of men and women aged > 50 years who were exposed to high levels of PFOA (means = 98.6 ng/mL and 24.3 ng/mL for women and men respectively) and background levels of PFOS (means = 25.7 ng/mL and 29.1 ng/mL for women and men respectively), PFOS and PFOA were positively associated with T4 (Knox et al. 2011). However, this study did not measure fT4 and T3 so as to compare with our results. In the same population (aged > 20 years), higher PFOA was associated with increased risk of hyperthyroidism among men and hypothyroidism among women (Winquist and Steenland 2014); however, no age-dependent associations were reported.

As for the other prior studies, due to the fact that our analysis exclusively focused on aging men and women, and that women were mostly postmenopausal, our results may not be comparable to theirs. Still, the results of these studies are not consistent, possibly due to difference in populations and exposure levels, and small sample sizes. For example, serum PFOS and PFOA were not associated with T4 and fT4, and positively associated with T3 in occupational groups from two facilities of the 3M Company that used PFOA salt and who were exposed to very high levels of PFOA and PFOS (Olsen et al. 2003). When this occupational data were reanalyzed including workers from a third facility (n = 506), serum PFOA was negatively associated with fT4 (Olsen and Zobel 2007). However, the authors considered the associations to be clinically irrelevant. At PFC levels lower than ours (GMs = 14.2 ng/mL for PFOS and 4.15 ng/mL for PFOA; age > 20 years), in the 1181 participants of the National Health and Nutrition Examination Survey (NHANES) 2007-2010, positive significant association between serum PFOA and T3 were detected among women (Wen et al. 2013). In a separate analysis of NHANES 2007-2008 participants, Jain (2013) reported positive association of serum PFOA with T3

and TSH. In a study of 623 Inuits, aged 18 to73 years and residing in Nunavik, Canada, serum PFOS (GM = 18.28 ng/mL) was positively associated with fT4 and negatively associated with T3 and TSH (Dallaire et al. 2009). Other studies that focused on background levels of the chemicals reported no association with fT4 (Bloom et al. 2010; Ji et al. 2012). As for TSH, most of studies have reported no association (Bloom et al. 2010; Lopez-Espinosa et al. 2012; Olsen et al. 2003; Olsen and Zobel 2007; Wen et al. 2013).

In this study, we did not detect main effects for TSH. Increases in THs, without significant change in TSH, as detected in our study, may indicate alterations in TTPs (Bloom et al. 2013). Generally, we would expect that increases in THs would prompt decrease in TSH due to negative feedback mechanism. However, it is possible that peripheral deiodination of excess THs might restore TH homeostasis without influencing the HPT axis, or subtle increases in THs might not be sufficient enough to suppress TSH secretion. Mechanisms by which PFCs may influence THs, including PFCs' interference with TTPs, are not well-understood. PFC-influenced increase in hepatic production of TTPs has also been implicated for increase in T4 (Knox et al. 2011). The results from a handful of human studies that have examined the associations of PFCs with measures of TTPs, including thyroxine-binding globulin (TBG), triiodothyronine uptake, and albumin, were not consistent (Benvenga 2012; Dallaire et al. 2009; Knox et al. 2011; Olsen et al. 2003). We also detected interaction effects between age and PFOA for fT4 and T4. The literature suggests that levels of TTPs as well as their binding capacity may change in older adults (Benvenga 2012; Braverman et al. 1966), possibly altering competitive binding of PFOA to TTPs.

Furthermore, PFCs have also been demonstrated to inhibit the activity of thyroperoxidase, an enzyme localized in thyroid gland responsible for oxidation of iodide to form THs, using *in vitro* assay procedure (Song et al. 2012); inhibition of thyroperoxidase and consequent decrease in organification of iodine may decrease TH secretion (Thalmann and Meier 2012). However, we detected the opposite findings.

No prior studies have reported effects of concurrent exposures to PFCs and other POPs on thyroid function. We detected that concurrent exposure to high levels of PFOS and total PCB exhibited no effect on T3 while exposure to PFOS among individuals with lower PCB level suggested potential elevation in T3. Prior literature indicates that PCBs decrease serum THs through several mechanisms that influence hormone synthesis and metabolism and transport proteins (Boas et al. 2012; Liu et al. 2012) which could explain the observed antagonistic effect. Similarly, multiplicative effects on TSH due to concurrent exposure to PFOA and PBDE may be due to biological interactions at several pathways including that involving serum TTPs (Boas et al. 2012; Meerts et al. 2000; Ren and Guo 2012).

It should also be noted that few toxicological studies have suggested association of PFOS with fT4 to be an experimental artifact. Specifically, it has been suggested that reduction in fT4 observed in prior animal studies following the PFOS exposure may be due to negative bias induced by analog hormone assay techniques, rather than a true reduction, in the presence of compounds that interfere with binding to serum protein analog including PFOS (Chang et al. 2007; Chang et al. 2008). However, negative bias was not observed in fT4 measurement due to the presence of PFOA and PFOS in a

human population with serum PFOS ranges similar to ours but with higher PFOA levels (Knox et al. 2011).

The current study was performed in aging individuals without a history of overt thyroid diseases and clinical neuropsychological conditions. It is likely that the currently reported alterations in THs have meaningful implications on neurocognitive well-being, particularly among hyperthyroid individuals or those individuals at the upper ends of TH distributions (Whybrow and Bauer 2005; Winquist and Steenland 2014). The associated public health impact may be significant given the heightened risk of thyroid diseases in elderly (Peeters 2008), ubiquity of PFCs (Kato et al. 2011), and rapidly growing aging populations (US Administration on Aging 2012).

It should be noted that levels of PFOS and PFOA in the current study were higher than those reported in the aging U.S. population (Kato et al. 2011). Given that serum PFOS was significantly correlated with serum total PCB and the current study population lived near a PCB-contaminated site, we could speculate that elevated levels may be due to common environmental or occupational sources in addition to the typical sources of PFCs.

However, the results should be carefully interpreted due to several limitations of the study. Due to cross-sectional nature of the study, it is not possible to determine temporal of order of PFC exposure and TH change, which limits from making etiological inferences. We did not have information on serum albumin and TBG which would have helped to tease out the detected associations. In addition, multiple statistical comparisons were made, which increased probability that the observed associations are chance findings due to inflation of Type I error rate. Small sample size limited the analysis in

several ways; for instance, we did not have enough sample size to assess the joint effects of age and sex on the THs, and we were not able to examine the associations with subclinical and clinical thyroid disorders due to small number of cases. Additionally, we used archived sera to estimate thyroid biomarkers and PFCs. However, stability of TSH and THs following 3-5 years of storage at -20°C and that of the PFCs following 6-8 years of storage may not be a concern (Mannisto et al. 2007; Martin et al. 2004).

The selection of the 87 participants was based on serum availability for PFC and TH determinations, thyroid disease status, and use of sex hormones and thyroid medications. Compared to those excluded, percentage reporting alcohol consumption was more whereas proportion of women was smaller in the 87. Difference in sex composition is due to exclusion of women taking sex hormones. Differential selection by alcohol consumption status is less likely to affect validity of the findings, given that PFCs are unlikely to be affected by alcohol.

2.6 Conclusions

Higher serum PFC levels were associated with increments in THs in general. Changes in levels of THs associated with the ranges of PFCs exposures seem to be relatively small but may still have substantial impact on cognitive function and neurobehavior. The results may be helpful in shedding light on the associations between other shorter chained PFCs which are still being used and understudied. Further studies, both epidemiologic and toxicological, that incorporate comprehensive sets of thyroid function end points and TTPs are warranted to support the findings of this study and to elucidate possible mechanisms involved.

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Figure 1: Study areas: Glens Falls, Hudson Falls, and Fort Edward

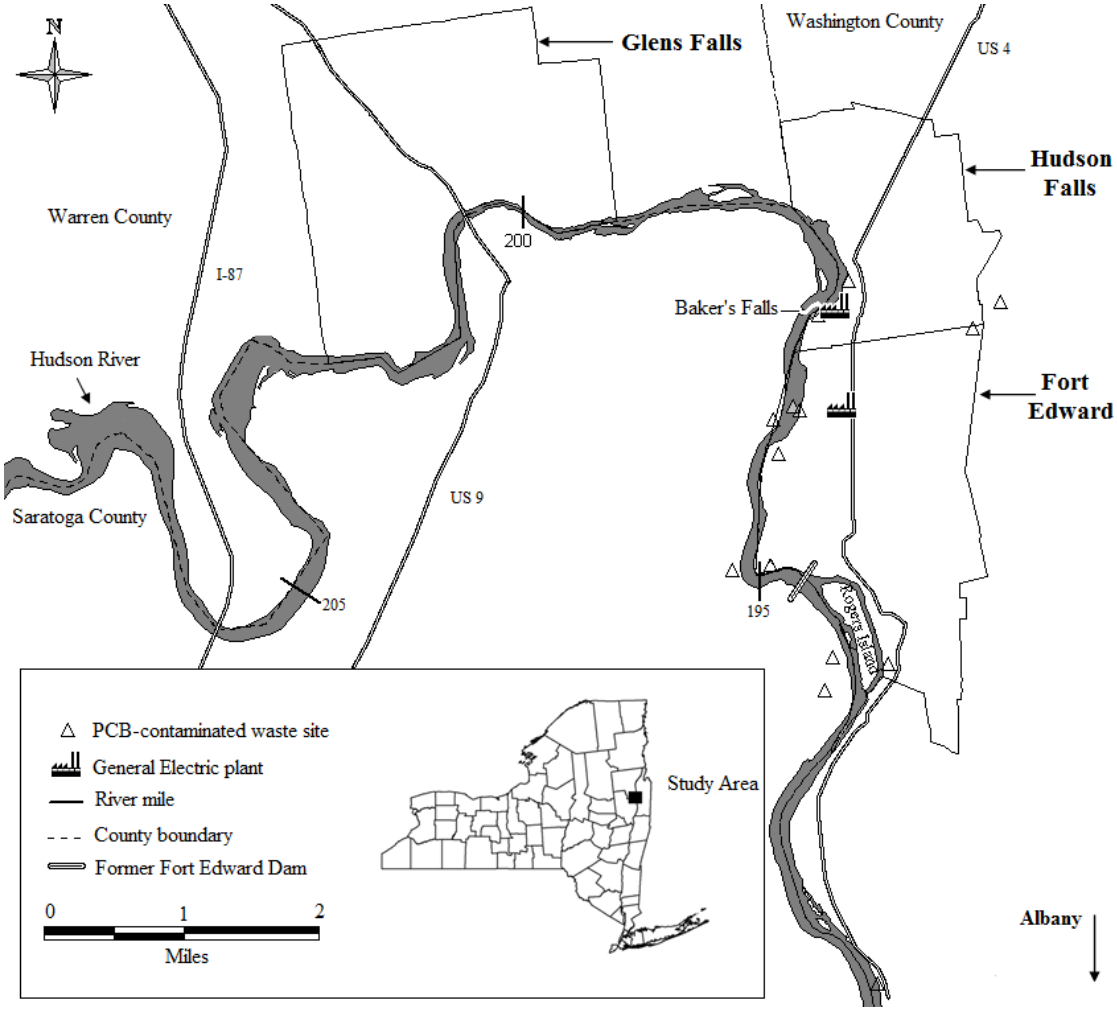
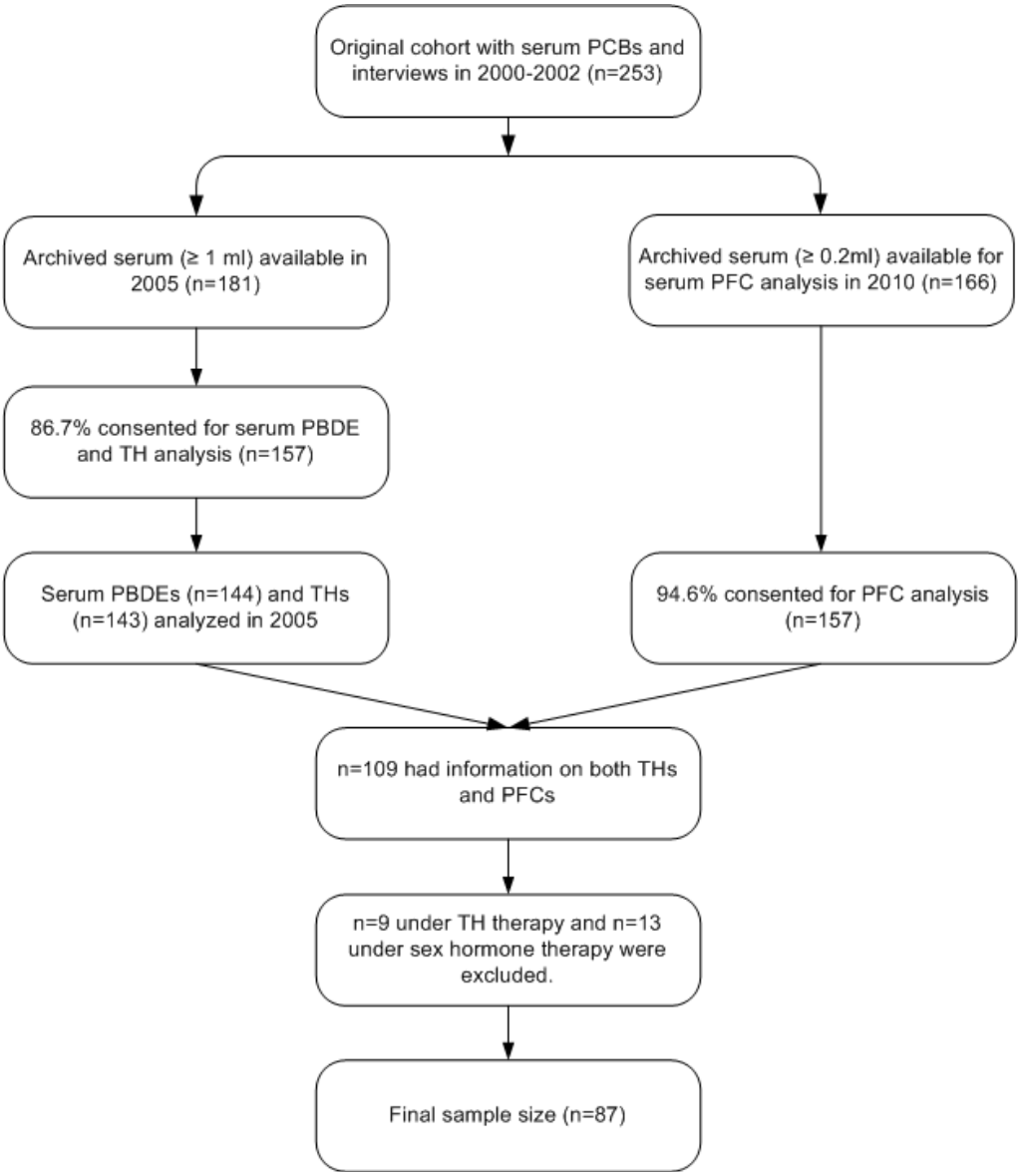


Figure 2: Sample selection process



Abbreviations: PCBs, Polychlorinated biphenyls; PBDEs Polybrominated Diphenyl Ethers; PFCs, Perfluorinated Compounds; THs, Thyroid Hormones

Table 1: Background characteristics of study participants (n = 87)

Variable	n	Mean (SD)	Median	Range
Age at interview (years)[a]	87	63.57 (6.06)	63	55, 74
Body mass index (kg/m^2)[a]	87	28.81 (5.76)	27.55	17.22, 49.6
Alcohol consumption (Amount over past year)	87	284.61 (377.36)	116	0, 2184
Among drinkers	78	317.45 (385.34)	187	1, 2184
Cigarette smoking (Total packs in last year)[a]	87	46.19 (144.06)	0	0, 730
Among smokers	14	287.08 (250.65)	273.75	0.65, 730
Years of education[a]	87	13.92 (2.78)	14	6, 20
Serum total PBDE (ng/g serum total lipids)[b]	87	30.52 (3.38)	23.51	4.95, 912.99
Serum total PCB (ng/g serum total lipids)[b]	87	458.12 (1.57)	445.25	139.3, 1638.19
Serum DDE+DDT (ng/g serum total lipids)[b]	87	506.45 (3.12)	612.98	3.03, 3593.04
Categories	**n**	**%**		
Sex				
Men	51	58.62		
Women	36	41.38		
Income category[c]				
< $15,000	4	4.88		
≥ $15,000 to $30,000	15	18.29		
> $30,000 to $45,000	23	28.05		
> $45,000 to $60,000	17	20.73		
> $60,000 to $75,000	13	15.85		
> $75,000	10	12.2		

Abbreviations: [a] Arithmetic Mean; [b] Geometric Mean; PBDE, Polybrominated Diphenyl Ethers; PCB, Polychlorinated Biphenyls; SD, Arithmetic Standard Deviation; [c] Frequency Missing = 5

Table 2: Descriptive statistics – serum PFCs and thyroid biomarkers (n = 87)

Categories	AM (SD)	GM (SD)	Median	Range
PFOS (ng/mL)	36.58 (22.8)	31.60 (1.70)	29.78	5.29, 139.53
PFOA (ng/mL)	10.42 (5.68)	9.17 (1.72)	9.32	0.58, 42.69
TSH (µIU/mL)	2.58 (1.47)	2.25 (1.72)	2.15	0.23, 9.05
fT4 (ng/dL)	1.24 (0.17)	1.23 (1.15)	1.26	0.86, 1.68
T4(µg/dL)	8.69 (1.47)	8.57 (1.19)	8.66	6.09, 12.08
T3(ng/dL)	125.69 (15.58)	124.71(1.14)	124.60	82.70, 172.40

Abbreviations: AM, Arithmetic Mean; GM: Geometric Mean; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; SD, Standard Deviation; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone

Table 3: Final multivariable models* for thyroid function markers with serum PFCs (ng/mL)† (n = 87)

Variable	β	95% LCI	95% UCI	P-value
TSH (μIU/mL)†				
PFOS	0.129	-0.023	0.281	0.094
PFOA	0.102	-0.047	0.250	0.176
fT4 (ng/dL)				
PFOS	0.054	0.002	0.106	0.044
PFOA	0.016	-0.036	0.069	0.536
T4(μg/dL)				
PFOS	0.766	0.327	1.205	0.001
PFOA	0.380	-0.070	0.830	0.097
T3 (ng/dL)				
PFOS	2.631	-2.248	7.510	0.287
PFOA	3.032	-1.725	7.789	0.208

Abbreviations: LCI, Lower Confidence Interval; PCB, Polychlorinated Biphenyls; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone; UCI, Upper Confidence Interval

* Adjusted for age, sex, years of education, and serum total PCB (ng/g serum total lipids)

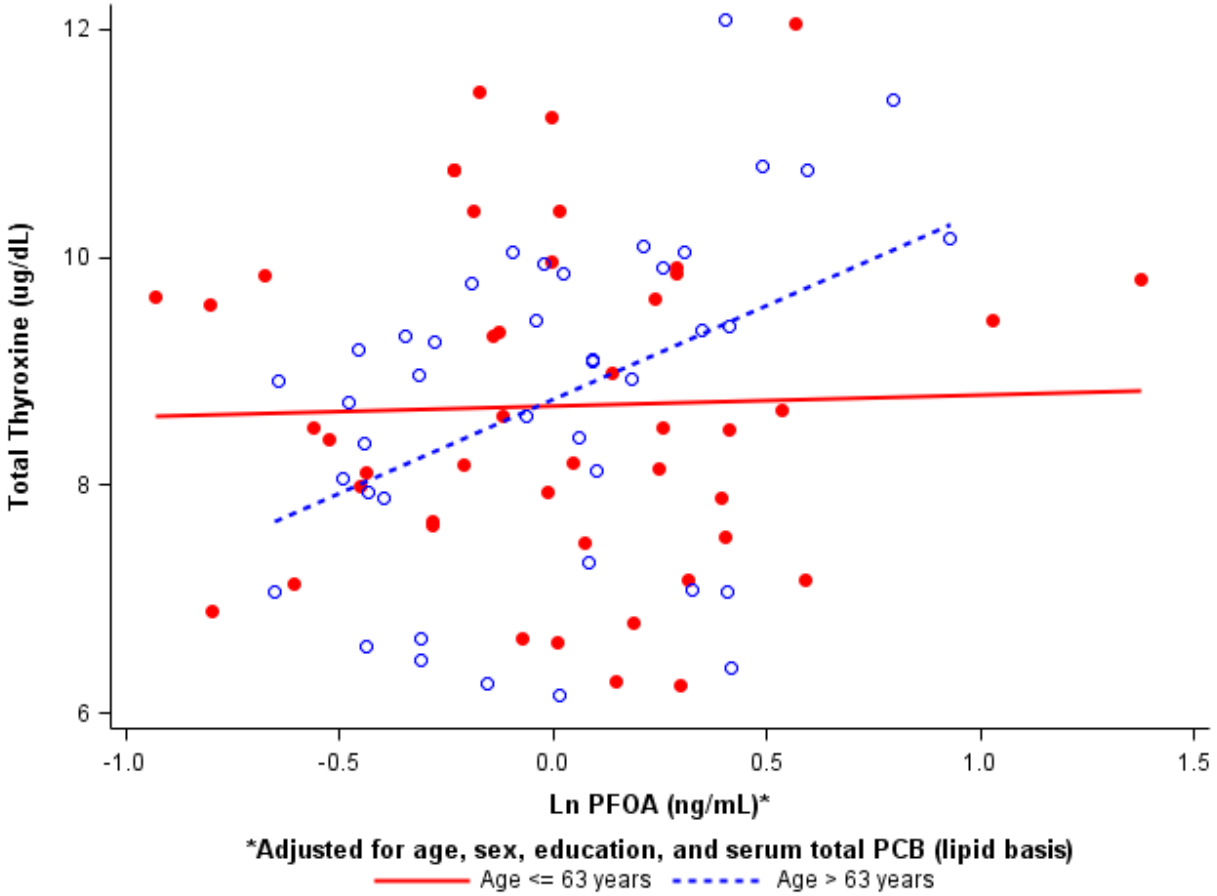
† Log-natural transformed

Table 4: Individual and joint effects* of PFOA† and age on thyroid hormones (n = 87)

	PFOA (β (CI)) [M1]	Age (β (CI)) [M2]	Joint effect (β (CI))[J]	p
TSH (μIU/mL) †	0.032 (-0.148, 0.212)	0.107 (-0.107, 0.321)	0.293 (0.035, 0.551)	0.225
fT4 (ng/dL)	-0.024 (-0.086, 0.038)	-0.034 (-0.108, 0.041)	0.031 (-0.058, 0.121)	0.043
T4(μg/dL)	0.005 (-0.530, 0.540)	-0.210 (-0.847, 0.428)	0.618 (-0.150, 1.387)	0.029
T3 (ng/dL)	1.557 (-4.233, 7.346)	-3.301 (-10.198, 3.596)	1.496 (-6.819, 9.812)	0.427

Abbreviations: CI, 95% Confidence Interval; PCB, Polychlorinated Biphenyls; PFOA, Perfluorooctanoic Acid; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone; * Adjusted for age, sex, years of education, and serum total PCB (ng/g serum total lipids); †Log-natural transformed; M1= Individual effect of PFOA (i.e., change in thyroid hormone level per IQR increase in PFOA among reference age group); M2 = Individual effect of age (i.e., change in thyroid hormone level per IQR increase in age among reference PFOA group); J = Joint effect of PFOA and age (i.e., change in thyroid hormone level score per IQR increments in both PFOA and age); p = p-value of a product term between PFOA and age

Figure 3: Associations between total thyroxine (µg/dL) and PFOA (ng/mL, log-transformed) stratified by age ≤ median value of 63 years (n = 87)



Supplemental Table 1: Final multivariable models* for thyroid function markers with serum PFCs (ng/mL)† (n = 100)

Variable	β	95% LCI	95% UCI	P-value
TSH (μIU/mL) [b]				
PFOS	0.027	-0.108	0.162	0.695
PFOA	0.070	-0.085	0.224	0.374
fT4 (ng/dL)				
PFOS	0.051	0.009	0.094	0.019
PFOA	0.025	-0.025	0.076	0.320
T4(μg/dL)				
PFOS	0.882	0.485	1.279	<0.001
PFOA	0.417	-0.047	0.882	0.078
T3 (ng/dL)				
PFOS	5.154	0.349	9.960	0.036
PFOA	4.666	-0.664	9.996	0.085

Abbreviations: LCI, Lower Confidence Interval; PCB, Polychlorinated Biphenyls; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone; UCI, Upper Confidence Interval

* Adjusted for age, years of education, and serum total PCB (ng/g serum total lipids)

†Log-natural transformed

Chapter 3: Thyroid Function and Neuropsychological Status in Older Adults

3.1 Abstract

Although overt thyroid dysfunction has been established as a risk factor for neuropsychological deficits in aging populations, evidence of whether subclinical changes in levels of markers of thyroid function are associated with such deficits is limited. Therefore, we assessed if changes in levels of thyroid stimulating hormone (TSH), total thyroxine (T4), free T4 (fT4), and total triiodothyronine (T3) are associated with neuropsychological function among men and women aged 55-74 years without overt thyroid diseases and living in upper Hudson River communities. We performed neuropsychological tests to assess various domains, including memory and learning, executive function, measures of attention, visuospatial function, reaction time, affective state, and motor function. Multivariable regression analyses were performed adjusting for age, sex, education, and cigarette smoking. Higher T4 and fT4 were associated with improved visuospatial function, as measured by Block Design Subtest total scores, in the overall study sample. We detected statistical interactions between age and thyroid hormones (THs) for effects in tasks of memory and learning and executive function. Concurrent increase in age and T4/fT4 was associated with deficits in memory and learning, as measured by subtests of the California Verbal Learning Test. Similarly, joint increase in age and T4/T3 was associated with impaired executive function, as measured by subtests of the Wisconsin Card Sorting Test. Our results indicate that associations

between TH and neuropsychological function could be domain-specific, and are modified by increasing age.

3.2 Introduction

Thyroid hormones (THs) play important roles in proper functioning of both the developing and adult brain. Overt clinical thyroid conditions affect neuropsychological function, including mood and neurocognition, in adults (Bauer et al. 2008). Generally, aging populations demonstrate substantial burdens of both neuropsychological impairments and thyroid dysfunction (Peters et al. 2008; Plassman et al. 2008). Given the aging-associated vulnerabilities to these health effects, even subclinical, or subtle changes in levels of circulating THs including thyroid stimulating hormone (TSH), may be critical for neurocognitive function in elderly (Davis et al. 2003; Joffe et al. 2013).

Current biomonitoring studies report widespread presence of persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and perfluorinated compounds (PFCs), in the environment (Centers for Disease Control and Prevention 2005). Body burdens of these potential thyroid disruptors and neurotoxicants (Boas et al. 2012) in general populations are still significant even though they are no longer used (i.e., PCBs), or their uses are being gradually discontinued (i.e., PBDEs and PFCs). Previously, we reported that exposures to such POPs were associated with subtle changes in THs, and with poor memory and learning in an aging population (Bloom et al. 2013; Fitzgerald et al. 2008; Fitzgerald et al. 2012). Changes in THs associated with these compounds may therefore have important implications on neuropsychological well-being of aging adults since their body burdens increase with

age, and aging individuals may have diminished ability to cope with the toxic effects (Geller and Zenick 2005).

A large number of studies have assessed association of THs with cognitive function in aging populations with euthyroid or subclinical thyroid conditions (Roberts et al. 2006; St John et al. 2009; van Boxtel et al. 2004; Volpato et al. 2002; Wahlin et al. 1998; Wahlin et al. 2005; Wijsman et al. 2013). However, the results are not consistent across studies (Joffe et al. 2013). For instance, subclinical hyperthyroidism was associated with improved cognition as well as with deficits (Ceresini et al. 2009; Wijsman et al. 2013), and both high and low serum TH levels were associated with poor cognition (Hogervorst et al. 2008; Prinz et al. 1999; Volpato et al. 2002). A better understanding of such associations in individuals without overt TH dysfunction is essential from a clinical perspective, and may provide insight on mediation of neurotoxic effects of POPs by THs.

In this study, we assessed associations between serum TSH and TH levels with neuropsychological function among older men and women, without overt thyroid diseases, and living in upper Hudson River communities. We predicted that aging would accelerate deficits in neuropsychological function associated with serum TSH and THs.

3.3 Methods

3.3.1 Sample Selection

The current study is derived from a larger project examining exposure to PCBs, THs, and neuropsychological function. The study population is comprised of men and women aged 55 to 74 years, who lived in three demographically similar communities near the Hudson River: Fort Edward, Hudson Falls, and Glens Falls of New York State

(NYS). The study areas were chosen because General Electric plants in Hudson Falls and Fort Edward used PCBs to manufacture electric capacitors from 1947 until 1977. These facilities discharged almost one million pounds of PCBs into upper Hudson River (U.S. EPA 2011).

The participant recruitment procedures have been described in detail elsewhere (Fitzgerald et al. 2008). We identified the source population using an online telephone directory search engine and a digital database (InfoUSA). A total of 2704 men and women aged 55 to 74 years and living in one of the three target communities were contacted by telephone to determine the study eligibility. Exclusion criteria included those: i) who had not lived in their respective areas for at least 25 years, ii) who had been involved in PCB-related job for ≥1 year, or iii) who had certain medical conditions, including a history of stroke, severe head injury, Parkinson's disease, Alzheimer's disease, or severe cognitive impairment. Of those who met the eligibility criteria and invited to participate, only 40% agreed.

The final cohort consisted of 253 participants from all three communities. During the years 2000-2002, structured in-person interviews were conducted to obtain information on socio-demographics, and histories on residence, fish consumption, occupation and medication use. Serum samples were also collected during 2002-2002 to measure levels of PCBs. Leftover samples were archived at -20°C. In 2005, participants with sufficient volume of the archived sera were asked for consent to determine THs, and 143 agreed. The final study sample for the current analysis included 130 participants after excluding 13 with clinical thyroid disease and/or who were under TH therapy.

3.3.2 Thyroid Function Biomarkers

Levels of TSH, total thyroxine (T4), free thyroxine (fT4), and total triiodothyronine (T3) in serum were measured using an immunoelectrochemiluminometric assay (Roche Elecsys 1010 system, Roche Diagnostics, U.S.A) in 2005. The analyses were performed in the Clinical Laboratory, Wadsworth Center, NYS Department of Health. The average inter-run coefficients of variation for TSH, T4, fT4, and T3 were 2.5% (5.1% at concentrations < 0.2 μIU/mL), 4.5%, 2.2%, and 5.9%, respectively. The laboratory reference intervals were 0.3-4.2 μIU/mL for TSH, 5.1-14.1 μg/dL for T4, 0.9-1.7 ng/dL for fT4, and 80-200 ng/dL for T3.

3.3.3 Neuropsychological Assessment

Neuropsychological tests were administered during 2000-2002, and the details can be found elsewhere (Fitzgerald et al. 2008). The California Verbal Learning Test (CVLT) (Delis et al. 2000) and the Wechsler Memory Scale (WMS) Form I-Russell's Revision tests (Russell 1975) were used to evaluate memory and learning. The CVLT consists of five learning trials for acquisition of a 16 item word list (i.e., List A) followed by an immediate recall after presentation of an interference word list (i.e., List B), a 20-minute delay recall trial, and a delayed recognition trial. Scores generated assess the ability to acquire, retain, and retrieve verbal information. In addition, CVLT provides an assessment of organizational strategies such as ability to organize words according to semantic features and learning efficiency. The WMS was used to assess immediate and delayed memory of verbal and visual material.

Measures of attention were assessed using the Trail Making Test (TMT)-Parts A and B, a subtest of the Halstead-Reitan Battery (Reitan and Wolfson 1993). The Stroop

Color Word Test (SCWT) (Trenerry et al. 1989) and the Wisconsin Card Sorting Test (WCST) (Heaton 1981) were used to assess executive function, a set of cognitive skills involved in anticipation, planning, and initiation of a number of goal-directed behaviors. In the SCWT, the participant is asked to name aloud the ink color in which a color name is typed while ignoring the verbal content. The SCWT assesses individuals' ability to shift a perceptual set, here the ability to suppress a dominant response (word reading) in order to provide the required response. In the WCST, the participant is presented with two decks of 64 response cards and is instructed to sort each card to one of four key stimulus cards, one at a time, based on one of three sorting principles (color, shape, or number). The participant, uninformed about a sorting principle, would have to determine underlying sorting principles by using corrective feedback ('correct'/'incorrect') that is provided by the examiner. As the sorting principle changes during the test, mental flexibility is required to shift cognitive strategies and to generate alternative sorting principles.

Visual and spatial functions were assessed by the Digit Symbol Substitution Test (DSST), a measure of visuomotor tracking and processing speed, and the Block Design Subtest (BDT), a measure of visuospatial organization (Wechsler 1981). The Simple Reaction Time Test was used to assess the ability of an individual to respond to a visual stimulus after an auditory warning.

The Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI) were used to assess presence and severity of depression, and state and trait anxiety, respectively (Beck et al. 1961; Speilberger et al. 1970). In the motor function domain, the Static Motor Steadiness Test (SMST) (Lezak et al. 2004), the Grooved

Pegboard Test (GPT) (Klove 1963), and the Finger Tapping Test (FTT) (Reitan and Wolfson 1993) were used to assess steadiness of hand and arm, complex visuomotor coordination and visuospatial orientation, and motor speed and coordination, respectively. In addition, we used the Test of Memory Malingering (TOMM) to differentiate the participants who are exhibiting an adequate level of effort from those who are not (Tombaugh 1996).

3.3.4 Statistical Analysis

We used Student t-test, analysis of variance, and non-parametric tests, such as Wilcoxon's two sample test, Kruskal-Wallis test, and Spearman correlation coefficients (r_s) to assess bivariate associations between THs and neuropsychological test scores, and with covariates. Serum TSH and some of the neuropsychological test scores were log-transformed to base 'e' for normality in statistical models. To assess associations of THs with neuropsychological tests adjusting for potential confounders, multivariable regression models were performed. We decided to select covariates for inclusion in the models based on the prior literature and based on the hypothesized causal associations between the covariates in directed acyclic graphs (Greenland et al. 1999). Regression models were adjusted for age, sex, years of education, and cigarette smoking (Ardila et al. 2000; Bertelsen and Hegedus 1994; Brann et al. 2007; Peeters 2008; Peters et al. 2008; Plassman et al. 2008; Saykin et al. 1995; Swan and Lessov-Schlaggar 2007; Tahboub and Arafah 2009).

For the continuous outcome variables with normal distribution, linear regression models were constructed. Linear regression assumptions, including linearity, homoscedasticity, normality, and independence of errors, were assessed. Cook's D,

dfbetas, and dffits were examined to identify influential observations during regression modeling (Kleinbaum et al. 1998). Regression models were rebuilt without influential observations. For highly skewed continuous outcome variables for which normality could not be achieved after log-transformation, quantile regression models (for the 25^{th}, 50^{th}, and 75^{th} quantiles of the test scores) were constructed (Hao and Naiman 2007). Standard errors were computed using a resampling method. Parameter estimates obtained from quantile regression indicate the change in a specified quantile of a neuropsychological test score per unit change in the exposure variable. Negative-binomial regression models were constructed for count outcomes. Here, we have reported the change in a neuropsychological test score per interquartile range (IQR) increase in a TH for all regression models.

In addition, two-way interactions were assessed by introducing a product term between age and a TH in regression models. A product term in a linear regression assesses departure from additivity; departure from additivity is implied if the joint effect of age and a TH differs from the sum of the individual effects. However, a product term in linear regression with log-transformed outcome assesses departure from multiplicativity (i.e., if the joint effect differs from the product of the individual effects). Here, for those neuropsychological test scores for which p-value (p) for a product term was < 0.10, we have reported individual effect of a TH (i.e., change in a neuropsychological test score per IQR increase in a TH among individuals in the first quartile of age), individual effect of age (i.e., change in a test score per IQR increase in age among individuals in the first quartile of a TH), and joint effects of age and a TH

(i.e., change in test score per concurrent IQR increase in age and a TH) (Knol et al. 2009).

Consistent with other studies (Hogervorst et al. 2008; Volpato et al. 2002), as a secondary analyses, we repeated regression analysis in 108 individuals with TSH and THs within the laboratory reference intervals. All the statistical tests were two-tailed, and considered significant for main effect at $p < 0.05$ and for product term at $p < 0.10$. All the analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC)

3.4 Results

Table 1 summarizes the background characteristics of the participants. The mean age (standard deviation (SD)) was 63.1 (6.1) years, and there were an equal proportion of men and women. Around 16 % (n = 21) reported to have smoked cigarettes in the previous year, and the median number of packs of cigarettes smoked in the previous year was 274. One hundred and nine reported to have consumed alcohol in the previous year, and the median number of drinks was 154. Five women and two men reported antidepressant use at the time of study. Around 11% were diabetics, and 20% reported use of β-blockers.

Table 2 presents the descriptive statistics of TSH and THs. Geometric means (SDs) of TSH, T4, fT4, and T3 were 2.5 (1.8) µIU/mL, 8.7 (1.2) µg/dL, 1.2 (1.2) ng/dL, and 126.0 (1.2) ng/dL, respectively. Levels of TSH, T4, fT4, and T3 ranged 0.2-14.8 µIU/mL, 5.6-12.1 µg/dL, 0.8-1.7 ng/dL, and 82.7-189 ng/dL, respectively. Twenty two individuals had TSH or THs outside the laboratory reference intervals (n = 21 for TSH and n = 2 for fT4). TSH was negatively correlated with THs ($r_s = -0.26$, $p = 0.0002$ for

fT4; and r_s = -0.16, p = 0.06 for T3), and T4 was positively correlated with fT4 (r_s = 0.56, p < 0.0001) and T3 (r_s = 0.57, p < 0.0001).

Unadjusted associations between THs and neuropsychological test scores in memory and learning domain, affective state, and motor function are presented in Supplemental Table 1; T4 was positively correlated with BDT total scores (r_s = 0.23, p < 0.01), and negatively correlated with FTT for non-dominant hand (r_s = -0.21, p = 0.02). In addition, fT4 was negatively correlated with trial 1 score (r_s = -0.21, p = 0.02), short delay free recall (r_s = -0.21, p = 0.02), and long delay free recall (r_s= -0.23, p = 0.01), and positively correlated with recognition hits vs. long delay free recall (r_s = 0.23, p = 0.01). We also detected positive correlations for fT4 with BDT total scores (r_s = 0.29, p < 0.01), and with SMST total time touching for dominant hand (r_s = 0.19, p = 0.03). TSH and T3 were also correlated with tasks in memory and learning.

After adjusting for age, sex, education, and cigarette smoking, higher T4 and fT4 were only associated with improved BDT total scores ((β = 4.051, 95% Confidence Intervals (CI) = 1.930, 6.172) for T4 and (β = 4.920, CI = 2.640, 7.200) for fT4 which correspond to 15% and 19% increases) in the overall study sample. No associations of TSH and T3 with neuropsychological test scores were statistically significant.

We detected statistical interactions between age and T4 for tasks in memory and learning and executive function. Table 3 presents individual and joint effects of age and THs on neuropsychological test scores in the selected domains. Both higher T4 and increasing age were associated with poorer performance in tasks of memory and learning. Subadditive interactions (i.e., joint effect < the sum of individual effects) were detected for subtests of CVLT. For example, individual effects of T4 and age indicated 11 % and

15% decreases in trial 1 score (i.e., number of words recalled from the first trial), respectively. However, concurrent increase in both age and T4 (i.e., joint effect) was associated with only 14% decrease (i.e., < 26%) in the score. Figure 1 shows the associations between T4 and CVLT, short delay free recall score stratified by median value of age (i.e., 62 years); decreasing slope for younger group and increasing slope for older group were detected. Qualitative multiplicative interactions were detected for WCST subtests. However, unlike with tasks of memory and learning, statistically significant, protective individual effects of T4 on perseverative errors and responses were detected (27% and 31% decreases respectively); however, no individual effect of age was detected. Yet, concurrent increase in age and T4 was associated with elevated perseverative errors and perseverative responses (~33% increases).

Similarly, we detected sub-additive interactions between age and fT4 for subtests of memory and learning, executive function, and motor function (Table 3). Overall patterns indicated that joint increase in fT4 and age was associated with impaired memory and learning (~0.01% to 18% decreases in CVLT subtests). Joint increase in age and fT4 was also associated with 5% decrease in FTT, average score of non-dominant hand, and 7% increase in SCWT. Except for executive function, the results of the individual effects indicated that higher fT4 and increasing age were associated with poor performances in these tasks.

Concurrent increase in age and T3 showed departure from additivity for effects in tasks of memory and learning and departure from multiplicativity for tasks of executive function. Joint increase in age and T3 was associated with decreases in CVLT, learning slope and WMS, logical memory immediate recall, and increases in WCST, perseverative

responses and errors. Particularly, for subtests of WCST, no individual effect of T3 was detected; however, individual effect of age and joint effect of age and T3 indicated 25% and 53% increases in the scores respectively. We also detected departure from multiplicativity between age and TSH for TMT-part A and GPT, time to completion (non-dominant hand).

In a secondary analysis of 108 individuals with TSH and THs within the laboratory reference intervals, higher TSH was significantly associated with CVLT, proactive interference in the overall study sample. The product terms between age and TSH for TMT-part A and GPT (i.e., for non-dominant hand) were no longer significant; instead, qualitative sub-multiplicative interaction for CVLT, perseverations ($p < 0.05$) was detected. Product terms between T4 and age for t-score and trial 1 score remained no longer significant. The results for fT4 were similar. In addition, multiplicative interaction between age and fT4 on subtests of WCST and reaction time were detected.

3.5 Discussion

In this study, we investigated the cross-sectional associations of TSH and THs with neuropsychological status among men and women aged 55 to 74 years, without overt thyroid diseases, and living in upper Hudson River communities in NYS. Our findings suggest that serum T4 and fT4 concentrations were associated with neuropsychological function, including memory and learning, executive function, and visuospatial function, and that the associations may be age-dependent and domain-specific.

Higher T4 and fT4 were associated with increases in BDT total scores in the overall study sample, which suggests that low serum THs may impair the ability to

analyze and synthesize spatial relationship (Lezak et al. 2004). We detected statistical interactions between THs and age for effects on tasks of memory and learning and executive function. Concurrent increases in age and THs (i.e., T4, or fT4) were associated with poor performance in CVLT subtests, including t-score, trial 1 and short and long delay free recall scores, indicating impairments in memory formation, consolidation, and retrieval. Poor performance in these tests has been linked with impairments in frontal cortex and medial temporal lobe (hippocampus) (Mitrushina et al. 2005). However, statistical interactions for CVLT were sub-additive in nature. One possible explanation for this finding could be that normative aging and THs affect the same brain region/s responsible for memory and learning; therefore deleterious effects (i.e., effect size) of higher THs on memory and learning gradually become smaller, as aging progresses, due to already deteriorating memory and learning.

Concurrent increases in age and THs (i.e., T4, or T3) were associated with elevated perseverations in the WCST, indicating diminished cognitive flexibility, concept formation, and abilities to execute tasks that require planning and organization skills etc. Studies indicate that poor executive functions including performance in the WCST are exhibited due to prefrontal cortex impairments (Mitrushina et al. 2005).

We detected that individual effects of THs on executive function were protective, whereas joint effects of age and THs were deleterious. Protective individual effects of THs on executive function and protective overall effect on BDT total score contradict with the findings for memory and learning. This is very surprising because we *a priori* expected associations for both domains to be in the same direction since executive function and memory are often impaired concurrently, and parts of the brain that affect

executive function also affect memory and learning (e.g., prefrontal cortex) (Duff et al. 2005; McCabe et al. 2010). One potential explanation for this discrepancy could be a 'multiple factor framework' for cognitive aging, which proposes that multiple distinctive factors may independently target different brain systems (Buckner 2004). For example, in advanced aging, decline in 'frontal-striatal systems', that involve prefrontal cortex, occur in a preferential manner leading to deficits in executive function and memory encoding. Whereas hippocampal memory system, that involve medial temporal lobe, are preferentially affected in Alzheimer's disease. It is possible that different causal factors involving THs resulted in disparate results for the two domains.

In addition, structures and functions of brain regions decline at different rates during aging process (Phillips and Dela Salla 1998). Furthermore, although TH receptors are predominant in the brain, they are not uniformly distributed throughout; for example, TH receptors are more concentrated in the hippocampus (Whybrow and Bauer 2005). It is possible that the pathways, direct or indirect, by which TH could affect the brain, may differ by regions leading to differential associations with memory and learning and executive function.

Unlike for T4 and fT4, our results did not provide sufficient evidence for the associations between TSH and other neuropsychological test scores. Serum TSH, also regarded as the best marker of thyroid function, is generally used for screening of thyroid dysfunction in aging adults. Our results that T4 and fT4, rather than TSH, were associated with neuropsychological function may have important clinical implications and provide support to the recommendations by clinical practice guidelines for the use of fT4/T4 in the screening (Garber et al. 2012).

In studies of participants with the age ranges similar to ours, inconsistent associations for THs and TSH have been reported. It should be noted that neuropsychological tests employed by prior studies differ from the ones that we used (for e.g., we did not use Mini Mental State Examination (MMSE), a measure of global cognition)). Hogervost et al. (2008) reported that higher fT4 was associated with worse MMSE scores in an aging population of England and Wales (age range = 64-94 years, n=1047). In a cross-sectional analysis of 177 Norwegian adults (mean age = 61/62 years), higher fT4 was associated with poor performance on the Stroop tests whereas higher fT3 was associated with improved visual recall (Jorde et al. 2006). Higher fT4 was associated with better Middlesex Elderly Assessment of Mental State and Folstein-MMSE scores in a study of aging adults (age range = 65-84 years) from central England (Roberts et al. 2006). However, the authors concluded that such increases do not have any clinical relevance. Volpato et al. (2002) reported no association between baseline serum T4 and cognitive function, as measured by MMSE, in euthyroid women aged > 65 years (n = 628); however, they reported an association for lower T4 with cognitive decline in a longitudinal analysis. Likewise, higher TSH was associated with worse (van Boxtel et al. 2004) as well as better performances (Jorde et al. 2006; Roberts et al. 2006) in measures of cognition and executive function. Other studies that focused on individuals with age > 70 years have also reported discrepant results (Gussekloo et al. 2004; Wahlin et al. 1998; Wahlin et al. 2005; Wijsman et al. 2013). Statistical interactions between age and THs have not been reported in prior studies.

We did not detect associations for THs with measures of affective state. Previous studies in aging populations reported inconsistent associations between subclinical

thyroid dysfunction and depression and anxiety (Joffe et al. 2013; Roberts et al. 2006). As for motor function, associations detected in the current study were not consistent. Motor function has not been studied much in relation to THs and has been suggested to be less impacted by thyroid dysfunction in adults (Bauer et al. 2008; Dugbartey 1998).

Previous investigators have suggested that THs affect mood and behavior by interacting with neurotransmitters (Bauer et al. 2008). In addition, exposure to THs may augment necrotic neuronal death (Chan et al. 1996) and TH-induced oxidative stress has been suggested to adversely affect neurons (Marcocci et al. 2012; Quinlan et al. 2010; Tan et al. 2008). Thyroid dysfunctions are also linked with clinical forms of dementia, including Alzheimer's disease (Tan et al. 2008), suggesting potential roles for TSH and THs in cognitive decline.

Strengths of this study include objective and comprehensive assessments of both exposures and outcomes. Assessment of the associations using multiple neuropsychological tests allowed identification of TH-specific effects. Many of the prior studies were limited in this regard as they used only global measures and/or limited measures of cognitive function as the neuropsychological end point (Hogervorst et al. 2008; Joffe et al. 2013; Roberts et al. 2006).

Several limitations of the study necessitate careful interpretation of the results. Due to the cross-sectional nature of the study, we could not determine temporality of exposure and outcome, and infer causality. Levels of THs tend to fluctuate over a short period of time and therefore use of single measurement could have biased our results towards the null due to non-differential exposure measurement error. We performed multiple statistical tests; it is possible that the detected associations were chance findings

due to inflation of Type I error rate. In addition, the consistent associations detected within memory and learning domain may be due to strong correlations between these subtests scores (Appendix B, Table B-1). We did not adjust for depression/mood in our analysis, though it has been suggested to confound and/or mediate associations (Roberts et al. 2006; van Boxtel et al. 2004), because depression does not affect TH imbalances to meet the criteria of confounding, and furthermore, we were interested in total effects of THs. Still, the results from supplementary analyses that adjusted for BDI scores were similar to the reported results (Appendix B, Tables B-2, B-3, B-4, and B-5). Participants included in the current analysis were slightly younger (63 vs. 64 years) and reported to consume alcohol more (84 vs.75%) than the excluded participants; however, the selection of the participants were based on serum availability rather than willingness to participate. Such differential selection is less likely to affect internal validity of our results.

3.6 Conclusion

In this aging population with euthyroid or subclinical thyroid dysfunction, changes in levels of fT4 and T4 were associated with changes in neurocognitive function, including memory and learning, executive function and visuospatial function, and the differences seem to be age-dependent for memory and learning domain and executive function. Serum TSH, which is used for screening of thyroid dysfunctions in aging adults, was not associated with neuropsychological function. So the results provide support to the usefulness of fT4/T4 in the screening. In addition, these findings hint that even subtle changes in THs resulted by exposures to POPs may have important implications in neuropsychological function, although further studies are required to establish this hypothesis.

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Table 1: Background characteristics of study participants (n = 130)

Variable	AM (SD)	Median	Range
Age at interview (years)	63.12 (6.13)	62	55, 74
Body mass index (kg/m^2)	28.21 (5.79)	27.27	16.7, 49.6
Alcohol consumption			
(Amount over past year)	249.76 (347.75)	94	0, 2184
Among drinkers only (n=109)	297.88 (360.53)	154	12,184
Cigarette smoking			
(Total packs in last year)	43.33 (134.58)	0	0, 730
Among smokers only (n=21)	268.23 (231.25)	273.75	0.65, 730
Years of education	14.02 (2.72)	14	6, 20
	n (%)		
Sex			
Women	65 (50)		
Men	65 (50)		
Income category[a]			
< $15,000	6 (4.84)		
≥ $15,000 to $30,000	26 (20.97)		
> $30,000 to $45,000	29 (23.39)		
> $45,000 to $60,000	27 (21.77)		
> $60,000 to $75,000	22 (17.74)		
> $75,000	14 (11.29)		

Abbreviations: AM, Arithmetic Mean; SD, Standard Deviation; [a]Missing = 6

Table 2: Descriptive statistics of serum levels of thyroid markers (n=130)

Variable	AM (SD)	GM (SD)	Median	Range
TSH (μIU/mL)	2.89 (1.87)	2.46 (1.76)	2.46	0.23, 14.78
T4 (μg/dL)	8.8 (1.54)	8.66 (1.19)	8.85	5.57, 12.08
fT4 (ng/dL)	1.22 (0.17)	1.21 (1.15)	1.22	0.79, 1.68
T3 (ng/dL)	126.96 (19.32)	125.53 (1.16)	124.85	82.7, 189

Abbreviations: AM, Arithmetic Mean; GM, Geometric Mean; SD, Standard Deviation; TSH, Thyroid Stimulating Hormone; T4, Total Thyroxine; fT4, Free Thyroxine; T3, Total Triiodothyronine

Table 3: Individual and joint effects* of THs and age on selected neuropsychological tests (n = 130)

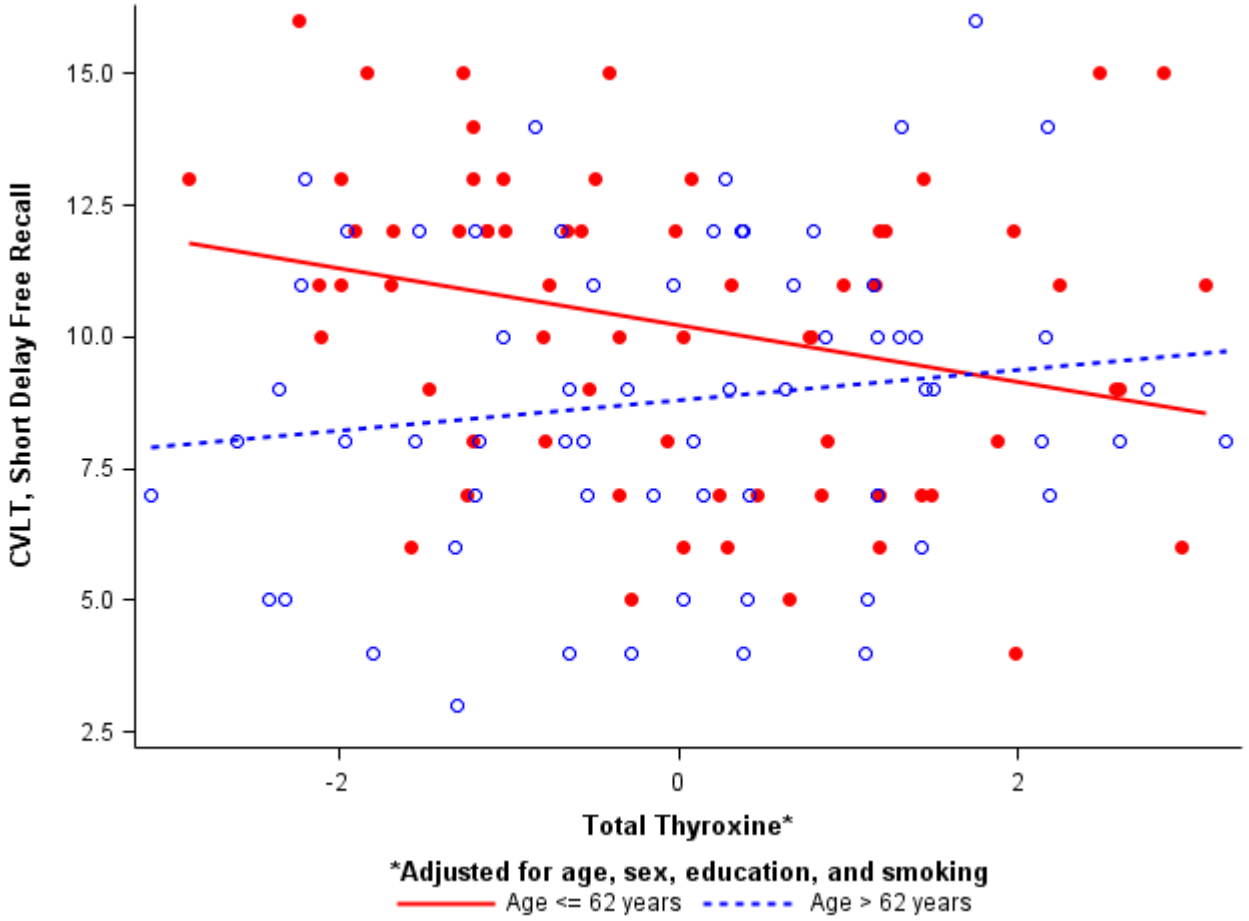
Neuropsychological Test	Individual Thyroid Effect (β (CI)) [M1]	Individual Age Effect (β (CI)) [M2]	Joint Effect (β (CI))[J]	p[p]
Total Thyroxine				
Memory and Learning				
CVLT, t-score [A,L]	-4.042 (-7.497, -0.587)	0.078 (-3.488, 3.644)	0.637 (-3.094, 4.367)	0.043
CVLT, trial 1 score [A,L]	-0.739 (-1.307, -0.171)	-0.995 (-1.600, -0.390)	-0.941 (-1.575, -0.306)	0.036
CVLT, short delay free recall [A,L]	-0.948 (-1.851, -0.046)	-1.529 (-2.49, -0.568)	-1.170 (-2.178, -0.162)	0.030
CVLT, discriminability (75^{th} quantile) [B,L]	-0.310 (-1.111, 0.491)	-0.297 (-0.486, -0.108)	0.147 (0.010, 0.285)	0.036
Executive Function				
WCST, perseverative errors† [A,H]	-0.276 (-0.505, -0.047)	0.183 (-0.059, 0.424)	0.329 (0.071, 0.587)	0.007
WCST, perseverative responses† [A,H]	-0.312 (-0.560, -0.064)	0.155 (-0.107, 0.417)	0.333 (0.054, 0.612)	0.004
Free Thyroxine				
Memory and Learning				
CVLT, t-score [A,L]	-4.758 (-8.415, -1.101)	-1.408 (-5.201, 2.386)	-0.008 (-3.964, 3.949)	0.007
CVLT, trial 1 score [A,L]	-0.747 (-1.375, -0.118)	-1.019 (-1.670, -0.369)	-1.058 (-1.738, -0.378)	0.068
CVLT, short delay free recall [A,L]	-1.582 (-2.554, -0.609)	-1.866 (-2.873, -0.859)	-1.745 (-2.798, -0.693)	0.005
CVLT, long delay free recall [A,L]	-1.347 (-2.356, -0.338)	-1.523 (-2.567, -0.478)	-1.654 (-2.745, -0.562)	0.051
CVLT, semantic cluster ratio [A,L]	-0.225 (-0.499, 0.048)	-0.128 (-0.411, 0.155)	-0.041 (-0.337, 0.255)	0.064
CVLT, discriminability (75^{th} quantile) [B,L]	-6.66 (-14.121, 0.801)	-0.328 (-0.623, -0.032)	1.066 (-0.158, 2.290)	0.087
Executive Function				
Stroop Color Word Test, t-score [A,L]	2.724 (0.292, 5.156)	3.951 (1.459, 6.444)	3.561 (0.884, 6.239)	0.044
Motor Function				
FTT (non-dominant hand), average score [A,L]	-2.447 (-4.205, -0.689)	-1.910 (-3.711, -0.110)	-2.398 (-4.300, -0.496)	0.068
Total Triiodothyronine				
Memory and Learning				
CVLT, learning slope [A,L]	0.077 (-0.098, 0.252)	0.124 (-0.070, 0.318)	-0.088 (-0.302, 0.125)	0.021
WMS, logical memory immediate recall score [A,L]	-1.777 (-3.244, -0.31)	-1.994 (-3.620, -0.369)	-1.811 (-3.604, -0.017)	0.062
Executive Function				
WCST, perseverative errors† [A,H]	-0.031 (-0.245, 0.184)	0.254 (0.018, 0.491)	0.539 (0.265, 0.812)	0.044

Table 3: Individual and joint effects* of THs and age on selected neuropsychological tests (n = 130)

Neuropsychological Test	Individual Thyroid Effect (β (CI)) [M1]	Individual Age Effect (β (CI)) [M2]	Joint Effect (β (CI))[J]	p[p]
WCST, perseverative responses† [A,H]	-0.041 (-0.274, 0.191)	0.242 (-0.015, 0.499)	0.556 (0.259, 0.853)	0.037
Thyroid Stimulating Hormone				
Measures of Attention Trail Making Test Part A-time to complete† [A,H]	0.061 (-0.012, 0.134)	0.180 (0.092, 0.268)	0.122 (0.028, 0.217)	0.035
Motor Function GPT (non-dominant hand), time to completion† [A,H]	0.024 (-0.040, 0.088)	0.210 (0.141, 0.279)	0.130 (0.056, 0.203)	0.021

Abbreviations: CVLT, California Verbal Learning Test; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; *Adjusted for age, sex, education, and cigarette smoking; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; M1 = Individual effect of a TH (i.e., change in a neuropsychological test score per IQR increase in TH among reference age group); M2 = Individual effect of age (i.e., change in a neuropsychological test score per IQR increase in age among reference TH group); J = Joint effect of a TH and age (i.e., change in neuropsychological test score per concurrent IQR increment in both TH and age); p = p-value of a product term between TH and age

Figure 1: Associations between total thyroxine (T4, µg/dL) and California Verbal Learning Test, short free delayed recall score stratified by age ≤ median value of 62 years (n = 130)



Supplemental Table 1: Spearman correlation coefficients (p-value) between thyroid markers and neuropsychological test scores (n = 130)

Neuropsychological Tests	n	TSH (p)	T4 (p)	fT4 (p)	T3 (p)
Memory and Learning					
CVLT, t-score [L]	127	0.16 (0.07)	-0.09 (0.32)	-0.11 (0.22)	-0.04 (0.69)
CVLT, trial 1 score [L]	130	0.12 (0.17)	-0.12 (0.17)	-0.21 (0.02)	0.08 (0.37)
CVLT, short delay free recall [L]	130	0.18 (0.04)	-0.10 (0.24)	-0.21 (0.02)	-0.07 (0.43)
CVLT, long delay free recall [L]	130	0.21 (0.01)	-0.08 (0.36)	-0.23 (0.01)	-0.04 (0.64)
CVLT, proactive interference (list B adjusted for trial 1) [L]	130	0.12 (0.17)	-0.10 (0.28)	-0.04 (0.66)	-0.18 (0.05)
CVLT, semantic cluster ratio [L]	130	0.11 (0.19)	-0.08 (0.38)	-0.11 (0.20)	-0.08 (0.37)
CVLT, learning slope [L]	130	0.05 (0.54)	0.02 (0.83)	-0.03 (0.74)	-0.18 (0.04)
CVLT, perseverations [H]	130	0.01 (0.92)	-0.13 (0.16)	-0.04 (0.64)	-0.04 (0.66)
CVLT, discriminability [L]	130	0.25 (<0.01)	0.00 (0.99)	-0.12 (0.17)	-0.06 (0.50)
CVLT, recognition hits vs. long delay free recall [L]	130	-0.19 (0.03)	0.09 (0.33)	0.23 (0.01)	0.01 (0.95)
WMS, logical memory immediate recall score [L]	130	0.13 (0.15)	-0.15 (0.10)	-0.12 (0.17)	-0.19 (0.03)
WMS, logical memory delayed recall score [L]	130	0.08 (0.39)	-0.07 (0.44)	-0.04 (0.69)	-0.17 (0.06)
WMS, visual reproduction immediate recall score [L]	130	-0.04 (0.63)	0.09 (0.30)	0.00 (0.98)	-0.04 (0.66)
WMS, visual reproduction delayed recall score [L]	130	-0.03 (0.73)	0.06 (0.49)	0.03 (0.72)	-0.05 (0.57)
Measures of Attention					
Trail making test Part A-time to complete [H]	129	-0.07 (0.42)	0.07 (0.41)	0.07 (0.43)	0.02 (0.86)
Trail making test Part B-time to complete [H]	126	-0.05 (0.58)	0.16 (0.08)	0.01 (0.90)	0.07 (0.44)
Executive Function					
Stroop Color Word Test, t-score [L]	130	0.06 (0.49)	0.06 (0.49)	0.1 (0.25)	0.02 (0.82)
WCST, perseverative errors [H]	124	-0.14 (0.12)	-0.01 (0.93)	0.02 (0.79)	0.13 (0.16)
WCST, perseverative responses [H]	124	-0.13 (0.16)	-0.01 (0.89)	0.02 (0.82)	0.12 (0.17)
WCST, number of categories completed [L]	124	0.17 (0.06)	0.01 (0.94)	-0.07 (0.42)	-0.08 (0.37)
WCST, failure to maintain set [H]	124	0.00 (1.00)	-0.14 (0.12)	-0.13 (0.14)	0.01 (0.89)
Visual and Spatial function					
Block Design Subtest, total score [L]	130	0.11 (0.22)	0.23 (<0.01)	0.29 (<0.01)	0.03 (0.73)
Digit Symbol Coding, total score [L]	130	0.05 (0.60)	-0.03 (0.76)	0.01 (0.94)	-0.04 (0.62)
Reaction Time					

Supplemental Table 1: Spearman correlation coefficients (p-value) between thyroid markers and neuropsychological test scores (n = 130)

Neuropsychological Tests	n	TSH (p)	T4 (p)	fT4 (p)	T3 (p)
Reaction time (dominant hand) [H]	128	0.05 (0.55)	0.05 (0.57)	0.02 (0.83)	-0.06 (0.53)
Affective State					
BDI, total score [H]	130	-0.03 (0.76)	0.11 (0.21)	0.13 (0.16)	0.07 (0.42)
STAI, state anxiety t-score [H]	130	-0.07 (0.41)	0.00 (0.99)	-0.05 (0.57)	0.04 (0.62)
STAI, trait anxiety t-score [H]	130	0.02 (0.80)	0.02 (0.80)	-0.02 (0.78)	0.08 (0.37)
Motor Function					
FTT (dominant hand), average score [L]	129	0.02 (0.82)	-0.05 (0.55)	0.04 (0.62)	-0.01 (0.88)
FTT (non-dominant hand), average score [L]	128	-0.04 (0.68)	-0.21 (0.02)	-0.07 (0.45)	-0.11 (0.22)
GPT (dominant hand), time to completion [H]	130	-0.08 (0.39)	-0.03 (0.77)	0.01 (0.93)	0.00 (0.98)
GPT (non-dominant hand), time to completion [H]	128	-0.10 (0.26)	0.09 (0.29)	0.07 (0.41)	0.06 (0.48)
SMST (dominant hand), total number of contacts [H]	129	-0.18 (0.04)	-0.09 (0.31)	0.16 (0.06)	-0.02 (0.82)
SMST (dominant hand), total time touching [H]	129	-0.22 (0.01)	-0.08 (0.39)	0.19 (0.03)	-0.07 (0.40)
SMST (non-dominant hand), total number of contacts [H]	128	-0.16 (0.08)	-0.08 (0.36)	0.15 (0.10)	0.00 (0.97)
SMST (non-dominant hand), total time touching [H]	128	-0.13 (0.14)	-0.05 (0.59)	0.14 (0.11)	0.03 (0.75)

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; WCST, Wisconsin Card Sorting Test; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test; SMST, Static Motor Steadiness Test; BDI, Beck Depression Inventory; STAI, State-Trait Anxiety Inventory H: High Score = Impairment, L: Low Score = Impairment; p = p value

Chapter 4: Perfluorinated Compounds, Thyroid Function, Neuropsychological Status in Older Adults

4.1 Abstract

Minimal data exist regarding the neurotoxicity of perfluorinated compounds (PFCs) in aging populations and the possible mediating effects of thyroid hormones (THs). Hence, the aims of this study were to: (i) assess associations between PFCs and neuropsychological function, and (ii) determine if such associations are mediated by changes in THs in an aging population. We measured perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in serum and performed neuropsychological tests in 157 men and women aged 55-74 years and living in upper Hudson River communities. Multivariable linear and quantile regressions were conducted to assess associations between PFCs and neuropsychological test scores. Mediation analyses were performed in a subset of 87 participants for whom information was available on both PFCs and THs. We obtained thyroid-mediated, non-thyroid mediated, and total effects of a PFC on a neuropsychological test score. The overall results suggested protective effects of PFCs in tasks of memory and learning and executive function. For instance, a one interquartile range increase in PFOA was associated with 16% to 18% decreases in scores (i.e., improved performance) of the Wisconsin Card Sorting Test (p-value = 0.04). Total thyroxine partially mediated the protective effect of PFOS on Block Design Subtest total scores, a measure of visuospatial function (proportion mediated = 51%, p-value = 0.04). However, the protective effects of PFCs on memory, learning and executive function were mostly mediated via pathways other than those involving alterations in THs. These

findings provide insight regarding the impact of PFCs on neuropsychological function and the role of THs.

4.2 Introduction

Perfluorinated compounds (PFCs) are a class of persistent, bioaccumulative, and toxic compounds which have been widely used in the variety of consumer products and industrial applications as liquid repellants and processing aids (Agency for Toxic Substances and Disease Registry (ATSDR) 2009; Lau et al. 2007), and have become pervasive in the environment (Giesy and Kannan 2001; Kato et al. 2011). Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the two most predominant long-chained PFCs in the environment. Animal and human studies have linked PFCs with a spectrum of adverse health effects including endocrine disruption, metabolic disorders, and immunotoxicity (Lau et al. 2007; Steenland et al. 2010). PFCs have also been implicated as potential neurotoxicants in toxicological studies. For instance, *in-vitro* models and animal studies suggest that PFCs may induce developmental neurotoxicity (Johansson et al. 2009; Liu et al. 2010; Onishchenko et al. 2011; Pinkas et al. 2010; Slotkin et al. 2008; Zhang et al. 2011) and that exposure to PFC during adulthood might also lead to an impairment in memory retention (Fuentes et al. 2007). Only a handful of studies, however, have investigated PFCs' neurotoxic effects in humans, and the majority of those were focused on children (Fei and Olsen 2010; Hoffman et al. 2010; Stein and Savitz 2011; Stein et al. 2013). To date, only two studies have examined the association of PFCs with cognition in aging adults (Gallo et al. 2013; Power et al. 2013); however, the results suggested that PFCs may be neuroprotective.

Further studies using more sensitive neurocognitive endpoints may help to elucidate and characterize the associated risks of PFCs.

PFCs may alter neuropsychological function via (i) disruption of thyroid homeostasis, one of the putative mechanisms by which other persistent organic pollutants may cause neurotoxicity (Kodavanti 2005), (ii) direct effects on the nervous system (Johansson et al. 2008; Mariussen 2012), and (iii) activation of peroxisome proliferator activated receptors (PPARs)(Gallo et al. 2013). Optimal function of the hypothalamus-pituitary-thyroid system is important to maintain proper neuropsychological function (Bauer et al. 2008). Previous studies suggest that PFCs may alter levels of thyroid hormones (THs) in human adults (Knox et al. 2011a; Steenland et al. 2010; Wen et al. 2013). In our analysis of the aging participants from the current study, we also detected positive associations of PFCs with THs, and that alterations in THs affect neuropsychological function, including memory and learning, executive function, and visuospatial function. Yet, the role of thyroid function in mediating the PFC-neuropsychological function effects has not been evaluated in prior research.

To help address these research gaps, we performed a cross-sectional study among men and women in New York State (NYS). The study had two objectives: (i) to examine associations between PFOA and PFOS and neuropsychological status; and (ii) to evaluate whether the effects of PFCs on neuropsychological function were mediated by markers of thyroid function.

4.3 Methods

4.3.1 Sample Selection

The source population consisted of men and women aged 55 to 74 years, who lived in three demographically similar communities near the Hudson River in NYS: Fort Edward, Hudson Falls, and Glens Falls. Study participants were recruited between 2000 and 2002 for a larger parent project designed to examine associations between polychlorinated biphenyls (PCBs) and neuropsychological function. The study areas were chosen because General Electric plants, located in Hudson Falls and Fort Edward, used PCBs to manufacture electric capacitors from 1947 until 1977 and discharged almost one million pounds of PCBs into the upper Hudson River (U.S. EPA 2011).

Details including study population and participant recruitment procedures have been described elsewhere (Fitzgerald et al. 2007; Fitzgerald et al. 2008). The source population was identified using an online telephone directory search engine and a digital database (InfoUSA). A total of 2704 men and women aged 55 to 74 years living in one of the three communities were invited to participate in the study. Individuals were excluded if: i) they had not lived in their respective areas for at least 25 years, ii) they had been involved in PCB-related job for $\geq$ 1 year, or iii) they had certain medical conditions, including a history of stroke, severe head injury, Parkinson's disease, Alzheimer's disease, or severe cognitive impairment. Of those who met the eligibility criteria and invited to participate, only 40% (i.e., n = 253) agreed to participate.

Structured in-person interviews were conducted during 2000 - 2002 to collect data on sociodemographics and histories of residence, fish consumption, occupation and medication use. Serum samples were also collected during the same years to measure

levels of PCBs, and residual samples were archived at -20°C. Of 253 participants recruited to the parent PCB-neurocognitive function study (Fitzgerald et al. 2008), 144 had sufficient serum remaining (≥ 1.0 mL), and agreed to analysis for TSH, THs, and polybrominated diphenyl ethers (PBDEs) in 2005. In 2010, 157 participants had adequate archived serum samples (volume > 0.2 mL) and consented to analysis for serum PFCs. We thus examined associations between PFCs and neuropsychological function in 157 individuals. However, only 109 of the 157 had information on both thyroid biomarkers and PFCs. We excluded participants with clinically diagnosed thyroid disease, who were taking any thyroid-related medications (n = 9), and who were under sex hormone therapy (n = 13) (Surks and Sievert 1995; Tahboub and Arafah 2009), and thus assessed the mediating effect of thyroid function in only n = 87.

4.3.2 Neuropsychological Assessment

The details on neuropsychological tests, conducted 2000-2002, can be found elsewhere (Fitzgerald et al. 2008). Memory and learning were assessed using the California Verbal Learning Test (CVLT) (Delis et al. 2000) and the Wechsler Memory Scale (WMS) Form I-Russell's Revision tests (Russell 1975). The CVLT consists of five learning trials for acquisition of a 16 item word list (i.e., List A) followed by an immediate recall after presentation of an interference word list (i.e., List B), a 20-minute delay recall trial, and a delayed recognition trial. The variables generated from the tests provide an assessment of the ability to acquire, retain, and retrieve verbal information, and information regarding the learning process such as organizational strategies and learning efficiency. The WMS assesses immediate and delayed memory of verbal and visual material.

Measures of attention were assessed using the Trail Making Test (TMT) - Parts A and B (Reitan and Wolfson 1993). The Stroop Color Word Test (SCWT) (Trenerry et al. 1989) and the Wisconsin Card Sorting Test (WCST) (Heaton 1981) were used to assess executive function (i.e., a set of cognitive skills involved in anticipation, planning, and initiation of a number of goal-directed behaviors). The SCWT assesses individuals' ability to shift a perceptual set. In the WCST, the participant is presented with two decks of 64 response cards and is instructed to sort each card to one of four key stimulus cards, one at a time, based on one of three sorting principles (color, shape, or number). However, the participant is not informed about a sorting principle and would have to determine the underlying sorting principles by using corrective feedback ("correct"/"incorrect") that is provided by the examiner. As the sorting principle changes during the test, mental flexibility is required to shift cognitive strategies and to generate alternative sorting principles.

The Digit Symbol Substitution Test (DSST), a measure of visuomotor tracking and processing speed, and the Block Design Subtest (BDT), a measure of visuospatial organization, were used to assess the visual and spatial function; both are subtests of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler 1981). The Simple Reaction Time Test was used to assess the ability of an individual to respond to a visual stimulus after an auditory warning.

The Beck Depression Inventory (BDI) and the State-Trait Anxiety Inventory (STAI) were used to assess the presence and severity of depression, and state and trait anxiety, respectively (Beck et al. 1961; Speilberger et al. 1970). In the motor function domain, the Static Motor Steadiness Test (SMST) (Lezak et al. 2004), the Grooved

Pegboard Test (GPT) (Klove 1963), and the Finger Tapping Test (FTT) (Reitan and Wolfson 1993) were used to assess steadiness of hand and arm, complex visuomotor coordination and visuospatial orientation, and motor speed and coordination, respectively. In the SMST, tremor-like movements in the participants are assessed for both dominant and non-dominant hands. In addition, we used the Test of Memory Malingering (TOMM) to differentiate the participants who were exhibiting an adequate level of effort from those who were not (Tombaugh 1996).

4.3.3 Serum Chemical Analysis

A fasting sample of 25 mL of venous blood was drawn during 2000-2002 and centrifuged to obtain serum which was pipetted into a glass bottle. All the biomarker determinations including those of PCBs, PFCs, thyroid function markers, cholesterol, and triglycerides were performed at the Wadsworth Center of the NYS Department of Health (Albany, NY). The analytical and quality control/assurance procedures for the serum PCB analyses have been detailed in the previous articles (Fitzgerald et al. 2007; Fitzgerald et al. 2008). Thirty PCB congeners that constitute above 95% of the total PCB residue in human serum were analyzed and summed to obtain total PCB. Serum total lipids (2.27 × cholesterol + triglycerides + 0.623) was estimated, and serum total PCB was expressed on a lipid basis, i.e. ng/g of serum total lipids (Phillips et al. 1989).

The analytical procedure for the analysis of PFOS and PFOA is described in detail elsewhere (Kannan et al. 2004). Briefly, the chemicals were initially extracted from serum using an ion-pairing method with a subsequent subjection into a high performance liquid chromatograph-tandem mass spectrometer (HPLC-MS/MS). The limit of quantitation (LOQ) ranged from 0.5 to 1 ng/mL, which was determined based on the

linear range of the calibration curve prepared at a concentration range of 0.5 ng/mL to 100 ng/mL. There was one observation below the LOQ for PFOA, for which the machine-read value was assigned.

4.3.4 Thyroid Function Biomarkers

Thyroid hormones were measured at the Wadsworth Center Clinical Laboratory (Albany NY). Levels of TSH, total thyroxine (T4), free T4 (fT4), and total triiodothyronine (T3) in serum were measured using an immunoelectrochemiluminometric assay (Roche Elecsys 1010 system, Roche Diagnostics, U.S.A). The average inter-run coefficients of variation for TSH, T4, T4, and T3 were 2.5% (5.1% at concentrations < 0.2 μIU/mL), 4.5%, 2.2%, and 5.9%, respectively. The laboratory reference intervals were 0.3-4.2 μIU/mL for TSH, 5.1-14.1 μg/dL for T4, 0.9-1.7 ng/dL for fT4, and 80-200 ng/dL for T3.

4.3.5 Statistical Analysis

Serum PFOS, PFOA, TSH and some of the neuropsychological test scores were log transformed to base 'e' to achieve normality before multivariable analyses were conducted. Multivariable regression was performed to assess associations between PFCs and neuropsychological test scores adjusted for age, sex, education, and serum total PCB (lipid basis) using data from 157 participants. These covariates were considered for inclusion in models based on the literature (Davis et al. 2003; Fitzgerald et al. 2008; Greenland et al. 1999; Kato et al. 2011; Tahboub and Arafah 2009). Linear regressions were used for continuous outcome variables that were normally distributed. Negative binomial and quantile regressions (for the 25^{th}, 50^{th}, and 75^{th} quantiles of the test scores) were performed for count data and for highly skewed continuous outcome variables,

respectively. Parameter estimates obtained from quantile regression indicate the change in a specified quantile of a neuropsychological test score per unit change in the exposure variable (Hao and Naiman 2007). Here, we have reported the change in a neuropsychological test score per interquartile range (IQR) increase in ln PFC for all regression models.

Two-way interactions were tested by constructing regression models with a product term between sex and PFC. A product term in a linear regression assesses departure from additivity (i.e., if the joint effect of a PFC and sex differs from the sum of the individual effects). A product term in a linear regression with log-transformed outcome assesses departure from multiplicativity (i.e., if joint effect differs from the product of the individual effects). For those neuropsychological test scores with p-value (p) for a product term < 0.10, we have reported individual and joint effects (Knol et al. 2009). Individual effect of a PFC indicates the change in a neuropsychological test score per IQR increase in ln PFC among men. Individual effect of sex indicates the change in a test score in women as compared to men among the individuals with ln PFC at the first quartile. Joint effect of a PFC and sex indicates the change in a test score in women with ln PFC at the third quartile as compared to men with ln PFC at the first quartile.

We used the SAS Macro developed by Valeri and Vanderweele (2013) to assess if the associations of PFCs with neuropsychological test scores were mediated by TSH, T4, fT4, and T3 in 87 participants. In the absence of interaction between a PFC and a thyroid function marker, the total effect of a PFC on a neuropsychological test score decomposes into a non-TH-mediated effect (NTE) and a TH-mediated effect (TME). Briefly, the mediation analysis involves two steps: i) building a regression model with an exposure

predicting a mediator ($E(M|X = x, C = c) = \beta_0 + \beta_1 x + \beta_2 c + \varepsilon$); and ii) building a regression model with both the exposure and the mediator predicting an outcome ($E(Y|X = x, M = m, C = c) = \theta_0 + \theta_1 x + \theta_2 m + \theta_3 c + \varepsilon$). Here X, Y, M, and C indicate exposure, outcome, potential mediator, and covariate, respectively. When there is no interaction between a PFC & a TH, the direct effect can be estimated by θ_1 & the indirect effect by a product of θ_2 and β_1. We also performed mediation analysis allowing for an interaction between PFCs and THs ($E(Y|X = x, M = m, C = c) = \theta_0 + \theta_1 x + \theta_2 m + \theta_3 c + \theta_4 (m \times x) + \varepsilon$).

The NTE reported in the results section represents an average change in test score per IQR increase in ln PFC at the mean TH value. The TME estimate indicates change in test score for those with ln PFC level at the third quartile, but if TH were changed from the level it would take if ln PFC were at the first quartile to the level it would take if ln PFC were at the third quartile. The total effect indicates the average change in test per IQR increase in a PFC. The NTE, TME, and total effects were adjusted for age, sex, years of education, cigarette smoking, and serum total PCB (lipid basis). Standard errors were estimated using the delta method. We also repeated the analyses using bootstrapping techniques; effect estimates and 95 percentile confidence intervals (CIs) were obtained using1000 bootstrap samples.

Lipid standardization of PCBs has been suggested to produce biased estimates (Schisterman et al. 2005), and so we repeated the analysis using serum total PCBs on a wet-weight basis while adjusting for total lipids as a covariate. Statistical tests were two-tailed, and considered statistically significant for main effect at $p < 0.05$ and for product term at $p < 0.10$. All the analyses were performed using SAS v. 9.3 (SAS Institute, Cary, NC).

4.4 Results

Table 1 presents the background characteristics for 157 study participants. Mean age (standard deviation (SD)) of the participants was 63.7 (6.0) years. There were an equal proportion of women and men. Nineteen percent and 83% of the participants, respectively, reported cigarette smoking and alcohol consumption in the year before the study. Geometric mean (SD) of serum total PCB concentration was 470.11 (1.58) ng/g of serum total lipids. Geometric means (SDs) of serum PFOS and PFOA were 34.20 (1.80) ng/mL and 8.10 (1.72) ng/mL, respectively (for n = 157) and were similar to the analogous values for the 87 participants included in the mediation analysis (31.60 (1.70) ng/mL and 9.17 (1.72) ng/mL, respectively). Geometric means (SDs) for serum TSH, T4, fT4, and T3 were 2.25 (1.72) μIU/mL, 8.57 (1.19) μg/dL, 1.23 (1.15) ng/dL, and 124.71 (1.14) ng/dL, respectively. Serum PFOS was significantly correlated with serum total PCB (spearman correlation = 0.24, p = 0.002).

The multivariable results in the overall sample (n = 157) indicated that higher PFOA was significantly associated with low perseverative errors (β = -0.156, CI = -0.302, -0.009) and perseverative responses (β = -0.168, CI = -0.327, -0.009) in the WCST, adjusted for age, sex, education, and serum total PCB (lipid basis); perseverative errors and responses are measures of repetitive errors. Higher PFOA was also associated with improved motor function, as measured by the SMST, total number of contacts (β = -0.150, CI = -0.313, 0.014) and total time touching (β = -0.194, CI = -0.405, 0.018); however, the associations did not reach significance. Increasing PFOS was associated with improved performance in subtests of WMS, WCST, and SMST ($p < 0.10$); yet these associations were also not significant.

We detected evidence of statistical interactions between PFOA and sex for nine neuropsychological tests (p for the interaction term < 0.10); the results for selected tests are presented in Table 2. The individual effects of sex indicated that women performed better in a few neuropsychological tests compared to men except for the STAI, state anxiety t-score and the CVLT, recognition hits vs. long delay free recall (i.e., total number of words identified correctly from List A on recognition testing) ($p < 0.05$). The interactions were sub-additive, except for CVLT, learning slope and SMST, total number of contacts for which the interactions were super-additive and super-multiplicative, respectively. For example, individual effects of PFOA and sex were 4.7% and 27.5% increases in CVLT, trial 1 score, respectively, whereas joint effect indicated only 21% increase (i.e., < 32.2%, implying subadditivity). Figure 1 shows regression models between PFOA and CVLT, trial 1 score, stratified by sex; although women appeared to have better test scores than men, increasing PFOA was associated with poor performance among women. We detected statistical interactions between PFOS and sex for only three neuropsychological tests; joint exposure was associated with increases in WMS, visual reproduction delayed recall score, and reaction time.

Table 3 presents thyroid-mediated-, non-thyroid- and total effects of PFOS and PFOA on selected neuropsychological test scores, where p for total effect < 0.10 (see also Appendix C, Tables C-1 and C-2 for all neuropsychological tests). The effects were adjusted for age, sex, education, cigarette smoking, and serum total PCB (lipid basis). The general patterns of the results indicated that PFCs were associated with improved performance on neuropsychological tests in the domains including executive function, visuospatial function, and memory and learning. The effects were not mediated by THs

or TSH, except for BDT total score. A protective effect of PFOS on BDT total score was partially mediated by T4 (proportion mediated = 51%). The results of mediation analysis using bootstrapping technique are presented in Appendix C (Tables C-3 and C-4). Mediation analysis allowing interaction between PFCs and thyroid function markers were performed, and there was no evidence of interaction (Appendix C, Table C-5). The regression analyses were repeated adjusting for age, sex, education, serum total PCB (wet weight basis), and total lipids; the results were similar (Appendix C, Table C-6). We did not detect statistical interactions between age and PFCs.

4.5 Discussion

The overall results of this study of men and women aged 55 to74 years and living in upper Hudson River communities indicated that higher levels of PFOS and PFOA were significantly associated with improved performance in tasks of memory and learning and executive function. The results also indicated subadditive interactions between sex and PFOA, particularly in tasks of memory and learning, meaning the joint effect of increasing levels of PFOA and sex (with low PFOA levels and men being the respective comparison groups) was less than the sum of the individual effects. Our findings also suggested that the improved performances in memory and learning and executive function associated with increasing levels of PFCs were mediated through pathways that may not involve TH. However, we only detected partial T4 mediation for the protective effect of PFOS on BDT total scores, a measure of visuospatial function.

To the best of our knowledge, this is the first study to evaluate associations of PFOS using wide-ranging neuropsychological tests. The results from two sets of analyses, one in 157 individuals and another in a subsample of 87 individuals with

information on THs, indicated that PFOS and PFOA may positively affect memory and learning, as measured by the CVLT, and executive function, as measured by the WCST. The CVLT subtests have been used to assess memory and learning impairments in patient with dementia and brain injury in the clinical settings (Delis et al. 2000). Here, in this group of individuals without a history of clinical neuropsychological conditions, improved performances in the selected CVLT scores indicate improvements in memory formation, consolidation, retrieval, and learning efficiency. Clinically, poor performances in the tasks of the WCST including perseverative errors and responses have been detected mainly among individuals with impairments in prefrontal cortex or frontal lobe (Mitrushina et al. 2005). Low perseverative responses and errors, measures of repetitive errors, detected in relation to elevated PFCs indicate better concept formation and improved ability to shift cognitive strategies.

Our finding that PFCs may be neuroprotective in aging populations is in line with the findings from previous two studies (Gallo et al. 2013; Power et al. 2013). In National Health and Nutrition Examination Survey participants 60 to 85 years of age in 1999-2000 and 2003-2008, high levels of PFOS but not PFOA was associated with reduced odds of self-reported cognitive limitations among diabetics; the association was stronger among non-medicated diabetics (Power et al. 2013). Another study was performed among 21,024 adults, aged > 50 years, who lived in contaminated water districts near a chemical plant that used PFOA in the manufacture of fluoropolymers (i.e., the C8 cohort); increasing levels of both PFOS and PFOA were associated with reduced odds of a self-reported short term memory impairment (Gallo et al. 2013). However, no association among diabetics or differential association by treatment was detected in contrary to the

findings by Power et al (2013). PFOA levels in the C8 cohort were 10 to 13 fold higher whereas PFOS levels were comparable to ours. Unfortunately, the limited number of individuals with diabetes in our study (n = 8) precluded a stratified analysis.

We are also the first to report joint associations between PFCs and sex (Gallo et al. 2013; Power et al. 2013); our findings suggest that women with greater PFC burden may experience less neuroprotective effect. The literature suggests that estrogen is neuroprotective, and have important implications for brain regions responsible for memory, learning, and executive functions, including hippocampus and frontal lobes (Brann et al. 2007). Given women are mostly postmenopausal in this study and thereof lack estrogen, one impression would be that the effects on men and women would be similar. Nevertheless, these women may have already had experienced neurocognitive deficits when they were premenopausal as a consequence of PFC associated loss in estradiol (Knox et al. 2011b). As little is known about the underlying mechanisms including PFC-estradiol associations, we can only make speculations regarding associations. On the other hand, PFOS and PFOA have also been shown to act as estrogen receptor (ER) agonists and as androgen receptor antagonists (Kjeldsen and Bonefeld-Jorgensen 2013). Contrary to our *a priori* hypothesis, this suggests that ER agonism may mediate the apparent neuroprotective effect of PFCs.

Although we detected PFC-associated increases in THs, and TH-associated changes in memory and learning and executive function in our prior work, our current findings suggest that the PFC-associated TH changes may not be sufficient to bring about significant changes in those neuropsychological domains. In our earlier work, we detected sub-additive interactions between age and fT4 and T4 for effects on memory and

learning and executive function, but in the current analyses, we did not detect any interaction between age and PFCs. It is possible that we could have failed to detect mediation by THs because we could not accommodate such complex interactions in our mediation analyses.

The investigators of the prior studies (Gallo et al. 2013; Power et al. 2013) postulated that protective associations could be due to the ability of PFCs to activate PPARs, ligand-activated transcription factors that regulate genes involved in lipid metabolism and inflammation (Vanden Heuvel et al. 2006). The hypothesis was based on the findings that PPAR γ agonists including thiazolidinediones elicit neuroprotective effects, potentially due to their anti-inflammatory property (Kaundal and Sharma 2010). A recent study indicated that PFOS upregulates activity of PPAR γ, providing further support to the hypothesis (Wan Ibrahim et al. 2013). On the other hand, evidence that PFCs may be neurotoxic is growing as well (Lee et al. 2012; Reistad et al. 2013). It is possible that effects mediated via pathways that lead to toxicity are comparatively small relative to effects mediated by pathways that lead to neuroprotection, resulting in a protective effect overall. In addition, studies of children and adolescents have reported mixed associations (Hoffman et al. 2010; Stein and Savitz 2011; Stein et al. 2013), so it is possible that the timing of exposure may also determine the chemodynamics of PFCs producing divergent associations across populations (Gallo et al. 2013).

It should be noted that the levels of PFOS and PFOA in the current study were higher than those reported in the general U.S. population of similar age range (Kato et al. 2011). We are unsure as to the source of elevated PFC levels in our study population. However, given the fact that serum PFOS was significantly correlated with serum total

PCB, we can speculate that the elevated levels may be due to common environmental sources. The study population lived in close proximity to PCB-contaminated areas, and PCB levels in men were 30% higher than that in general U.S. population (Centers for Disease Control and Prevention 2005). However, we did not find a correlation between Hudson River fish consumption and serum PFCs, indicating that other environmental or occupational exposure sources may be important.

The distributions of socio-demographics for 157 participants included in this study were similar to 96 participants in the original parent study that were excluded, except the proportion of participants reporting alcohol consumption was greater in the current study (83% vs. 74%). Differences in alcohol consumption between these groups are unlikely to affect associations between PFCs and neuropsychological function, and consequently the internal validity of the findings is unlikely to be compromised.

Strengths of the current study include the sensitive and objective measures of environmental exposures, mediators, and study outcomes we employed. We also addressed the limitations of neurocognitive outcome self-report used by previous studies (Gallo et al. 2013; Power et al. 2013); we employed batteries of well-known and widely accepted tests to assess neuropsychological tests in clinical populations and general populations exposed to various neurotoxicants (Fitzgerald et al. 2008; Schantz et al. 2001). In addition, our comprehensive evaluation of the wide-ranging domains of neuropsychological function allowed us to identify specific PFC associated effects. Furthermore, ours is the first study to assess mediating effects of THs for the associations between PFCs and neuropsychological function.

However, the results of this study should be interpreted carefully due to several limitations. We made multiple statistical comparisons, which increased the likelihood that the results are spurious due to inflation of the Type I error rate. Yet, consistent protective associations in the subtests of the memory and learning domain and the executive function suggest that these findings were not due to chance. The small sample size limited the extent and complexity of our statistical analysis; for example, we were unable to assess effect modification by diabetes and diabetic medications, which were suggested to be potential effect modifiers (Power et al. 2013). In addition, we could not determine the temporal order of exposure, mediator, and outcome in this cross-sectional study; so due to the possibility that detected associations could be due to reverse causation, etiologic inferences could not be made without reservation.

4.6 Conclusions

Consistent with prior studies, our findings suggest that PFCs are associated with improved memory learning and executive function, and visuospatial function. The results also suggest interactions between sex and PFCs. There was limited evidence of mediation for the effects of PFCs on neuropsychological function by thyroid function, although our results mostly suggested non-thyroid pathways. These findings provide insight regarding the biological relevance of THs in the effects of PFCs on neuropsychological function.

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Table 1: Background characteristics of study participants

Variable	n	AM (SD)	Median	Range	GM (SD)
Age at interview (years)	157	63.74 (5.99)	64	55, 74	
Body mass index (kg/m^2)	157	28.88 (5.76)	27.7	16.72, 49.61	
Alcohol consumption (Number of drinks over past year)	157	240.77 (341.94)	84	0, 2184	
Among drinkers (Number of drinks over past year)	130	290.78 (356.01)	156.40	1,2184.00	
Cigarette smoking (Total packs in last year)	156	56.05 (143.11)	0	0, 730	
Among smokers (Total packs in last year)	30	291.48 (186.17)	365	0.65,730	
Years of education	157	13.8 (2.6)	13	6, 20	
Serum total PCB (ng/g of total lipids)	155	523.53 (264.23)	462.13	139.3, 1638.19	470.11 (1.58)
Serum PFOS (ng/mL)	157	40.73 (27.93)	32.63	4.58, 216.96	34.24 (1.79)
Serum PFOA (ng/mL)	157	9.3 (5.23)	8.1	0.58, 42.69	8.1 (1.72)
Serum PFOS (ng/mL)	87	36.58 (22.8)	29.78	5.29, 139.53	31.60 (1.70)
Serum PFOA (ng/mL)	87	10.42 (5.68)	9.32	0.58, 42.69	9.17 (1.72)
Thyroid Stimulating Hormone (μIU/mL)	87	2.58 (1.47)	2.15	0.23, 9.05	2.25 (1.72)
Free Thyroxine (ng/dL)	87	1.24 (0.17)	1.26	0.86, 1.68	1.23 (1.15)
Total Thyroxine(μg/dL)	87	8.69 (1.47)	8.66	6.09, 12.08	8.57 (1.19)
Total Triiodothyronine (ng/dL)	87	125.69 (15.58)	124.60	82.70, 172.40	124.71(1.14)
Categories	n	%			
Sex					
Women	79	50.32			
Men	78	49.68			
Income					
< $15,000	10	6.62			
≥ $15,000 to $30,000	34	22.52			
> $30,000 to $45,000	35	23.18			
> $45,000 to $60,000	31	20.53			
> $60,000 to $75,000	23	15.23			
> $75,000	18	11.92			

Abbreviations: AM, Arithmetic Mean; GM, Geometric Mean; PCB, Polychlorinated Biphenyls; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; SD, Standard Deviation

Table 2: Individual and joint effects* of PFCs (ng/mL)† and sex on the selected neuropsychological tests (n = 157)

Neuropsychological Test	PFC (β (CI)[I1]	Sex (β (CI) [I2]	Joint Exposure (β (CI)[J]	p[p]
PFOA (ng/mL)†				
Memory and Learning				
CVLT, trial 1 score [A,L]	0.306 (-0.182, 0.794)	1.801 (1.155, 2.447)	1.400 (0.721, 2.079)	0.064
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	-7.664 (-16.888, 1.599)	-10.810 (-21.688, 0.066)	-1.595 (-12.969, 9.778)	0.009
CVLT, learning slope [A,L]	-0.032 (-0.181, 0.117)	0.060 (-0.138, 0.258)	0.226 (0.018, 0.434)	0.089
CVLT, discriminability [B,L] (75^{th} quantile)	2.986 (-0.472, 6.444)	4.222 (1.769, 6.676)	-3.664 (-7.659, 0.33)	0.072
CVLT, recognition hits vs. long delay free recall [B,L] (75^{th} quantile)	-36.354 (-64.565, -8.143)	-73.442 (-100.322, -46.562)	30.779 (-5.394, 66.951)	0.095
WMS, visual reproduction delayed recall score [A,L]	0.569 (-0.271, 1.409)	1.227 (0.115, 2.339)	0.337 (-0.833, 1.507)	0.027
Executive function				
WCST, number of categories completed [B,L] (50^{th} quantile)	-0.859 (-2.313, 0.595)	-0.002 (-1.173, 1.17)	2.15 (0.293, 4.008)	0.024
Affective State				
STAI, state anxiety t-score [A,H]	1.828 (-0.822, 4.478)	4.093 (0.585, 7.600)	2.046 (-1.644, 5.736)	0.062
Motor function				
SMST (dominant hand), total number of contacts† [A,H]	0.086 (-0.129, 0.301)	-0.265 (-0.549, 0.020)	-0.464 (-0.764, -0.165)	0.089
PFOS (ng/mL)†				
Memory and Learning				
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	-5.638 (-14.639, 3.364)	-8.151 (-19.118, 2.816)	-2.816 (-14.064, 8.433)	0.077
WMS, visual reproduction delayed recall score [A,L]	1.288 (0.336, 2.24)	1.254 (0.094, 2.415)	1.393 (0.203, 2.583)	0.080
Reaction time				
Reaction time (dominant hand)† [A,H]	-0.027 (-0.082, 0.028)	0.014 (-0.052, 0.080)	0.077 (0.009, 0.145)	0.017

Abbreviations: CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; STAI, State-Trait Anxiety Inventory; SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, and serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; I1 = reported individual effect of a PFC; I2 = individual effect of sex; J = joint effect of a PFC and sex; p = p-value of a product term between a PFC and sex

Figure 1: Associations between PFOA (ng/mL) and California Verbal Learning Test, trial 1 score stratified by sex

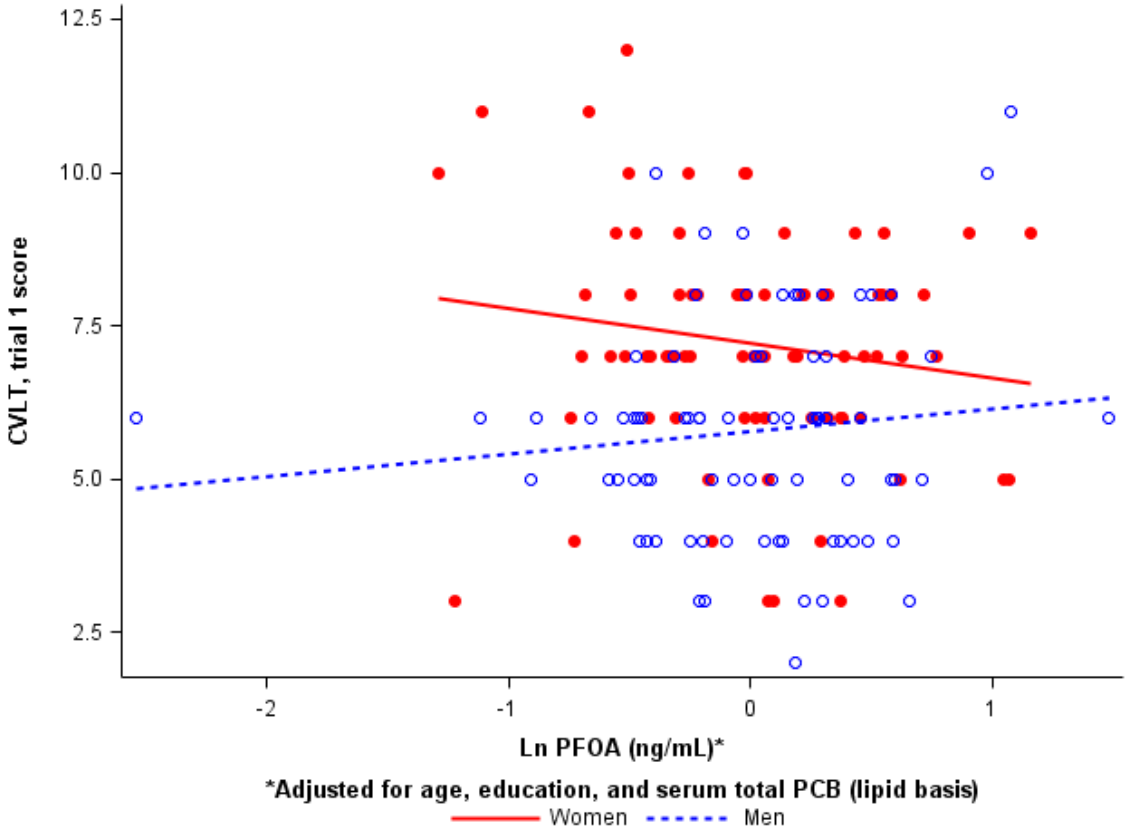

Table 3: Thyroid, Non-Thyroid, and Total Effects* of PFCs on the Selected Neuropsychological Tests (n = 87)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
PFOS (ng/mL)†							
Executive Function							
WCST, perseverative errors†[H]	M1	-0.014 (-0.053, 0.025)	0.475	-0.246 (-0.480, -0.012)	0.039	-0.259 (-0.494, -0.024)	0.031
	M2	-0.011 (-0.083, 0.061)	0.768	-0.248 (-0.492, -0.004)	0.047		
	M3	-0.007 (-0.047, 0.032)	0.711	-0.252 (-0.489, -0.015)	0.037		
	M4	0.005 (-0.032, 0.043)	0.788	-0.261 (-0.493, -0.029)	0.028		
WCST, perseverative responses†[H]	M1	-0.011 (-0.046, 0.024)	0.538	-0.283 (-0.538, -0.029)	0.029	-0.293 (-0.548, -0.039)	0.024
	M2	-0.009 (-0.086, 0.069)	0.827	-0.285 (-0.550, -0.020)	0.035		
	M3	-0.006 (-0.048, 0.036)	0.778	-0.288 (-0.545, -0.031)	0.028		
	M4	0.005 (-0.034, 0.045)	0.788	-0.296 (-0.547, -0.044)	0.021		
Visuospatial Function							
Block Design Subtest, total score[L]	M1	0.409 (-0.299, 1.118)	0.257	1.886 (-0.915, 4.687)	0.187	2.643 (-0.149, 5.436)	0.064
	M2	1.349 (0.093, 2.606)	0.035	1.294 (-1.523, 4.111)	0.368		
	M3	0.895 (-0.221, 2.011)	0.116	1.749 (-0.913, 4.410)	0.198		
	M4	0.054 (-0.214, 0.322)	0.691	2.589 (-0.209, 5.388)	0.070		
PFOA (ng/mL)†							
Memory and Learning							
CVLT, t-score [L]	M1	0.247 (-0.297, 0.791)	0.373	2.199 (-0.539, 4.937)	0.115	2.458 (-0.271, 5.188)	0.078
	M2	0.070 (-0.532, 0.673)	0.820	2.388 (-0.401, 5.177)	0.093		
	M3	-0.186 (-0.669, 0.298)	0.451	2.635 (-0.078, 5.347)	0.057		
	M4	0.295 (-0.299, 0.890)	0.330	2.155 (-0.583, 4.892)	0.123		
CVLT, short delay free recall [L]	M1	0.074 (-0.080, 0.228)	0.346	0.794 (0.057, 1.531)	0.035	0.868 (0.131, 1.605)	0.021

Table 3: Thyroid, Non-Thyroid, and Total Effects* of PFCs on the Selected Neuropsychological Tests (n = 87)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
	M2	-0.015 (-0.175, 0.145)	0.853	0.883 (0.129, 1.637)	0.022		
	M3	-0.095 (-0.311, 0.121)	0.387	0.963 (0.251, 1.675)	0.008		
	M4	0.037 (-0.093, 0.167)	0.578	0.831 (0.086, 1.576)	0.029		
CVLT, long delay free recall [L]	M1	0.052 (-0.084, 0.188)	0.457	0.595 (-0.166, 1.355)	0.126	0.646 (-0.110, 1.402)	0.094
	M2	-0.011 (-0.174, 0.153)	0.899	0.657 (-0.117, 1.430)	0.096		
	M3	-0.092 (-0.303, 0.119)	0.391	0.739 (0.005, 1.472)	0.048		
	M4	0.023 (-0.105, 0.150)	0.729	0.624 (-0.142, 1.389)	0.110		
CVLT, proactive interference[L]	M1	1.013 (-0.924, 2.950)	0.305	-8.015 (-16.517, 0.487)	0.065	-7.002 (-15.496, 1.491)	0.106
	M2	-0.337 (-1.646, 0.973)	0.614	-6.665 (-15.227, 1.897)	0.127		
	M3	-0.078 (-0.683, 0.526)	0.800	-6.924 (-15.405, 1.557)	0.110		
	M4	-0.356 (-1.508, 0.796)	0.545	-6.647 (-15.143, 1.850)	0.125		
CVLT, semantic cluster ratio[L]	M1	-0.007 (-0.037, 0.023)	0.659	0.156 (-0.045, 0.357)	0.129	0.178 (-0.025, 0.382)	0.086
	M2	-0.009 (-0.054, 0.036)	0.683	0.188 (-0.020, 0.395)	0.077		
	M3	-0.007 (-0.033, 0.019)	0.580	0.186 (-0.018, 0.389)	0.075		
	M4	-0.001 (-0.034, 0.033)	0.975	0.179 (-0.028, 0.385)	0.089		

Abbreviations: CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, and serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

Chapter 5: Discussion

5.1 Discussion

An epidemiological investigation was conducted to elucidate relationships of perfluorinated compounds (PFCs), thyroid function, and neuropsychological status in an aging population residing in upper Hudson River communities. The investigation intended to help address several research gaps, including underrepresentation of aging populations in prior studies.

Here I reported i) associations of serum perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) with serum thyroid stimulating hormone (TSH) and thyroid hormones (THs); ii) associations between thyroid function and neuropsychological status; iii) associations between serum PFOS and PFOA and neuropsychological status; and iv) mediation of effects of the PFCs on neuropsychological function by TSH and THs. Additionally, I reported effects of other persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), on associations of the PFCs with thyroid function.

5.2 PFCs and Thyroid Function

Consistent with the results of a prior study for an aging group (Knox et al. 2011a), our study results indicated positive association between serum PFOS and total thyroxine (T4) and free thyroxine (fT4). Participants from the prior study were exposed to PFOS levels similar to ours, but their PFOA levels were 10 to 13 fold higher than ours. We did not detect individual effects for PFOA, but detected statistical interactions between age

and PFOA for T4 and fT4; concurrent increase in age and PFOA was associated with very small increases in the THs.

5.3 Thyroid Function and Neuropsychological Function

Associations of THs with neuropsychological status were age-dependent and domain-specific. Higher T4 and fT4 were associated with increases in Block Design Subtest (BDT) total scores, a measure of visuospatial function, in the overall study sample. We detected statistical interactions between THs and age for tasks of memory and learning and executive function. Concurrent increases in age and THs (i.e., T4, or fT4) were associated with poor performance in California Verbal Learning Test (CVLT) subtests, indicating impairments in memory formation, consolidation, and retrieval. Concurrent increases in age and THs (i.e., T4, or total triiodothyronine (T3)) were associated with poor performance in subtests of the Wisconsin Card Sorting Test (WCST), indicating diminished cognitive flexibility and other abilities to execute tasks that require planning and organization skills. We did not detect associations for TSH. This is one of the few studies to report and elaborate statistical interactions between age and THs (Osterweil et al. 1992; St John et al. 2009).

5.4 PFCs, Thyroid Function, and Neuropsychological Function

The overall patterns of the results indicated that PFCs are neuroprotective, which is consistent with the findings from two previous studies of PFCs and cognitive function in adults (Gallo et al. 2013; Power et al. 2013). Serum PFCs were associated with improved performance in memory and learning, executive function, and visuospatial

function. The results also indicated statistical interactions between sex and PFOA, particularly in tasks of memory and learning.

Interestingly, although we detected that PFCs alter THs and that both PFCs and THs might affect the same neuropsychological domains (i.e., memory and learning and executive function), formal tests of mediation suggested that improved performances in those domains associated with increasing levels of PFCs were not mediated through pathways that involve THs. Only a protective effect of PFOS on BDT total scores was partially mediated by T4.

The experimental literature suggests that PFCs increase THs by interfering with serum TH transport proteins (TTPs) rather than altering the hypothalamus-pituitary-thyroid axis (Chang et al. 2007; Chang et al. 2008; Han et al. 2003; Jones et al. 2003). It is possible that small increases in circulating THs associated with PFCs might be normalized by peripheral deiodination (Bloom et al. 2013) and might not be sufficient to bring about significant changes in TSH as well as in neuropsychological function. In addition, our findings suggested that age and sex modify relationships of PFCs, TH, and neuropsychological function. However, we could not accommodate such complex interactions in our mediation analyses, and therefore, we could have failed to detect mediation by THs. Yet, we did examine mediation by THs in the strata of sex, or age (i.e., stratified by median age of 63 years); the results were essentially the same (Appendix D, Tables D-1 to D-4). The only differences were that we detected stronger mediation for BDT by T4 and fT4 and partial 'inconsistent mediation' by T3 for PFOS and CVLT, learning slope among those aged > 63 years.

It is interesting that early animal and toxicological studies indicated that PFCs were neurotoxic in nature (Johansson et al. 2009; Liu et al. 2010; Onishchenko et al. 2011; Pinkas et al. 2010; Slotkin et al. 2008; Zhang et al. 2011), but recent human studies suggest that PFCs could have neuroprotective effects (Gallo et al. 2013; Power et al. 2013). Researchers have postulated that protective associations could be due to the ability of PFCs to activate peroxisome proliferator activated receptors (PPARs) (Gallo et al. 2013; Power et al. 2013; Vanden Heuvel et al. 2006; Wan Ibrahim et al. 2013) since PPAR γ agonists including thiazolidinediones have been shown to elicit neuroprotective effects, potentially due to their anti-inflammatory property (Kaundal and Sharma 2010). One possible explanation could be that PFCs may affect nervous system via several different pathways, neurotoxic as well as neuroprotective, but the overall effect is protective in nature.

5.5 Effect of Aging on Associations of PFCs, Thyroid Function, and Neuropsychological Function

Our results suggested that aging may affect the relationships between PFOA and THs, and between THs and neuropsychological function. The effects of age on PFOA-TH associations appeared very subtle, whereas those for TH-neuropsychological function associations were more obvious. Aging was negatively associated with performance in memory and learning among individuals with lower THs while THs were negatively associated with memory and learning, and positively associated with executive function among younger individuals. Yet, joint increases of age and THs were associated with decrements in both memory and learning and executive function.

The opposite directions of associations for individual effects on memory and learning and executive function is intriguing because both domains are often impaired concurrently and affected by common brain regions (Duff et al. 2005; McCabe et al. 2010). Although speculative, such findings could be explained by several factors including differential distribution of TH receptors in brain regions (Whybrow and Bauer 2005a), differential rates at which structures and functions of brain may decline (Phillips and Dela Salla 1998), and multiple distinctive factors that may independently target different brain systems (Buckner 2004).

For memory and learning, joint effects were subadditive (i.e., less than the sum of individual effects of age and a TH) which is contrary to the general notion that aging may exacerbate TH-neuropsychological association. One possible explanation could be that brain regions that are affected by normative aging and THs overlap, and as aging progresses, deleterious effects (i.e., effect sizes) of higher THs on memory and learning gradually become less significant, or smaller to detect, due to already deteriorating memory and learning. However, aging exacerbated effects of THs on executive function, partially supporting our aging hypothesis.

5.6 Sex Differences in the Associations of PFCs with Thyroid and Neuropsychological Function

We did not detect any evidence of effect modification by sex for PFC-thyroid and thyroid-neuropsychological associations. However, we detect sub-additive interactions between PFOA and sex for neuropsychological function. The individual effects of sex indicated that women performed better in a few neuropsychological tests as compared to men among the individuals exposed to low PFOA levels. However, concurrent effect of

higher PFOA and sex (lower PFOA and men being the reference group) was lower than the sum of individual effects of PFOA and sex. Estrogen is neuroprotective and neurotrophic in nature (Brann et al. 2007). So, we could speculate that women with greater PFCs burden may experience neuroprotective effect to a lesser extent given that PFCs may negatively affect estradiol levels (Knox et al. 2011b). Further evidences are required to support this hypothesis, because we are unsure of causal associations between PFCs and estradiol levels.

5.7 Exposure to Other POPs

We detected that both PCB and PBDE might alter the associations of PFCs with thyroid and neuropsychological function. For PCB, we detected that concurrent exposure to high levels of PFOS and total PCB exhibited no effect on T3 while exposure to PFOS among individuals with lower PCB level suggested potential elevation in T3. Similarly, we detected consistent multiplicative interactions between PFOA and PCB for tests in executive function (Appendix D, Table D-5). Specifically, individual effects of PFOA (i.e., among low levels of total PCB) on executive function were protective; however, concurrent exposure to total PCB and PFOA indicated no neurotoxic effects.

Total PBDE exhibited statistical interaction with PFOA for TSH; total PBDE, however, did not exhibit consistent patterns of statistical interactions with PFCs for neuropsychological function. The literature indicates that PCBs and PBDEs affect serum THs and TSH through several mechanisms that influence hormone synthesis and metabolism and transport proteins (Boas et al. 2012; Liu et al. 2012) which could explain observed statistical interaction for THs. Likewise, PCBs have also been linked with

neurotoxicity (Fitzgerald et al. 2008), which may explain their antagonistic effects on PFC-neuropsychological associations.

5.8 Strengths and Limitations

Strengths of the study include the sensitive and objective measures of environmental exposures, mediators, and study outcomes. Furthermore, comprehensive assessment of multiple neuropsychological domains allowed us to identify specific PFC- and TH-associated effects. We employed neuropsychological tests that have been widely used in clinical populations (Mitrushina et al. 2005), and general populations exposed to various neurotoxicants (Fitzgerald et al. 2008). In addition, in order to discriminate true memory impairment from malingering, the Test of Memory Malingering was also administered, and no association of PFCs and THs with memory malingering was detected (Appendix D, Table D-6), providing support to the validity of our results. Furthermore, we were also able to assess interactions between PFCs and other POPs.

There are several limitations of the study - presented by cross-sectional nature, multiple testing, and limited sample size. Due to lack of information on temporal order of exposure, mediator, and outcome, we could not make causal inferences on detected associations. Since we tested multiple hypotheses, the likelihood that detected associations were false positives increased due to inflation of the Type I error rate. Yet, consistent associations across the specific aims support that findings may not due to chance. Small sample size limited the analyses in several ways; for instance, we did not have enough sample size to assess statistical interactions with several covariates that were suggested to interact with the exposures, and to assess potential non-linear associations.

Levels of PFOS and PFOA in the current study were higher than those reported in the aging U.S. population (Kato et al. 2011). Although we are unsure as to the sources of elevated PFC levels in our study population, given that serum PFOS was modestly correlated with serum total PCB and the current study population lived near a PCB-contaminated site, we could speculate that elevated levels may be due to common environmental or occupational sources in addition to the typical environmental sources of PFCs. Therefore, the results may not be generalized to other aging populations.

The analyses were conducted among subsamples of the participants of the original cohort and sample sizes differed for each specific aims. However, the internal validity of the study results is less likely to be affected as sample selection was based on the availability of enough serum samples for PFC and TH measurements, rather than participants' willingness to participate, and use of sex hormones. Moreover, the distributions of characteristics of participants in each aim were similar to those in the original parent study that were excluded, except the proportion of participants reporting alcohol consumption was greater among those included.

5.9 Research Implications and Future Directions

Our results that T4 and fT4, rather than TSH, were associated with neuropsychological function may have important clinical implications. Generally serum TSH alone is used for screening of thyroid dysfunction in aging adults. Our results however provide support to the recommendations by clinical practice guidelines for the use of fT4/T4 in the screening of thyroid disease in adults (Garber et al. 2012). In addition, our findings provide insight regarding the impact of PFCs on neuropsychological function and the role of THs in aging populations.

However, it should be noted that the current study was performed in aging individuals without a history of overt thyroid diseases and clinical neuropsychological conditions. So although we did not find strong evidence of mediation by THs in these presumably healthy aging individuals, PFC-associated alterations in THs may have meaningful implications on neurocognitive well-being among hyperthyroid individuals or those individuals at the upper ends of TH distributions (Whybrow and Bauer 2005b; Winquist and Steenland 2014). Assessment of the associations among individuals with clinical thyroid and neuropsychological impairments in future may help to illustrate the potential links.

Furthermore, to provide more valid and robust evidence regarding some of our interesting findings including neuroprotective effect of PFCs, longitudinal studies with larger sample sizes and repetitive assessments of both the exposures and outcomes are warranted. In addition, future studies of PFCs and THs also need to focus on other thyroid function markers including thyroglobulin and serum TTPs so as to understand underlying biology, as recent studies hint involvement of these biomarkers in PFC-associated TH imbalance (Han et al. 2003; Wen et al. 2013).

To our best knowledge, this is the first study to assess mediating effects of THs for the associations between PFCs and neuropsychological function. Besides these PFCs, other POPs have also been hypothesized to alter neuropsychological function via thyroid function disruption (Kodavanti 2005); however, mediating effects have not been formally assessed. Our findings, i.e., limited mediation by THs, may justify the need to assess if TH changes brought about by the ranges of POP exposures at present are meaningful and can actually affect neuropsychological function.

5.10 Conclusions

Changes in levels of THs associated with the ranges of PFCs exposures in our study population seem to be relatively small. Consistent with prior studies, our findings suggest that PFCs are neuroprotective and may specifically affect memory learning, executive function, and visuospatial function. The results also suggest that THs and age interact to affect memory and learning and executive function. However, our findings suggest that protective effects of PFCs on neuropsychological function are mainly due to the pathways that do not involve THs. Apparent increases in THs associated with PFCs seem very small to bring about changes in memory and learning and executive function.

The results require careful interpretation, and further studies warranted to support the findings of this study and to elucidate possible mechanisms involved. Although the results suggests that PFCs may be neuroprotective, potential links with the other adverse health outcomes including metabolic disorders still advise against the use of such chemicals.

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6 Appendix

6.1 Appendix A: Supplementary Results for Chapter 2

Table A-1: Final multivariable models* for thyroid function markers with serum PFCs (with both PFOS and PFOA in the models)† (n = 87)

	PFOS		PFOA	
Thyroid Markers	**β (CI)**	**P-value**	**β (CI)**	**P-value**
TSH (μIU/ml)†	0.108 (-0.071, 0.286)	0.234	0.063 (-0.098, 0.225)	0.436
fT4 (ng/dl)	0.048 (-0.013, 0.109)	0.124	-0.001 (-0.057, 0.055)	0.975
T4 (μg/dl)	0.660 (0.144, 1.175)	0.013	0.142 (-0.331, 0.615)	0.552
T3 (ng/dl)	0.374 (-5.292, 6.040)	0.896	2.897 (-2.306, 8.101)	0.271

Abbreviations: CI, 95% Confidence Interval; PCB, Polychlorinated Biphenyls; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone

*Adjusted for age, sex, years of education, serum PCB (ng/g serum total lipids), and other PFC

†Log-natural transformed

Table A-2: Final multivariable models* for thyroid function markers with serum PFCs (ng/mL)† (n = 87)

Variable	**β**	**95% LCI**	**95% UCI**	**P-value**
TSH (μIU/mL)†				
PFOS	0.128	-0.026	0.282	0.103
PFOA	0.102	-0.048	0.252	0.180
fT4 (ng/dL)				
PFOS	0.050	-0.001	0.101	0.054
PFOA	0.013	-0.038	0.064	0.615
T4(μg/dL)				
PFOS	0.737	0.307	1.167	0.001
PFOA	0.355	-0.090	0.800	0.117
T3 (ng/dL)				
PFOS	2.483	-2.408	7.375	0.315
PFOA	2.945	-1.840	7.730	0.224

Abbreviations: LCI, Lower Confidence Interval; PCB, Polychlorinated Biphenyls; PFCs, Perfluorinated Compounds; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone; UCI, Upper Confidence Interval

*Adjusted for age, sex, years of education, serum PCB (wet basis), and total lipids

Table A-3: Individual and joint effects[a] of PFOA† and age on thyroid hormones (n = 87)

	PFOA (β (CI)) [M1]	Age (β (CI)) [M2]	Joint effect (β (CI))[J]	p
TSH (μIU/mL) †	0.031 (-0.149, 0.211)	0.104 (-0.111, 0.319)	0.292 (0.032, 0.551)	0.218
fT4 (ng/dL)	-0.026 (-0.087, 0.035)	-0.035 (-0.108, 0.037)	0.025 (-0.063, 0.113)	0.044
T4(μg/dL)	-0.010 (-0.535, 0.515)	-0.222 (-0.849, 0.406)	0.573 (-0.185, 1.331)	0.030
T3 (ng/dL)	1.495 (-4.281, 7.271)	-3.304 (-10.200, 3.593)	1.386 (-6.949, 9.722)	0.433

Abbreviations: CI, 95% Confidence Interval; PCB, Polychlorinated Biphenyls; PFOA, Perfluorooctanoic Acid; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone; [a]Adjusted for sex, years of education, serum total PCB (wet basis), and total lipids; †Log-natural transformed; M1 = Individual effect of PFOA (i.e., change in thyroid hormone level per IQR increase in PFOA among reference age group); M2 = Individual effect of age (i.e., change in thyroid hormone level per IQR increase in age among reference PFOA group); J= Joint effect of PFOA and age (i.e., change in thyroid hormone level score per IQR increments in both PFOA and age); p = p-value of a product term between PFOA and age

Table A-4: Individual and joint effects of PFC† and POP on thyroid hormones (n = 87)

	PFC (β (CI)) [M1]	POP (β (CI)) [M2]	Joint effect (β (CI))[J]	p
PBDE (wet basis)† and TSH (μIU/mL)† for PFOA†[a]	0.167 (-0.005, 0.340)	0.038 (-0.116, 0.191)	0.053 (-0.139, 0.244)	0.091
PCB (wet basis)† and T3 (ng/dL) for PFOS†[b]	3.665 (-1.550, 8.880)	0.627 (-3.591, 4.845)	-0.617 (-5.972, 4.739)	0.051

Abbreviations: CI, 95% Confidence Interval; PBDE, Polybrominated Diphenyl Ethers; PCB, Polychlorinated Biphenyls; PFOA, Perfluorooctanoic Acid; PFOS, Perfluorooctane Sulfonate; POP, Persistent Organic Pollutant; fT4, Free Thyroxine; T3, Total Triiodothyronine; T4, Total Thyroxine; TSH, Thyroid Stimulating Hormone; [a]Adjusted for age, sex, years of education, serum total PCB (wet basis) and total lipids; [a]Adjusted for age, sex, years of education, and total lipids;†Log-natural transformed; M1 = Individual effect of PFC (i.e., change in thyroid hormone level per IQR increase in PFC among reference POP group); M2 = Individual effect of POP (i.e., change in thyroid hormone level per IQR increase in POP among reference PFC group); J = Joint effect of PFC and POP (i.e., change in thyroid hormone level score per IQR increments in both PFC and POP); p = p-value of a product term between PFC and POP

6.2 Appendix B: Supplementary Results for Chapter 3

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130)

	CVLT				
Neuropsychological Test	**T- score**	**Trial 1 score**	**Short delay free recall**	**Long delay free recall**	**Proactive interference**
Memory and Learning					
CVLT, t-score [L]	-	0.62 (<0.0001)	0.71 (<0.0001)	0.68 (<0.0001)	-0.07 (0.46)
CVLT, trial 1 score [L]	0.62 (<0.0001)	-	0.49 (<0.0001)	0.46 (<0.0001)	-0.37 (<0.0001)
CVLT, short delay free recall [L]	0.71 (<0.0001)	0.49 (<0.0001)	-	0.83 (<0.0001)	0.04 (0.64)
CVLT, long delay free recall [L]	0.68 (<0.0001)	0.46 (<0.0001)	0.83 (<0.0001)	-	0.00 (1.00)
CVLT, proactive interference [L]	-0.07 (0.46)	-0.37 (<0.0001)	0.04 (0.64)	0.00 (1.00)	-
CVLT, semantic cluster ratio [L]	0.59 (<0.0001)	0.40 (<0.0001)	0.54 (<0.0001)	0.53 (<0.0001)	-0.08 (0.35)
CVLT, learning slope [L]	0.31 (<0.01)	-0.20 (0.02)	0.45 (<0.0001)	0.51 (<0.0001)	0.42 (<0.0001)
CVLT, perseverations [H]	0.26 (<0.01)	0.29 (<0.01)	0.26 (<0.01)	0.21 (0.02)	0.1 (0.28)
CVLT, discriminability [L]	0.6 (<0.0001)	0.42 (<0.0001)	0.65 (<0.0001)	0.66 (<0.0001)	-0.09 (0.34)
CVLT, recognition hits vs. long delay free recall [L]	-0.62 (<0.0001)	-0.37 (<0.0001)	-0.77 (<0.0001)	-0.93 (<0.0001)	-0.06 (0.47)
WMS, logical memory immediate recall [L]	0.28 (<0.01)	0.2 (0.02)	0.36 (<0.0001)	0.39 (<0.0001)	0.11 (0.22)
WMS, logical memory delayed recall [L]	0.30 (<0.01)	0.19 (0.03)	0.37 (<0.0001)	0.41 (<0.0001)	0.16 (0.08)
WMS, visual reproduction immediate recall [L]	0.07 (0.43)	0.01 (0.94)	0.13 (0.15)	0.15 (0.08)	0.08 (0.36)
WMS, visual reproduction delayed recall [L]	0.14 (0.11)	0.05 (0.56)	0.23 (0.01)	0.24 (0.01)	0.12 (0.19)
Measures of Attention					
TMT Part A-time to complete [H]	-0.02 (0.78)	-0.05 (0.57)	-0.17 (0.05)	-0.12 (0.18)	-0.18 (0.04)
TMT Part B-time to complete [H]	-0.19 (0.04)	-0.15 (0.1)	-0.19 (0.03)	-0.18 (0.04)	-0.07 (0.46)

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130)

	CVLT				
Neuropsychological Test	**T- score**	**Trial 1 score**	**Short delay free recall**	**Long delay free recall**	**Proactive interference**
Executive Function					
SCWT, t-score [L]	0.17 (0.06)	0.01 (0.89)	-0.02 (0.82)	-0.02 (0.85)	0.07 (0.45)
WCST, perseverative errors [H]	-0.06 (0.48)	-0.1 (0.25)	-0.16 (0.09)	-0.11 (0.23)	0 (0.99)
WCST, perseverative responses [H]	-0.07 (0.45)	-0.1 (0.29)	-0.15 (0.1)	-0.11 (0.23)	0 (0.98)
WCST, number of categories completed [L]	0.02 (0.82)	0.1 (0.26)	0.17 (0.07)	0.09 (0.32)	0.05 (0.61)
WCST, failure to maintain set [H]	-0.08 (0.38)	0.07 (0.44)	-0.1 (0.25)	-0.09 (0.33)	-0.05 (0.6)

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; TMT, Trail Making Test; SCWT, Stroop Color Word Test; WCST, Wisconsin Card Sorting Test; H: High Score = Impairment, L: Low Score = Impairment

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130) (continued)

	CVLT				
Neuropsychological Test	**Semantic Cluster Ratio**	**Learning Slope**	**Perseverations**	**Discriminability**	**Recognition hits**
Memory and Learning					
CVLT, t-score [L]	0.59 (<0.0001)	0.31 (<0.01)	0.26 (<0.01)	0.6 (<0.0001)	-0.62 (<0.0001)
CVLT, trial 1 score [L]	0.4 (<0.0001)	-0.2 (0.02)	0.29 (<0.01)	0.42 (<0.0001)	-0.37 (<0.0001)
CVLT, short delay free recall [L]	0.54 (<0.0001)	0.45 (<0.0001)	0.26 (<0.01)	0.65 (<0.0001)	-0.77 (<0.0001)
CVLT, long delay free recall [L]	0.53 (<0.0001)	0.51 (<0.0001)	0.21 (0.02)	0.66 (<0.0001)	-0.93 (<0.0001)
CVLT, proactive interference [L]	-0.08 (0.35)	0.42 (<0.0001)	0.1 (0.28)	-0.09 (0.34)	-0.06 (0.47)
CVLT, semantic cluster ratio [L]	-	0.36 (<0.0001)	-0.05 (0.6)	0.53 (<0.0001)	-0.5 (<0.0001)
CVLT, learning slope [L]	0.36 (<0.0001)	-	-0.03 (0.76)	0.36 (<0.0001)	-0.5 (<0.0001)
CVLT, perseverations [H]	-0.05 (0.6)	-0.03 (0.76)	-	0.17 (0.06)	-0.23 (0.01)
CVLT, discriminability [L]	0.53 (<0.0001)	0.36 (<0.0001)	0.17 (0.06)	-	-0.5 (<0.0001)
CVLT, recognition hits vs. long delay free recall [L]	-0.5 (<0.0001)	-0.5 (<0.0001)	-0.23 (0.01)	-0.5 (<0.0001)	-
WMS, logical memory immediate recall [L]	0.29 (<0.01)	0.18 (0.04)	0.13 (0.14)	0.3 (<0.01)	-0.34 (<0.0001)
WMS, logical memory delayed recall [L]	0.34 (<0.0001)	0.2 (0.02)	0.1 (0.23)	0.29 (<0.01)	-0.38 (<0.0001)
WMS, visual reproduction immediate recall [L]	0.08 (0.34)	0.11 (0.2)	-0.12 (0.16)	0.15 (0.09)	-0.16 (0.07)
WMS, visual reproduction delayed recall [L]	0.09 (0.31)	0.18 (0.04)	-0.06 (0.47)	0.19 (0.03)	-0.24 (0.01)
Measures of Attention					
TMT Part A-time to complete [H]	-0.04 (0.62)	-0.05 (0.61)	0.14 (0.12)	-0.07 (0.46)	0.12 (0.18)
TMT Part B-time to complete [H]	-0.16 (0.08)	-0.15 (0.09)	0.06 (0.52)	-0.23 (0.01)	0.15 (0.09)
Executive Function					
SCWT, t-score [L]	0.02 (0.83)	0.01 (0.89)	-0.04 (0.66)	-0.09 (0.33)	0 (0.98)
WCST, perseverative errors [H]	-0.12 (0.18)	-0.12 (0.18)	0.06 (0.53)	-0.19 (0.03)	0.06 (0.53)

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130) (continued)

	CVLT				
Neuropsychological Test	**Semantic Cluster Ratio**	**Learning Slope**	**Perseverations**	**Discriminability**	**Recognition hits**
WCST, perseverative responses [H]	-0.12 (0.19)	-0.13 (0.15)	0.06 (0.54)	-0.19 (0.04)	0.06 (0.5)
WCST, number of categories completed [L]	0.03 (0.73)	0.1 (0.27)	0.02 (0.86)	0.12 (0.18)	-0.07 (0.45)
WCST, failure to maintain set [H]	-0.16 (0.07)	-0.11 (0.21)	0.13 (0.14)	-0.01 (0.89)	0.08 (0.36)

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; TMT, Trail Making Test; SCWT, Stroop Color Word Test; WCST, Wisconsin Card Sorting Test; H: High Score = Impairment, L: Low Score = Impairment

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130) (continued)

	WMS, logical memory		WMS, visual memory		TMT
Neuropsychological Test	**immediate recall**	**delayed recall**	**immediate recall**	**delayed recall**	**Part A, time to complete**
Memory and Learning					
CVLT, t-score [L]	0.28 (<0.01)	0.3 (<0.01)	0.07 (0.43)	0.14 (0.11)	-0.02 (0.78)
CVLT, trial 1 score [L]	0.2 (0.02)	0.19 (0.03)	0.01 (0.94)	0.05 (0.56)	-0.05 (0.57)
CVLT, short delay free recall [L]	0.36 (<0.0001)	0.37 (<0.0001)	0.13 (0.15)	0.23 (0.01)	-0.17 (0.05)
CVLT, long delay free recall [L]	0.39 (<0.0001)	0.41 (<0.0001)	0.15 (0.08)	0.24 (0.01)	-0.12 (0.18)
CVLT, proactive interference [L]	0.11 (0.22)	0.16 (0.08)	0.08 (0.36)	0.12 (0.19)	-0.18 (0.04)
CVLT, semantic cluster ratio [L]	0.29 (<0.01)	0.34 (<0.0001)	0.08 (0.34)	0.09 (0.31)	-0.04 (0.62)
CVLT, learning slope [L]	0.18 (0.04)	0.2 (0.02)	0.11 (0.2)	0.18 (0.04)	-0.05 (0.61)
CVLT, perseverations [H]	0.13 (0.14)	0.1 (0.23)	-0.12 (0.16)	-0.06 (0.47)	0.14 (0.12)
CVLT, discriminability [L]	0.3 (<0.01)	0.29 (<0.01)	0.15 (0.09)	0.19 (0.03)	-0.07 (0.46)
CVLT, recognition hits vs. long delay free recall [L]	-0.34 (<0.0001)	-0.38 (<0.0001)	-0.16 (0.07)	-0.24 (0.01)	0.12 (0.18)
WMS, logical memory immediate recall [L]	-	0.81 (<0.0001)	0.22 (0.01)	0.25 (<0.01)	-0.03 (0.7)
WMS, logical memory delayed recall [L]	0.81 (<0.0001)	-	0.25 (<0.01)	0.36 (<0.0001)	-0.1 (0.27)
WMS, visual reproduction immediate recall [L]	0.22 (0.01)	0.25 (<0.01)	-	0.82 (<0.0001)	-0.37 (<0.0001)
WMS, visual reproduction delayed recall [L]	0.25 (<0.01)	0.36 (<0.0001)	0.82 (<0.0001)	-	-0.35 (<0.0001)
Measures of Attention					
TMT Part A-time to complete [H]	-0.03 (0.7)	-0.1 (0.27)	-0.37 (<0.0001)	-0.35 (<0.0001)	-
TMT Part B-time to complete [H]	-0.33 (<0.01)	-0.27 (<0.01)	-0.25 (0.01)	-0.25 (<0.01)	0.53 (<0.0001)
Executive Function					
SCWT, t-score [L]	0.15 (0.09)	0.1 (0.24)	0.1 (0.28)	0.11 (0.23)	-0.01 (0.87)

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130) (continued)

	WMS, logical memory		WMS, visual memory		TMT
Neuropsychological Test	**immediate recall**	**delayed recall**	**immediate recall**	**delayed recall**	**Part A, time to complete**
WCST, perseverative errors [H]	-0.1 (0.29)	-0.08 (0.39)	-0.37 (<0.0001)	-0.35 (<0.0001)	0.17 (0.05)
WCST, perseverative responses [H]	-0.1 (0.28)	-0.07 (0.42)	-0.37 (<0.0001)	-0.35 (<0.0001)	0.16 (0.07)
WCST, number of categories completed [L]	-0.01 (0.9)	0.02 (0.87)	0.28 (<0.01)	0.3 (<0.01)	-0.19 (0.04)
WCST, failure to maintain set [H]	-0.14 (0.13)	-0.21 (0.02)	-0.12 (0.19)	-0.1 (0.29)	0.1 (0.26)

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; TMT, Trail Making Test; SCWT, Stroop Color Word Test; WCST, Wisconsin Card Sorting Test; H: High Score = Impairment, L: Low Score = Impairment

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130) (continued)

	TMT		SCWT		WCST	
Neuropsychological Test	**Part B, time to complete**	**T-score**	**Perseverative error**	**Perseverative response**	**Categories completed**	**Failure to maintain set**
Memory and Learning						
CVLT, t-score [L]	-0.19 (0.04)	0.17 (0.06)	-0.06 (0.48)	-0.07 (0.45)	0.02 (0.82)	-0.08 (0.38)
CVLT, trial 1 score [L]	-0.15 (0.10)	0.01 (0.89)	-0.10 (0.25)	-0.10 (0.29)	0.10 (0.26)	0.07 (0.44)
CVLT, short delay free recall [L]	-0.19 (0.03)	-0.02 (0.82)	-0.16 (0.09)	-0.15 (0.1)	0.17 (0.07)	-0.1 (0.25)
CVLT, long delay free recall [L]	-0.18 (0.04)	-0.02 (0.85)	-0.11 (0.23)	-0.11 (0.23)	0.09 (0.32)	-0.09 (0.33)
CVLT, proactive interference [L]	-0.07 (0.46)	0.07 (0.45)	0.00 (0.99)	0.00 (0.98)	0.05 (0.61)	-0.05 (0.6)
CVLT, semantic cluster ratio [L]	-0.16 (0.08)	0.02 (0.83)	-0.12 (0.18)	-0.12 (0.19)	0.03 (0.73)	-0.16 (0.07)
CVLT, learning slope [L]	-0.15 (0.09)	0.01 (0.89)	-0.12 (0.18)	-0.13 (0.15)	0.1 (0.27)	-0.11 (0.21)
CVLT, perseverations [H]	0.06 (0.52)	-0.04 (0.66)	0.06 (0.53)	0.06 (0.54)	0.02 (0.86)	0.13 (0.14)
CVLT, discriminability [L]	-0.23 (0.01)	-0.09 (0.33)	-0.19 (0.03)	-0.19 (0.04)	0.12 (0.18)	-0.01 (0.89)
CVLT, recognition hits vs. long delay free recall [L]	0.15 (0.09)	0 (0.98)	0.06 (0.53)	0.06 (0.5)	-0.07 (0.45)	0.08 (0.36)
WMS, logical memory immediate recall [L]	-0.33 (<0.01)	0.15 (0.09)	-0.1 (0.29)	-0.10 (0.28)	-0.01 (0.9)	-0.14 (0.13)
WMS, logical memory delayed recall [L]	-0.27 (<0.01)	0.1 (0.24)	-0.08 (0.39)	-0.07 (0.42)	0.02 (0.87)	-0.21 (0.02)
WMS, visual reproduction immediate recall [L]	-0.25 (0.01)	0.1 (0.28)	-0.37 (<0.0001)	-0.37 (<0.0001)	0.28 (<0.01)	-0.12 (0.19)
WMS, visual reproduction delayed recall [L]	-0.25 (<0.01)	0.11 (0.23)	-0.35 (<0.0001)	-0.35 (<0.0001)	0.3 (<0.01)	-0.1 (0.29)
Measures of Attention						
TMT Part A-time to complete [H]	0.53 (<0.0001)	-0.01 (0.87)	0.17 (0.05)	0.16 (0.07)	-0.19 (0.04)	0.1 (0.26)
TMT Part B-time to complete [H]	-	-0.11 (0.22)	0.39 (<0.0001)	0.38 (<0.0001)	-0.3 (<0.01)	0.21 (0.02)

Table B-1: Spearman correlation coefficients (p-value) between neuropsychological test scores (n = 130) (continued)

	TMT		SCWT		WCST	
Neuropsychological Test	**Part B, time to complete**	**T-score**	**Perseverative error**	**Perseverative response**	**Categories completed**	**Failure to maintain set**
Executive Function						
SCWT, t-score [L]	-0.11 (0.22)	-	-0.07 (0.42)	-0.06 (0.48)	0.13 (0.16)	-0.19 (0.04)
WCST, perseverative errors [H]	0.39 (<0.0001)	-0.07 (0.42)	-	0.99 (<0.0001)	-0.83 (<0.0001)	0.29 (<0.01)
WCST, perseverative responses [H]	0.38 (<0.0001)	-0.06 (0.48)	0.99 (<0.0001)	-	-0.81 (<0.0001)	0.28 (<0.01)
WCST, number of categories completed [L]	-0.3 (<0.01)	0.13 (0.16)	-0.83 (<0.0001)	-0.81 (<0.0001)	-	-0.28 (<0.01)
WCST, failure to maintain set [H]	0.21 (0.02)	-0.19 (0.04)	0.29 (<0.01)	0.28 (<0.01)	-0.28 (<0.01)	-

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; TMT, Trail Making Test; SCWT, Stroop Color Word Test; WCST, Wisconsin Card Sorting Test; H: High Score = Impairment, L: Low Score = Impairment

Table B-2: Individual and joint effects* of total thyroxine (µg/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Total Thyroxine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
Memory and Learning				
CVLT, t-score [A,L]	-4.021 (-7.504, -0.538)	0.094 (-3.488, 3.675)	0.662 (-3.105, 4.428)	0.044
CVLT, trial 1 score [A,L]	-0.717 (-1.289, -0.145)	-0.975 (-1.583, -0.367)	-0.913 (-1.553, -0.272)	0.041
CVLT, short delay free recall [A,L]	-1.042 (-1.943, -0.141)	-1.614 (-2.572, -0.657)	-1.289 (-2.298, -0.281)	0.023
CVLT, long delay free recall [A,L]	-0.731 (-1.667, 0.206)	-1.256 (-2.251, -0.261)	-1.194 (-2.243, -0.146)	0.201
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	-3.638 (-13.750, 6.474)	8.984 (-1.765, 19.732)	3.576 (-7.748, 14.900)	0.791
CVLT, semantic cluster ratio [A,L]	-0.221 (-0.470, 0.028)	-0.048 (-0.313, 0.217)	-0.029 (-0.307, 0.250)	0.145
CVLT, learning slope [A,L]	0.101 (-0.090, 0.291)	0.042 (-0.161, 0.244)	0.035 (-0.178, 0.248)	0.394
CVLT, perseverations† [A,H]	-0.219 (-0.504, 0.066)	-0.067 (-0.370, 0.235)	-0.292 (-0.611, 0.027)	0.976
CVLT, discriminability [B,L]				
25th quantile	-0.532 (-2.124, 1.061)	-0.444 (-0.773, -0.114)	0.109 (-0.076, 0.295)	0.247
50th quantile	-0.554 (-1.409, 0.300)	-0.339 (-0.586, -0.092)	0.094 (-0.012, 0.200)	0.083
75th quantile	-0.413 (-1.286, 0.461)	-0.279 (-0.477, -0.082)	0.166 (0.018, 0.314)	0.028
CVLT, recognition hits vs. long delay free recall [B,L]				
25th quantile	4.423 (-1.607, 10.453)	1.403 (0.036, 2.770)	-0.146 (-0.864, 0.573)	0.689
50th quantile	0.665 (-4.454, 5.783)	0.573 (-0.992, 2.138)	0.260 (-0.404, 0.924)	0.439
75th quantile	7.897 (-7.381, 23.175)	1.839 (-0.962, 4.641)	-0.677 (-2.210, 0.857)	0.384
WMS, logical memory immediate recall score [A,L]	-1.218 (-2.803, 0.367)	-1.643 (-3.328, 0.042)	-1.596 (-3.371, 0.179)	0.227
WMS, logical memory delayed recall score [A,L]	-0.786 (-2.255, 0.684)	-1.398 (-2.959, 0.164)	-1.180 (-2.825, 0.466)	0.301
WMS, visual reproduction immediate recall score [A,L]	0.898 (-0.010, 1.806)	-0.845 (-1.811, 0.120)	-0.416 (-1.433, 0.601)	0.435
WMS, visual reproduction delayed recall score [A,L]	0.761 (-0.201, 1.722)	-1.419 (-2.441, -0.397)	-1.08 (-2.157, -0.003)	0.506

Table B-2: Individual and joint effects* of total thyroxine (µg/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Total Thyroxine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
Measures of Attention				
Trail Making Test Part A-time to complete† [A,H]	0.028 (-0.062, 0.118)	0.130 (0.034, 0.226)	0.164 (0.063, 0.265)	0.919
Trail Making Test Part B-time to complete† [A,H]	0.010 (-0.090, 0.111)	0.099 (-0.008, 0.206)	0.180 (0.067, 0.294)	0.285
Executive Function				
Stroop Color Word Test, t-score [A,L]	1.493 (-0.860, 3.845)	3.071 (0.584, 5.558)	4.174 (1.535, 6.813)	0.801
WCST, perseverative errors† [A,H]	-0.293 (-0.523, -0.063)	0.164 (-0.079, 0.407)	0.302 (0.041, 0.563)	0.006
WCST, perseverative responses† [A,H]	-0.332 (-0.581, -0.084)	0.133 (-0.130, 0.396)	0.301 (0.018, 0.583)	0.004
WCST, number of categories completed [B,L]				
25th quantile	0.158 (-0.314, 0.629)	-0.081 (-0.178, 0.017)	-0.04 (-0.100, 0.021)	0.197
50th quantile	0.210 (-0.153, 0.574)	-0.126 (-0.221, -0.032)	-0.016 (-0.067, 0.036)	0.550
75th quantile	---	---	--	---
WCST, failure to maintain set [C,H]	1.022 (0.641, 1.630	1.369 (0.874, 2.145)	1.146 (0.681, 1.929)	0.510
Visual and Spatial Function				
Block Design Subtest, total score [A,L]	3.324 (0.645, 6.002)	-4.068 (-6.915, -1.220)	0.738 (-2.262, 3.738)	0.402
Digit Symbol Coding, total score [A,L]	-0.505 (-3.456, 2.447)	-5.863 (-8.913, -2.814)	-4.494 (-7.707, -1.281)	0.331
Reaction Time				
Reaction time (dominant hand)† [A,H]	-0.001 (-0.061, 0.058)	0.091 (0.027, 0.155)	0.067 (0.000, 0.133)	0.555
Affective State				
STAI, state anxiety t-score [A,H]	-2.077 (-4.926, 0.771)	0.219 (-2.809, 3.247)	0.121 (-3.069, 3.311)	0.293
STAI, trait anxiety t-score† [A,H]	-0.009 (-0.059, 0.042)	-0.018 (-0.071, 0.036)	-0.037 (-0.093, 0.02)	0.749
Motor Function				
FTT (dominant hand), average score [A,L]	-0.659 (-2.595, 1.278)	-1.992 (-4.047, 0.062)	-1.242 (-3.411, 0.927)	0.270
FTT (non-dominant hand), average score [A,L]	-1.635 (-3.254, -0.015)	-1.163 (-2.872, 0.546)	-2.063 (-3.881, -0.246)	0.489
GPT (dominant hand), time to completion† [A,H]	-0.016 (-0.075, 0.042)	0.205 (0.143, 0.267)	0.163 (0.097, 0.229)	0.510
GPT (non-dominant hand), time to completion† [A,H]	0.008 (-0.065, 0.081)	0.187 (0.111, 0.262)	0.178 (0.098, 0.258)	0.722

Table B-2: Individual and joint effects* of total thyroxine (µg/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Total Thyroxine Effect (β (CI))^{M1}	Individual Age Effect (β (CI))^{M2}	Joint Effect (β (CI))^{J}	p^{p}
SMST (dominant hand), total number of contacts† ^{A,H}	0.016 (-0.232, 0.264)	0.216 (-0.041, 0.472)	-0.022 (-0.288, 0.243)	0.116
SMST (dominant hand), total time touching† ^{A,H}	-0.051 (-0.234, 0.133)	0.173 (-0.027, 0.372)	-0.007 (-0.213, 0.199)	0.291
SMST (non-dominant hand), total number of contacts† ^{A,H}	0.026 (-0.233, 0.285)	0.232 (-0.034, 0.497)	-0.011 (-0.289, 0.268)	0.110
SMST (non-dominant hand), total time touching† ^{A,H}	0.031 (-0.295, 0.357)	0.304 (-0.045, 0.653)	0.097 (-0.269, 0.463)	0.269

Abbreviations: CVLT, California Verbal Learning Test; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; *Adjusted for age, sex, education, cigarette smoking, and Beck Depression Inventory; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; M1 = Individual effect of total thyroxine (i.e., change in a neuropsychological test score per IQR increase in total thyroxine among reference age group); M2 = Individual effect of age (i.e., change in a neuropsychological test score per IQR increase in age among reference total thyroxine group); J = Joint effect of total thyroxine and age (i.e., change in neuropsychological test score per concurrent IQR increment in both total thyroxine and age); p = p-value of a product term between total thyroxine and age

Table B-3: Individual and joint effects* of free thyroxine (ng/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Free Thyroxine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
Memory and Learning				
CVLT, t-score [A,L]	-4.739 (-8.424, -1.054)	-1.391 (-5.204, 2.421)	0.017 (-3.981, 4.014)	0.007
CVLT, trial 1 score [A,L]	-0.721 (-1.354, -0.089)	-0.996 (-1.65, -0.342)	-1.026 (-1.713, -0.339)	0.076
CVLT, short delay free recall [A,L]	-1.697 (-2.666, -0.728)	-1.972 (-2.974, -0.970)	-1.892 (-2.945, -0.840)	0.003
CVLT, long delay free recall [A,L]	-1.440 (-2.450, -0.430)	-1.608 (-2.652, -0.564)	-1.772 (-2.869, -0.675)	0.041
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	2.232 (-8.868, 13.331)	13.091 (1.617, 24.566)	6.588 (-5.466, 18.642)	0.200
CVLT, semantic cluster ratio [A,L]	-0.201 (-0.475, 0.073)	-0.106 (-0.389, 0.178)	-0.01 (-0.307, 0.288)	0.079
CVLT, learning slope [A,L]	-0.035 (-0.245, 0.175)	-0.083 (-0.300, 0.134)	0.003 (-0.225, 0.230)	0.347
CVLT, perseverations† [A,H]	-0.006 (-0.323, 0.311)	0.004 (-0.323, 0.332)	-0.143 (-0.487, 0.201)	0.466
CVLT, discriminability [B,L]				
25th quantile	-2.474 (-17.728, 12.780)	-0.364 (-0.728, -0.001)	0.413 (-1.07, 1.896)	0.583
50th quantile	-5.362 (-13.614, 2.890)	-0.381 (-0.634, -0.128)	0.62 (-0.391, 1.632)	0.227
75th quantile	-9.751 (-17.957, -1.545)	-0.262 (-0.532, 0.009)	0.927 (-0.23, 2.084)	0.115
CVLT, recognition hits vs. long delay free recall [B,L]				
25th quantile	37.735 (-36.259, 111.728)	1.193 (-0.292, 2.678)	-1.613 (-8.454, 5.228)	0.642
50th quantile	61.386 (-31.404, 154.175)	1.284 (-0.421, 2.988)	-1.865 (-10.093, 6.362)	0.654
75th quantile	119.175 (17.927, 220.423)	1.634 (-0.838, 4.106)	-8.9 (-22.328, 4.528)	0.192
WMS, logical memory immediate recall score [A,L]	-1.012 (-2.764, 0.740)	-1.416 (-3.228, 0.395)	-1.747 (-3.650, 0.156)	0.525
WMS, logical memory delayed recall score [A,L]	-0.691 (-2.310, 0.928)	-1.473 (-3.147, 0.201)	-1.192 (-2.95, 0.567)	0.327
WMS, visual reproduction immediate recall	0.683 (-0.325, 1.690)	-0.579 (-1.620, 0.462)	-0.701 (-1.794, 0.393)	0.193

Table B-3: Individual and joint effects* of free thyroxine (ng/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Free Thyroxine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
score [A,L]				
WMS, visual reproduction delayed recall score [A,L]	0.333 (-0.735, 1.400)	-1.489 (-2.592, -0.385)	-1.362 (-2.521, -0.203)	0.752
Measures of Attention				
Trail Making Test Part A-time to complete† [A,H]	-0.007 (-0.105, 0.091)	0.08 (-0.022, 0.181)	0.163 (0.056, 0.269)	0.134
Trail Making Test Part B-time to complete† [A,H]	-0.042 (-0.153, 0.069)	0.083 (-0.033, 0.198)	0.132 (0.011, 0.253)	0.181
Executive Function				
Stroop Color Word Test, t-score [A,L]	2.851 (0.395, 5.308)	4.035 (1.534, 6.536)	3.753 (1.021, 6.485)	0.043
WCST, perseverative errors† [A,H]	-0.197 (-0.465, 0.070)	0.206 (-0.065, 0.477)	0.294 (0.005, 0.584)	0.089
WCST, perseverative responses† [A,H]	-0.208 (-0.498, 0.083)	0.194 (-0.101, 0.489)	0.297 (-0.017, 0.612)	0.088
WCST, number of categories completed [B,L]				
25th quantile	1.634 (-2.645, 5.913)	-0.100 (-0.204, 0.005)	-0.339 (-0.804, 0.126)	0.152
50th quantile	0.755 (-2.173, 3.682)	-0.123 (-0.219, -0.027)	-0.299 (-0.685, 0.088)	0.129
75th quantile	-0.831 (-3.449, 1.787)	-0.019 (-0.117, 0.080)	-0.412 (-0.903, 0.079)	0.099
WCST, failure to maintain set [C,H]	1.130 (0.677, 1.885)	1.469 (0.898, 2.403)	1.23 (0.700, 2.162)	0.335
Visual and Spatial Function				
Block Design Subtest, total score [A,L]	4.333 (1.421, 7.244)	-3.881 (-6.89, -0.871)	1.569 (-1.592, 4.731)	0.531
Digit Symbol Coding, total score [A,L]	0.151 (-0.524, 0.826)	0.458 (-0.240, 1.155)	1.167 (0.434, 1.899)	0.399
Reaction Time				
Reaction time (dominant hand)† [A,H]	0.032 (-0.034, 0.097)	0.112 (0.045, 0.180)	0.085 (0.014, 0.157)	0.148
Affective State				
STAI, state anxiety t-score [A,H]	-1.833 (-4.978, 1.313)	0.361 (-2.890, 3.612)	-0.060 (-3.476, 3.355)	0.464
STAI, trait anxiety t-score† [A,H]	-0.027 (-0.082, 0.029)	-0.037 (-0.095, 0.020)	-0.041 (-0.101, 0.019)	0.494
Motor Function				

Table B-3: Individual and joint effects* of free thyroxine (ng/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Free Thyroxine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
FTT (dominant hand), average score [A,L]	-1.237 (-3.363, 0.890)	-2.172 (-4.368, 0.025)	-1.875 (-4.184, 0.434)	0.239
FTT (non-dominant hand), average score [A,L]	-2.616 (-4.388, -0.843)	-2.024 (-3.826, -0.223)	-2.607 (-4.532, -0.682)	0.059
GPT (dominant hand), time to completion† [A,H]	-0.004 (-0.069, 0.061)	0.206 (0.139, 0.274)	0.177 (0.106, 0.247)	0.508
GPT (non-dominant hand), time to completion† [A,H]	-0.004 (-0.083, 0.076)	0.173 (0.092, 0.253)	0.178 (0.092, 0.265)	0.847
SMST (dominant hand), total number of contacts† [A,H]	0.243 (-0.040, 0.526)	0.286 (-0.018, 0.589)	0.289 (-0.018, 0.596)	0.179
SMST (dominant hand), total time touching† [A,H]	0.148 (-0.055, 0.352)	0.159 (-0.059, 0.377)	0.201 (-0.02, 0.423)	0.409
SMST (non-dominant hand), total number of contacts† [A,H]	0.183 (-0.100, 0.465)	0.237 (-0.050, 0.524)	0.203 (-0.108, 0.514)	0.210
SMST (non-dominant hand), total time touching† [A,H]	0.223 (-0.139, 0.585)	0.210 (-0.158, 0.579)	0.370 (-0.029, 0.768)	0.772

Abbreviations: CVLT, California Verbal Learning Test; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; *Adjusted for age, sex, education, cigarette smoking, and Beck Depression Inventory; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; C: Negative binomial regression; M1 = Individual effect of free thyroxine (i.e., change in a neuropsychological test score per IQR increase in free thyroxine among reference age group); M2 = Individual effect of age (i.e., change in a neuropsychological test score per IQR increase in age among reference free thyroxine group); J = Joint effect of free thyroxine and age (i.e., change in neuropsychological test score per concurrent IQR increment in both free thyroxine and age); p = p-value of a product term between free thyroxine and age

Table B-4: Individual and joint effects* of total triiodothyronine (ng/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Total Triiodothyronine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
Memory and Learning				
CVLT, t-score [A,L]	-0.229 (-3.541, 3.084)	2.077 (-1.531, 5.685)	2.085 (-1.888, 6.058)	0.920
CVLT, trial 1 score [A,L]	0 (-0.55, 0.551)	-0.752 (-1.364, -0.14)	-0.403 (-1.076, 0.269)	0.375
CVLT, short delay free recall [A,L]	-0.23 (-1.101, 0.641)	-1.005 (-1.973, -0.038)	-1.11 (-2.174, -0.046)	0.840
CVLT, long delay free recall [A,L]	0.097 (-0.795, 0.989)	-0.738 (-1.729, 0.253)	-0.916 (-2.005, 0.173)	0.666
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	-7.683 (-17.164, 1.798)	6.382 (-4.153, 16.916)	0.916 (-10.665, 12.497)	0.743
CVLT, semantic cluster ratio [A,L]	-0.064 (-0.302, 0.174)	0.068 (-0.196, 0.332)	-0.002 (-0.292, 0.289)	0.973
CVLT, learning slope [A,L]	0.079 (-0.098, 0.255)	0.126 (-0.07, 0.322)	-0.087 (-0.302, 0.129)	0.022
CVLT, perseverations† [A,H]	-0.129 (-0.4, 0.143)	-0.084 (-0.385, 0.218)	-0.225 (-0.556, 0.106)	0.948
CVLT, discriminability [B,L]				
25th quantile	-1.184 (-4.785, 2.418)	-3.322 (-6.859, 0.216)	-4.9 (-16.043, 6.244)	0.846
50th quantile	-1.151 (-2.71, 0.408)	-2.899 (-4.855, -0.944)	-2.536 (-8.893, 3.821)	0.294
75th quantile	-0.477 (-2.341, 1.386)	-1.886 (-3.997, 0.224)	0.057 (-6.57, 6.684)	0.073
CVLT, recognition hits vs. long delay free recall [B,L]				
25th quantile	5.23 (-8.778, 19.238)	13.5 (-0.364, 27.364)	16.32 (-26.27, 58.909)	0.746
50th quantile	-0.463 (-12.85, 11.923)	5.164 (-9.263, 19.591)	13.383 (-29.376, 56.142)	0.283
75th quantile	-1.324 (-28.866, 26.22)	9.929 (-24.96, 44.818)	10.921 (-83.896, 105.739)	0.888
WMS, logical memory immediate recall score [A,L]	-1.816 (-3.292, -0.34)	-2.045 (-3.685, -0.405)	-1.856 (-3.658, -0.053)	0.058
WMS, logical memory delayed recall score	-1.436 (-2.808, -0.064)	-1.638 (-3.163, -0.114)	-1.635 (-3.311, 0.041)	0.143

Table B-4: Individual and joint effects* of total triiodothyronine (ng/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Total Triiodothyronine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
[A,L]				
WMS, visual reproduction immediate recall score [A,L]	-0.125 (-0.995, 0.746)	-1.158 (-2.125, -0.191)	-1.062 (-2.125, 0.001)	0.722
WMS, visual reproduction delayed recall score [A,L]	-0.136 (-1.052, 0.781)	-1.605 (-2.623, -0.587)	-1.76 (-2.88, -0.641)	0.976
Measures of Attention				
Trail Making Test Part A-time to complete† [A,H]	-0.001 (-0.086, 0.084)	0.138 (0.044, 0.233)	0.129 (0.024, 0.233)	0.889
Trail Making Test Part B-time to complete† [A,H]	-0.017 (-0.112, 0.079)	0.141 (0.035, 0.248)	0.114 (-0.004, 0.231)	0.872
Executive Function				
Stroop Color Word Test, t-score [A,L]	-0.012 (-2.166, 2.142)	1.731 (-0.657, 4.119)	3.005 (0.325, 5.684)	0.413
WCST, perseverative errors† [A,H]	-0.039 (-0.255, 0.176)	0.243 (0.004, 0.482)	0.523 (0.246, 0.801)	0.042
WCST, perseverative responses† [A,H]	-0.052 (-0.287, 0.182)	0.228 (-0.032, 0.487)	0.537 (0.235, 0.838)	0.034
WCST, number of categories completed [B,L]				
25^{th} quantile	0.116 (-0.982, 1.213)	-1.103 (-2.149, -0.057)	-1.514 (-5.168, 2.139)	0.491
50^{th} quantile	0.137 (-0.714, 0.988)	-1.728 (-2.65, -0.805)	-1.876 (-4.642, 0.89)	0.571
75^{th} quantile	0.11 (-0.327, 0.548)	-0.227 (-1.11, 0.655)	-0.732 (-2.938, 1.474)	0.172
WCST, failure to maintain set [C,H]	1.004 (0.662, 1.525)	1.2 (0.755, 1.908)	1.383 (0.834, 2.293)	0.642
Visual and Spatial Function				
Block Design Subtest, total score [A,L]	1.483 (-1.181, 4.148)	-2.745 (-5.706, 0.216)	-1.852 (-5.107, 1.403)	0.756
Digit Symbol Coding, total score [A,L]	0.618 (-2.323, 3.558)	-5.578 (-8.845, -2.311)	-5.075 (-8.666, -1.483)	0.957
Reaction Time				
Reaction time (dominant hand)† [A,H]	-0.038 (-0.094, 0.018)	0.067 (0.003, 0.13)	0.049 (-0.02, 0.117)	0.610
Affective State				

Table B-4: Individual and joint effects* of total triiodothyronine (ng/dL) and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual Total Triiodothyronine Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
STAI, state anxiety t-score [A,H]	0.172 (-2.539, 2.883)	1.25 (-1.762, 4.262)	1.277 (-2.034, 4.588)	0.940
STAI, trait anxiety t-score† [A,H]	0.019 (-0.029, 0.068)	-0.006 (-0.058, 0.047)	-0.022 (-0.082, 0.038)	0.292
Motor Function				
FTT (dominant hand), average score [A,L]	-0.116 (-1.947, 1.715)	-1.647 (-3.68, 0.387)	-0.948 (-3.185, 1.289)	0.533
FTT (non-dominant hand), average score [A,L]	-0.602 (-2.035, 0.83)	-0.633 (-2.225, 0.958)	-1.304 (-3.056, 0.447)	0.946
GPT (dominant hand), time to completion† [A,H]	-0.014 (-0.07, 0.042)	0.182 (0.12, 0.244)	0.185 (0.116, 0.253)	0.689
GPT (non-dominant hand), time to completion† [A,H]	0.039 (-0.028, 0.107)	0.209 (0.135, 0.282)	0.184 (0.103, 0.266)	0.185
SMST (dominant hand), total number of contacts† [A,H]	-0.07 (-0.305, 0.165)	0.104 (-0.148, 0.357)	-0.037 (-0.314, 0.24)	0.661
SMST (dominant hand), total time touching† [A,H]	-0.093 (-0.265, 0.08)	0.133 (-0.061, 0.327)	-0.05 (-0.262, 0.163)	0.465
SMST (non-dominant hand), total number of contacts† [A,H]	-0.057 (-0.296, 0.183)	0.155 (-0.116, 0.426)	0.004 (-0.289, 0.297)	0.585
SMST (non-dominant hand), total time touching† [A,H]	0.141 (-0.165, 0.446)	0.348 (0.002, 0.693)	0.138 (-0.236, 0.511)	0.113

Abbreviations: CVLT, California Verbal Learning Test; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; *Adjusted for age, sex, education, cigarette smoking, and Beck Depression Inventory; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; C: Negative binomial regression; M1= Individual effect of total triiodothyronine (i.e., change in a neuropsychological test score per IQR increase in total triiodothyronine among reference age group); M2 = Individual effect of age (i.e., change in a neuropsychological test score per IQR increase in age among reference total triiodothyronine group); J = Joint effect of total triiodothyronine and age (i.e., change in neuropsychological test score per concurrent IQR increment in both total triiodothyronine and age); p = p-value of a product term between total triiodothyronine and age

Table B-5: Individual and joint effects* of thyroid stimulating hormone (TSH) (μIU/mL)† and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual TSH Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
Memory and Learning				
CVLT, t-score [A,L]	1.79 (-1.077, 4.657)	2.704 (-0.665, 6.072)	3.096 (-0.583, 6.775)	0.513
CVLT, trial 1 score [A,L]	-0.012 (-0.491, 0.467)	-0.799 (-1.374, -0.225)	-0.351 (-0.971, 0.269)	0.212
CVLT, short delay free recall [A,L]	0.578 (-0.176, 1.332)	-0.882 (-1.785, 0.022)	-0.512 (-1.488, 0.464)	0.72
CVLT, long delay free recall [A,L]	0.694 (-0.077, 1.464)	-0.654 (-1.577, 0.27)	-0.547 (-1.545, 0.45)	0.434
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	5.314 (-2.99, 13.617)	7.273 (-2.677, 17.223)	13.047 (2.303, 23.792)	0.942
CVLT, semantic cluster ratio [A,L]	0.127 (-0.109, 0.363)	0.096 (-0.157, 0.348)	0.178 (-0.093, 0.448)	0.791
CVLT, learning slope [A,L]	0.072 (-0.085, 0.228)	0.063 (-0.125, 0.25)	-0.04 (-0.243, 0.162)	0.148
CVLT, perseverations† [A,H]	0.023 (-0.215, 0.26)	-0.18 (-0.46, 0.1)	0.061 (-0.248, 0.369)	0.229
CVLT, discriminability [B,L]				
25th quantile	2.561 (0.062, 5.059)	-3.125 (-6.712, 0.462)	-1.174 (-11.491, 9.143)	0.776
50th quantile	1.349 (-0.695, 3.394)	-2.242 (-5.036, 0.552)	-1.832 (-10.209, 6.545)	0.6
75th quantile	0.847 (-1.045, 2.74)	-1.024 (-3.331, 1.282)	-0.574 (-7.26, 6.112)	0.752
CVLT, recognition hits vs. long delay free recall [B,L]				
25th quantile	-5.13 (-14.498, 4.239)	10.541 (-3.887, 24.968)	4.983 (-30.438, 40.404)	0.942
50th quantile	-7.587 (-19.832, 4.657)	10.895 (-2.64, 24.43)	-0.053 (-43.702, 43.596)	0.71
75th quantile	1.758 (-20.493, 24.009)	20.392 (-5.119, 45.904)	0.911 (-80.061, 81.884)	0.208
WMS, logical memory immediate recall score [A,L]	0.928 (-0.382, 2.238)	-0.794 (-2.364, 0.776)	-0.53 (-2.225, 1.165)	0.51
WMS, logical memory delayed recall score	-0.113 (-1.331, 1.105)	-1.024 (-2.483, 0.436)	-0.842 (-2.419, 0.734)	0.753

Table B-5: Individual and joint effects* of thyroid stimulating hormone (TSH) (μIU/mL)† and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual TSH Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
[A,L]				
WMS, visual reproduction immediate recall score [A,L]	-0.242 (-0.998, 0.515)	-0.911 (-1.817, -0.004)	-1.428 (-2.407, -0.449)	0.636
WMS, visual reproduction delayed recall score [A,L]	0.083 (-0.712, 0.877)	-1.262 (-2.214, -0.31)	-1.961 (-2.988, -0.933)	0.202
Measures of Attention				
Trail Making Test Part A-time to complete† [A,H]	0.008 (-0.075, 0.091)	0.155 (0.066, 0.244)	0.104 (0.008, 0.199)	0.317
Trail Making Test Part B-time to complete† [A,H]	0.016 (-0.067, 0.1)	0.145 (0.045, 0.245)	0.142 (0.032, 0.251)	0.758
Executive Function				
Stroop Color Word Test, t-score [A,L]	-0.035 (-2.299, 2.229)	2.931 (0.556, 5.305)	3.149 (0.544, 5.754)	0.874
WCST, perseverative errors† [A,H]	-0.125 (-0.32, 0.069)	0.329 (0.097, 0.56)	0.323 (0.068, 0.578)	0.427
WCST, perseverative responses† [A,H]	-0.112 (-0.324, 0.1)	0.333 (0.081, 0.585)	0.334 (0.056, 0.611)	0.492
WCST, number of categories completed [B,L]				
25th quantile	0.356 (-0.629, 1.341)	-1.57 (-2.514, -0.625)	-0.894 (-4.131, 2.342)	0.63
50th quantile	0.564 (0.038, 1.091)	-1.609 (-2.339, -0.878)	-1.02 (-2.986, 0.945)	0.946
75th quantile	--	--	--	--
WCST, failure to maintain set [C,H]	0.861 (0.569, 1.302)	1.204 (0.775, 1.871)	1.208 (0.738, 1.976)	0.593
Visual and Spatial Function				
Block Design Subtest, total score [A,L]	1.044 (-1.596, 3.684)	-3.321 (-6.145, -0.496)	-1.584 (-4.61, 1.442)	0.716
Digit Symbol Coding, total score [A,L]	-0.993 (-3.744, 1.759)	-5.485 (-8.43, -2.541)	-4.866 (-8.01, -1.722)	0.414
Reaction Time				
Reaction time (dominant hand)† [A,H]	0.01 (-0.039, 0.059)	0.07 (0.011, 0.129)	0.096 (0.032, 0.16)	0.673
Affective State				
STAI, state anxiety t-score [A,H]	0.288 (-2.057, 2.633)	2.275 (-0.535, 5.085)	0.059 (-2.975, 3.094)	0.166

Table B-5: Individual and joint effects* of thyroid stimulating hormone (TSH) (μIU/mL)† and age on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual TSH Effect (β (CI))[M1]	Individual Age Effect (β (CI))[M2]	Joint Effect (β (CI))[J]	p[p]
STAI, trait anxiety t-score† [A,H]	0.035 (-0.006, 0.077)	-0.01 (-0.059, 0.04)	-0.011 (-0.065, 0.042)	0.245
Motor Function				
FTT (dominant hand), average score [A,L]	0.743 (-0.86, 2.346)	-0.99 (-2.871, 0.891)	-1.041 (-3.109, 1.027)	0.511
FTT (non-dominant hand), average score [A,L]	1.255 (-0.099, 2.608)	-0.343 (-1.94, 1.255)	-0.42 (-2.179, 1.34)	0.197
GPT (dominant hand), time to completion† [A,H]	0.002 (-0.054, 0.058)	0.217 (0.159, 0.276)	0.155 (0.09, 0.219)	0.103
GPT (non-dominant hand), time to completion† [A,H]	0.027 (-0.038, 0.091)	0.21 (0.141, 0.279)	0.129 (0.055, 0.202)	0.019
SMST (dominant hand), total number of contacts† [A,H]	-0.111 (-0.324, 0.102)	0.14 (-0.116, 0.396)	0.05 (-0.229, 0.329)	0.895
SMST (dominant hand), total time touching† [A,H]	-0.062 (-0.214, 0.09)	0.142 (-0.04, 0.325)	-0.013 (-0.212, 0.186)	0.425
SMST (non-dominant hand), total number of contacts† [A,H]	-0.045 (-0.258, 0.168)	0.152 (-0.1, 0.404)	0.024 (-0.254, 0.302)	0.607
SMST (non-dominant hand), total time touching† [A,H]	0 (-0.272, 0.272)	0.266 (-0.057, 0.589)	0.06 (-0.296, 0.416)	0.32

Abbreviations: CVLT, California Verbal Learning Test; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; *Adjusted for age, sex, education, cigarette smoking, and Beck Depression Inventory; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; C: Negative binomial regression; M1 = Individual effect of TSH (i.e., change in a neuropsychological test score per IQR increase in TSH among reference age group); M2 = Individual effect of age (i.e., change in a neuropsychological test score per IQR increase in age among reference TSH group); J = Joint effect of TSH and age (i.e., change in neuropsychological test score per concurrent IQR increment in both TSH and age); p = p-value of a product term between TSH and age

6.3 Appendix C: Supplementary Results for Chapter 4

Mediation Analysis Complete Results: Standard Error Estimation Using Delta Method

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
Memory and Learning						
CVLT, t-score [A,L]	0.241 (-0.377, 0.859)	0.445	0.842 (-2.566, 4.251)	0.628	1.150 (-2.274, 4.574)	0.510
	0.245 (-0.958, 1.448)	0.690	0.905 (-2.689, 4.499)	0.622		
	-0.427 (-1.242, 0.388)	0.304	1.543 (-1.905, 4.991)	0.380		
	0.208 (-0.422, 0.838)	0.518	0.963 (-2.416, 4.343)	0.576		
CVLT, trial 1 score [A,L]	0.053 (-0.073, 0.180)	0.409	-0.235 (-0.793, 0.323)	0.409	-0.182 (-0.748, 0.385)	0.530
	-0.013 (-0.21, 0.185)	0.901	-0.169 (-0.769, 0.431)	0.581		
	-0.055 (-0.182, 0.072)	0.398	-0.127 (-0.701, 0.447)	0.665		
	0.016 (-0.047, 0.080)	0.615	-0.198 (-0.764, 0.368)	0.493		
CVLT, short delay free recall [A,L]	0.077 (-0.112, 0.266)	0.424	0.036 (-0.887, 0.958)	0.940	0.113 (-0.819, 1.045)	0.813
	0.038 (-0.288, 0.364)	0.818	0.074 (-0.912, 1.061)	0.883		
	-0.211 (-0.504, 0.083)	0.160	0.323 (-0.596, 1.243)	0.491		
	0.035 (-0.086, 0.156)	0.572	0.078 (-0.852, 1.008)	0.870		
CVLT, long delay free recall [A,L]	0.055 (-0.096, 0.206)	0.477	-0.005 (-0.945, 0.935)	0.992	0.05 (-0.892, 0.992)	0.917
	0.034 (-0.295, 0.363)	0.840	0.016 (-0.981, 1.013)	0.975		
	-0.207 (-0.499, 0.085)	0.164	0.257 (-0.674, 1.188)	0.588		
	0.023 (-0.075, 0.122)	0.644	0.027 (-0.916, 0.969)	0.956		
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	0.545 (-0.912, 2.002)	0.464	-3.040 (-11.686, 5.606)	0.491	-2.495 (-11.173, 6.183)	0.573
	-0.638 (-3.688, 2.412)	0.682	-1.857 (-11.038, 7.324)	0.692		
	-0.321 (-2.076, 1.434)	0.720	-2.174 (-11.013, 6.666)	0.630		

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	-0.275 (-1.297, 0.748)	0.598	-2.220 (-10.892, 6.452)	0.616		
CVLT, semantic cluster ratio [A,L]	-0.004 (-0.029, 0.021)	0.744	0.134 (-0.113, 0.381)	0.287	0.171 (-0.080, 0.422)	0.181
	-0.023 (-0.112, 0.066)	0.611	0.194 (-0.071, 0.459)	0.152		
	-0.020 (-0.074, 0.034)	0.472	0.191 (-0.064, 0.446)	0.142		
	0.001 (-0.019, 0.022)	0.891	0.17 0(-0.082, 0.421)	0.187		
CVLT, learning slope [A,L]	-0.012 (-0.046, 0.022)	0.491	-0.007 (-0.183, 0.169)	0.937	-0.005 (-0.185, 0.175)	0.957
	0.011 (-0.052, 0.075)	0.724	-0.016 (-0.207, 0.174)	0.866		
	-0.018 (-0.059, 0.023)	0.387	0.013 (-0.170, 0.196)	0.889		
	0.000 (-0.015, 0.014)	0.961	-0.005 (-0.186, 0.176)	0.960		
CVLT, perseverations† [A,H]	0.009 (-0.027, 0.045)	0.620	-0.179 (-0.472, 0.113)	0.229	-0.17 (-0.462, 0.121)	0.252
	-0.025 (-0.128, 0.077)	0.630	-0.145 (-0.453, 0.163)	0.356		
	-0.015 (-0.075, 0.045)	0.628	-0.155 (-0.452, 0.141)	0.304		
	0.010 (-0.026, 0.045)	0.591	-0.180 (-0.471, 0.111)	0.226		
WMS, logical memory delayed recall score [A,L]	0.004 (-0.153, 0.161)	0.960	-0.243 (-1.765, 1.279)	0.755	-0.239 (-1.752, 1.275)	0.757
	0.039 (-0.489, 0.568)	0.884	-0.278 (-1.881, 1.325)	0.734		
	-0.108 (-0.43, 0.214)	0.511	-0.131 (-1.669, 1.408)	0.868		
	0.01 (-0.114, 0.134)	0.876	-0.249 (-1.767, 1.270)	0.748		
WMS, logical memory immediate recall score [A,L]	0.049 (-0.142, 0.239)	0.615	-1.232 (-2.786, 0.322)	0.120	-1.183 (-2.732, 0.366)	0.134
	0.038 (-0.503, 0.578)	0.892	-1.221 (-2.861, 0.420)	0.145		
	-0.143 (-0.487, 0.201)	0.415	-1.040 (-2.611, 0.531)	0.194		
	-0.004 (-0.129, 0.120)	0.944	-1.179 (-2.732, 0.375)	0.137		
WMS, visual reproduction delayed recall score [A,L]	0.007 (-0.087, 0.101)	0.889	0.611 (-0.294, 1.515)	0.186	0.617 (-0.283, 1.517)	0.179

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	0.065 (-0.251, 0.382)	0.685	0.552 (-0.400, 1.504)	0.256		
	-0.038 (-0.221, 0.145)	0.686	0.655 (-0.261, 1.572)	0.161		
	-0.039 (-0.167, 0.089)	0.553	0.656 (-0.240, 1.553)	0.151		
WMS, visual reproduction immediate recall score [A,L]	-0.019 (-0.117, 0.080)	0.713	0.405 (-0.474, 1.284)	0.366	0.387 (-0.488, 1.262)	0.386
	0.144 (-0.173, 0.460)	0.374	0.243 (-0.679, 1.165)	0.605		
	0.012 (-0.162, 0.186)	0.889	0.374 (-0.518, 1.266)	0.411		
	-0.043 (-0.181, 0.094)	0.537	0.430 (-0.439, 1.300)	0.332		
Measures of Attention Trail Making Test Part A-time to complete† [A,H]	-0.011 (-0.032, 0.010)	0.305	0.028 (-0.061, 0.118)	0.535	-0.001 (-0.092, 0.091)	0.99
	0.016 (-0.017, 0.049)	0.344	-0.017 (-0.113, 0.080)	0.735		
	0.006 (-0.013, 0.025)	0.533	-0.007 (-0.1, 0.086)	0.888		
	0.001 (-0.007, 0.008)	0.831	-0.001 (-0.093, 0.090)	0.976		
Trail Making Test Part B-time to complete† [A,H]	-0.001 (-0.012, 0.009)	0.836	0.057 (-0.041, 0.155)	0.256	0.056 (-0.042, 0.154)	0.260
	0.024 (-0.013, 0.061)	0.210	0.032 (-0.070, 0.135)	0.535		
	0.002 (-0.017, 0.022)	0.805	0.054 (-0.047, 0.154)	0.294		
	-0.004 (-0.016, 0.009)	0.574	0.059 (-0.039, 0.157)	0.235		
Executive Function						
Stroop Color Word Test, t-score [A,L]	-0.062 (-0.337, 0.212)	0.656	-1.711 (-4.050, 0.628)	0.152	-1.773 (-4.103, 0.557)	0.136
	0.722 (-0.195, 1.639)	0.123	-2.495 (-4.916, -0.075)	0.043		
	0.380 (-0.239, 0.998)	0.229	-2.153 (-4.489, 0.183)	0.071		
	0.141 (-0.288, 0.571)	0.518	-1.915 (-4.220, 0.390)	0.103		

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
WCST, perseverative errors† [A,H]	-0.014 (-0.053, 0.025)	0.475	-0.246 (-0.480, -0.012)	0.039	-0.259 (-0.494, -0.024)	0.031
	-0.011 (-0.083, 0.061)	0.768	-0.248 (-0.492, -0.004)	0.047		
	-0.007 (-0.047, 0.032)	0.711	-0.252 (-0.489, -0.015)	0.037		
	0.005 (-0.032, 0.043)	0.788	-0.261 (-0.493, -0.029)	0.028		
WCST, perseverative responses† [A,H]	-0.011 (-0.046, 0.024)	0.538	-0.283 (-0.538, -0.029)	0.029	-0.293 (-0.548, -0.039)	0.024
	-0.009 (-0.086, 0.069)	0.827	-0.285 (-0.550, -0.020)	0.035		
	-0.006 (-0.048, 0.036)	0.778	-0.288 (-0.545, -0.031)	0.028		
	0.005 (-0.034, 0.045)	0.788	-0.296 (-0.547, -0.044)	0.021		
Visual and Spatial Function						
Block Design Subtest, total score [A,L]	0.409 (-0.299, 1.118)	0.257	1.886 (-0.915, 4.687)	0.187	2.643 (-0.149, 5.436)	0.064
	1.349 (0.093, 2.606)	0.035	1.294 (-1.523, 4.111)	0.368		
	0.895 (-0.221, 2.011)	0.116	1.749 (-0.913, 4.410)	0.198		
	0.054 (-0.214, 0.322)	0.691	2.589 (-0.209, 5.388)	0.070		
Digit Symbol Coding, total score [A,L]	-0.179 (-0.657, 0.299)	0.464	0.493 (-2.346, 3.331)	0.734	-0.57 (-3.106, 1.966)	0.660
	0.109 (-0.725, 0.943)	0.797	-0.679 (-3.347, 1.988)	0.618		
	0.265 (-0.369, 0.898)	0.413	0.049 (-2.840, 2.938)	0.973		
	0.127 (-0.289, 0.543)	0.549	0.187 (-2.650, 3.023)	0.897		
Reaction Time						
Reaction time (dominant hand)† [A,H]	0.003 (-0.006, 0.012)	0.500	0.014 (-0.043, 0.072)	0.627	0.017 (-0.041, 0.074)	0.567
	-0.015 (-0.038, 0.007)	0.171	0.032 (-0.028, 0.092)	0.291		
	-0.002 (-0.014, 0.009)	0.686	0.020 (-0.039, 0.078)	0.511		

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	-0.005 (-0.019, 0.009)	0.497	0.023 (-0.033, 0.079)	0.419		
Affective State						
BDI, total score [A,H]	-0.039 (-0.187, 0.110)	0.610	0.952 (-0.250, 2.155)	0.121	0.914 (-0.285, 2.112)	0.135
	0.336 (-0.126, 0.799)	0.154	0.578 (-0.672, 1.827)	0.365		
	0.151 (-0.138, 0.439)	0.306	0.763 (-0.447, 1.973)	0.217		
	0.043 (-0.109, 0.196)	0.577	0.870 (-0.326, 2.067)	0.154		
STAI, state anxiety t-score [A,H]	-0.171 (-0.623, 0.281)	0.459	1.742 (-0.886, 4.370)	0.194	1.571 (-1.068, 4.210)	0.243
	0.117 (-0.806, 1.040)	0.804	1.454 (-1.340, 4.248)	0.308		
	0.005 (-0.518, 0.529)	0.984	1.566 (-1.125, 4.256)	0.254		
	0.081 (-0.224, 0.386)	0.604	1.490 (-1.148, 4.128)	0.268		
STAI, trait anxiety t-score† [A,H]	0.000 (-0.005, 0.006)	0.900	0.016 (-0.035, 0.068)	0.533	0.017 (-0.035, 0.068)	0.522
	-0.005 (-0.022, 0.013)	0.615	0.021 (-0.033, 0.076)	0.440		
	0.001 (-0.010, 0.012)	0.872	0.023 (-0.033, 0.079)	0.427		
	-0.001 (-0.006, 0.004)	0.755	0.024 (-0.031, 0.080)	0.386		
Motor Function						
FTT (dominant hand), average score [A,L]	0.224 (-0.288, 0.735)	0.391	-1.065 (-2.929, 0.799)	0.263	-0.837 (-2.753, 1.078)	0.392
	-0.226 (-0.909, 0.458)	0.518	-0.612 (-2.640, 1.416)	0.554		
	-0.357 (-0.899, 0.185)	0.196	-0.478 (-2.389, 1.433)	0.624		
	-0.040 (-0.229, 0.148)	0.676	-0.800 (-2.718, 1.119)	0.414		
FTT (non-dominant hand), average score [A,L]	0.117 (-0.175, 0.409)	0.433	-0.324 (-1.833, 1.185)	0.674	-0.200 (-1.722, 1.321)	0.796
	-0.391 (-0.972, 0.189)	0.186	0.191 (-1.403, 1.785)	0.814		
	-0.363 (-0.859, 0.132)	0.151	0.162 (-1.334, 1.658)	0.832		
	-0.070 (-0.297, 0.157)	0.545	-0.134 (-1.649, 1.380)	0.862		

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
GPT (dominant hand), time to completion† [A,H]	0.000 (-0.006, 0.005)	0.893	-0.030 (-0.081, 0.022)	0.254	-0.030 (-0.081, 0.021)	0.246
	-0.007 (-0.026, 0.011)	0.440	-0.023 (-0.077, 0.031)	0.404		
	-0.005 (-0.017, 0.007)	0.418	-0.020 (-0.069, 0.029)	0.426		
	0.000 (-0.004, 0.004)	0.950	-0.03 (-0.082, 0.021)	0.246		
GPT (non-dominant hand), time to completion† [A,H]	0.000 (-0.007, 0.007)	0.977	-0.041 (-0.110, 0.027)	0.237	-0.033 (-0.09, 0.025)	0.268
	0.004 (-0.016, 0.025)	0.660	-0.037 (-0.097, 0.023)	0.230		
	0.008 (-0.006, 0.022)	0.266	-0.041 (-0.098, 0.017)	0.167		
	-0.001 (-0.007, 0.005)	0.793	-0.041 (-0.109, 0.027)	0.24		
SMST (dominant hand), total number of contacts† [A,H]	-0.023 (-0.079, 0.032)	0.409	0.012 (-0.231, 0.255)	0.922	-0.011 (-0.258, 0.236)	0.930
	0.001 (-0.085, 0.087)	0.985	-0.012 (-0.274, 0.250)	0.929		
	0.032 (-0.028, 0.092)	0.293	-0.043 (-0.293, 0.206)	0.733		
	0.002 (-0.018, 0.023)	0.818	-0.014 (-0.261, 0.234)	0.914		
SMST (dominant hand), total time touching† [A,H]	-0.019 (-0.064, 0.025)	0.400	-0.037 (-0.218, 0.144)	0.686	-0.057 (-0.241, 0.128)	0.548
	0.010 (-0.055, 0.074)	0.768	-0.066 (-0.262, 0.129)	0.506		
	0.024 (-0.021, 0.068)	0.298	-0.080 (-0.266, 0.106)	0.398		
	0.001 (-0.014, 0.016)	0.920	-0.057 (-0.242, 0.128)	0.544		
SMST (non-dominant hand), total number of contacts† [A,H]	-0.018 (-0.063, 0.027)	0.440	0.049 (-0.193, 0.290)	0.692	0.031 (-0.212, 0.274)	0.802
	-0.022 (-0.108, 0.064)	0.611	0.053 (-0.204, 0.311)	0.685		
	0.021 (-0.032, 0.075)	0.434	0.010 (-0.237, 0.257)	0.938		
	-0.006 (-0.030, 0.019)	0.661	0.037 (-0.207, 0.280)	0.769		
SMST (non-dominant hand),	-0.020 (-0.073, 0.034)	0.472	-0.001 (-0.329, 0.326)	0.993	-0.021 (-0.349, 0.307)	0.900

Table C-1: Thyroid, non-thyroid, and total effects* of PFOS on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
total time touching† [A,H]						
	-0.013 (-0.128, 0.102)	0.826	-0.008 (-0.356, 0.339)	0.963		
	0.050 (-0.035, 0.135)	0.245	-0.071 (-0.401, 0.258)	0.671		
	-0.006 (-0.038, 0.025)	0.690	-0.015 (-0.344, 0.314)	0.930		

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; WCST, Wisconsin Card Sorting Test; BDI, Beck Depression Inventory; STAI, State Trait Anxiety Inventory; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test, SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, and serum total PCB (lipid basis); †Log-natural transformed, H: High score=Impairment, L: Low Score=Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
Memory and Learning						
CVLT, t-score [A,L]	0.247 (-0.297, 0.791)	0.373	2.199 (-0.539, 4.937)	0.115	2.458 (-0.271, 5.188)	0.078
	0.070 (-0.532, 0.673)	0.819	2.388 (-0.401, 5.177)	0.093		
	-0.186 (-0.669, 0.298)	0.451	2.635 (-0.078, 5.347)	0.057		
	0.295 (-0.299, 0.890)	0.330	2.155 (-0.583, 4.892)	0.123		
CVLT, trial 1 score [A,L]	0.055 (-0.050, 0.161)	0.303	0.217 (-0.240, 0.675)	0.352	0.273 (-0.187, 0.733)	0.244
	-0.030 (-0.133, 0.074)	0.574	0.303 (-0.167, 0.772)	0.206		
	-0.029 (-0.106, 0.048)	0.465	0.302 (-0.156, 0.760)	0.197		
	0.018 (-0.061, 0.098)	0.649	0.255 (-0.211, 0.720)	0.283		
CVLT, short delay free recall [A,L]	0.074 (-0.080, 0.228)	0.346	0.794 (0.057, 1.531)	0.035	0.868 (0.131, 1.605)	0.021
	-0.015 (-0.175, 0.145)	0.853	0.883 (0.129, 1.637)	0.022		
	-0.095 (-0.311, 0.121)	0.387	0.963 (0.251, 1.675)	0.008		
	0.037 (-0.093, 0.167)	0.578	0.831 (0.086, 1.576)	0.029		
CVLT, long delay free recall [A,L]	0.052 (-0.084, 0.188)	0.457	0.595 (-0.166, 1.355)	0.126	0.646 (-0.11, 1.402)	0.094
	-0.011 (-0.174, 0.153)	0.899	0.657 (-0.117, 1.430)	0.096		
	-0.092 (-0.303, 0.119)	0.391	0.739 (0.005, 1.472)	0.048		
	0.023 (-0.105, 0.150)	0.729	0.624 (-0.142, 1.389)	0.110		
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	1.013 (-0.924, 2.950)	0.305	-8.015 (-16.517, 0.487)	0.065	-7.002 (-15.496, 1.491)	0.106
	-0.337 (-1.646, 0.973)	0.614	-6.665 (-15.227, 1.897)	0.127		
	-0.078 (-0.683, 0.526)	0.800	-6.924 (-15.405, 1.557)	0.110		
	-0.356 (-1.508, 0.796)	0.545	-6.647 (-15.143, 1.850)	0.125		
CVLT, semantic cluster ratio [A,L]	-0.007 (-0.037, 0.023)	0.659	0.156 (-0.045, 0.357)	0.129	0.178 (-0.025, 0.382)	0.086

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	-0.009 (-0.054, 0.036)	0.683	0.188 (-0.020, 0.395)	0.077		
	-0.007 (-0.033, 0.019)	0.580	0.186 (-0.018, 0.389)	0.075		
	-0.001 (-0.034, 0.033)	0.975	0.179 (-0.028, 0.385)	0.089		
CVLT, learning slope [A,L]	-0.016 (-0.049, 0.016)	0.327	0.046 (-0.098, 0.190)	0.531	0.039 (-0.108, 0.186)	0.602
	0.003 (-0.029, 0.035)	0.832	0.036 (-0.115, 0.186)	0.642		
	-0.008 (-0.031, 0.015)	0.491	0.047 (-0.100, 0.194)	0.530		
	-0.002 (-0.026, 0.023)	0.889	0.041 (-0.108, 0.190)	0.591		
CVLT, perseverations† [A,H]	0.007 (-0.030, 0.045)	0.710	0.059 (-0.183, 0.301)	0.635	0.066 (-0.174, 0.305)	0.590
	-0.026 (-0.083, 0.032)	0.385	0.091 (-0.152, 0.335)	0.462		
	-0.010 (-0.042, 0.022)	0.554	0.075 (-0.164, 0.315)	0.538		
	0.013 (-0.030, 0.056)	0.558	0.053 (-0.189, 0.295)	0.668		
WMS, logical memory delayed recall score [A,L]	-0.002 (-0.189, 0.184)	0.981	0.180 (-1.070, 1.430)	0.778	0.178 (-1.059, 1.414)	0.778
	-0.003 (-0.271, 0.264)	0.981	0.181 (-1.084, 1.446)	0.779		
	-0.051 (-0.218, 0.116)	0.550	0.229 (-1.009, 1.467)	0.717		
	0.009 (-0.194, 0.213)	0.928	0.168 (-1.085, 1.421)	0.792		
WMS, logical memory immediate recall score [A,L]	0.040 (-0.162, 0.242)	0.698	0.115 (-1.18, 1.411)	0.862	0.155 (-1.127, 1.437)	0.813
	-0.059 (-0.343, 0.224)	0.682	0.214 (-1.096, 1.525)	0.749		
	-0.081 (-0.297, 0.135)	0.464	0.236 (-1.042, 1.513)	0.718		
	-0.025 (-0.237, 0.188)	0.821	0.180 (-1.120, 1.479)	0.786		
WMS, visual reproduction delayed recall score [A,L]	0.014 (-0.100, 0.128)	0.813	0.085 (-0.666, 0.836)	0.825	0.099 (-0.645, 0.842)	0.795
	0.064 (-0.109, 0.237)	0.467	0.034 (-0.723, 0.792)	0.929		
	-0.007 (-0.084, 0.071)	0.866	0.105 (-0.642, 0.852)	0.782		

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	-0.062 (-0.208, 0.085)	0.409	0.160 (-0.588, 0.909)	0.674		
WMS, visual reproduction immediate recall score [A,L]	-0.015 (-0.126, 0.095)	0.785	-0.067 (-0.792, 0.659)	0.857	-0.082 (-0.8, 0.636)	0.823
	0.097 (-0.085, 0.279)	0.297	-0.179 (-0.907, 0.548)	0.629		
	0.012 (-0.066, 0.091)	0.757	-0.095 (-0.816, 0.627)	0.797		
	-0.067 (-0.215, 0.080)	0.371	-0.015 (-0.736, 0.707)	0.968		
Measures of Attention						
Trail Making Test Part A-time to complete† [A,H]	-0.008 (-0.024, 0.008)	0.324	0.047 (-0.025, 0.118)	0.199	0.041 (-0.033, 0.115)	0.280
	0.006 (-0.011, 0.023)	0.497	0.035 (-0.041, 0.111)	0.365		
	0.002 (-0.007, 0.011)	0.639	0.039 (-0.036, 0.113)	0.307		
	0.000 (-0.012, 0.012)	0.961	0.041 (-0.035, 0.116)	0.290		
Trail Making Test Part B-time to complete† [A,H]	-0.001 (-0.013, 0.011)	0.885	0.012 (-0.071, 0.095)	0.776	0.012 (-0.071, 0.094)	0.779
	0.014 (-0.008, 0.037)	0.211	-0.003 (-0.085, 0.080)	0.948		
	0.002 (-0.007, 0.011)	0.693	0.009 (-0.074, 0.092)	0.831		
	-0.006 (-0.021, 0.010)	0.455	0.016 (-0.066, 0.099)	0.697		
Executive Function Stroop Color Word Test, t-score [A,L]	-0.062 (-0.363, 0.238)	0.684	-1.481 (-3.400, 0.437)	0.130	-1.544 (-3.443, 0.355)	0.111
	0.320 (-0.199, 0.838)	0.227	-1.864 (-3.778, 0.051)	0.056		
	0.146 (-0.22, 0.5110)	0.435	-1.689 (-3.573, 0.194)	0.079		
	0.262 (-0.203, 0.727)	0.269	-1.806 (-3.698, 0.086)	0.061		
WCST, perseverative errors† [A,H]	-0.023 (-0.071, 0.025)	0.348	-0.074 (-0.298, 0.151)	0.519	-0.097 (-0.321, 0.127)	0.398
	-0.012 (-0.049, 0.025)	0.514	-0.084 (-0.309, 0.140)	0.462		

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	-0.002 (-0.020, 0.016)	0.795	-0.094 (-0.318, 0.129)	0.407		
	0.016 (-0.026, 0.057)	0.457	-0.108 (-0.330, 0.113)	0.338		
WCST, perseverative responses† [A,H]	-0.018 (-0.065, 0.029)	0.447	-0.094 (-0.339, 0.151)	0.453	-0.112 (-0.355, 0.131)	0.366
	-0.012 (-0.052, 0.027)	0.537	-0.100 (-0.344, 0.144)	0.423		
	-0.002 (-0.020, 0.015)	0.799	-0.110 (-0.353, 0.133)	0.374		
	0.017 (-0.027, 0.061)	0.460	-0.125 (-0.366, 0.116)	0.310		
Visual and Spatial Function						
Block Design Subtest, total score [A,L]	0.332 (-0.250, 0.913)	0.264	-0.516 (-2.790, 1.757)	0.656	-0.23 (-2.559, 2.098)	0.846
	0.828 (-0.134, 1.789)	0.092	-1.058 (-3.280, 1.164)	0.351		
	0.419 (-0.502, 1.340)	0.373	-0.649 (-2.810, 1.512)	0.556		
	0.127 (-0.290, 0.544)	0.551	-0.357 (-2.712, 1.998)	0.766		
Digit Symbol Coding, total score [A,L]	-0.221 (-0.695, 0.252)	0.359	0.709 (-1.62, 3.038)	0.551	0.458 (-1.593, 2.509)	0.662
	0.003 (-0.446, 0.453)	0.988	0.454 (-1.645, 2.554)	0.672		
	0.110 (-0.224, 0.445)	0.518	0.377 (-1.948, 2.703)	0.750		
	0.099 (-0.263, 0.461)	0.592	0.359 (-1.716, 2.433)	0.735		
Reaction Time Reaction time (dominant hand)† [A,H]	0.004 (-0.005, 0.013)	0.387	-0.015 (-0.062, 0.032)	0.529	-0.011 (-0.058, 0.036)	0.646
	-0.006 (-0.018, 0.006)	0.318	-0.005 (-0.052, 0.043)	0.839		
	-0.001 (-0.006, 0.004)	0.808	-0.010 (-0.057, 0.037)	0.665		
	-0.008 (-0.021, 0.005)	0.231	-0.003 (-0.049, 0.043)	0.892		
Affective State						
BDI, total score [A,H]	-0.044 (-0.206, 0.118)	0.596	0.453 (-0.545, 1.451)	0.374	0.409 (-0.579, 1.398)	0.417

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	0.200 (-0.091, 0.491)	0.179	0.210 (-0.780, 1.199)	0.678		
	0.074 (-0.113, 0.260)	0.439	0.336 (-0.646, 1.317)	0.502		
	0.072 (-0.116, 0.261)	0.451	0.337 (-0.660, 1.334)	0.508		
STAI, state anxiety t-score [A,H]	-0.210 (-0.654, 0.234)	0.354	1.323 (-0.839, 3.486)	0.230	1.113 (-1.047, 3.273)	0.312
	0.099 (-0.378, 0.576)	0.686	1.015 (-1.193, 3.222)	0.368		
	0.016 (-0.208, 0.241)	0.887	1.097 (-1.074, 3.268)	0.322		
	0.124 (-0.266, 0.514)	0.534	0.989 (-1.193, 3.172)	0.374		
STAI, trait anxiety t-score† [A,H]	0.000 (-0.006, 0.007)	0.915	0.003 (-0.043, 0.048)	0.913	0.009 (-0.034, 0.051)	0.691
	-0.002 (-0.012, 0.008)	0.709	0.011 (-0.033, 0.054)	0.634		
	0.001 (-0.004, 0.006)	0.769	0.002 (-0.043, 0.048)	0.925		
	-0.002 (-0.010, 0.005)	0.565	0.011 (-0.032, 0.053)	0.619		
Motor Function						
FTT (dominant hand), average score [A,L]	0.266 (-0.183, 0.716)	0.245	-0.644 (-2.182, 0.893)	0.411	-0.379 (-1.949, 1.191)	0.636
	-0.143 (-0.511, 0.224)	0.445	-0.235 (-1.833, 1.362)	0.773		
	-0.159 (-0.533, 0.215)	0.405	-0.220 (-1.760, 1.319)	0.779		
	-0.068 (-0.340, 0.205)	0.626	-0.309 (-1.897, 1.278)	0.703		
FTT (non-dominant hand), average score [A,L]	0.126 (-0.134, 0.386)	0.342	0.362 (-0.877, 1.601)	0.567	0.485 (-0.754, 1.724)	0.443
	-0.225 (-0.573, 0.123)	0.205	0.710 (-0.534, 1.954)	0.263		
	-0.160 (-0.522, 0.202)	0.387	0.646 (-0.55, 1.842)	0.290		
	-0.134 (-0.403, 0.135)	0.329	0.621 (-0.620, 1.863)	0.327		
GPT (dominant hand), time to completion† [A,H]	-0.001 (-0.007, 0.006)	0.824	-0.004 (-0.046, 0.039)	0.872	-0.004 (-0.046, 0.038)	0.844
	-0.005 (-0.016, 0.005)	0.333	0.001 (-0.042, 0.044)	0.964		

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	-0.002 (-0.009, 0.004)	0.471	-0.002 (-0.041, 0.038)	0.937		
	0.000 (-0.007, 0.007)	0.999	-0.004 (-0.047, 0.038)	0.846		
GPT (non-dominant hand), time to completion† [A,H]	0.000 (-0.007, 0.007)	0.994	-0.008 (-0.056, 0.040)	0.743	-0.008 (-0.055, 0.039)	0.739
	0.001 (-0.010, 0.012)	0.874	-0.009 (-0.057, 0.039)	0.718		
	0.004 (-0.005, 0.012)	0.418	-0.012 (-0.058, 0.035)	0.629		
	-0.004 (-0.012, 0.005)	0.437	-0.004 (-0.052, 0.043)	0.853		
SMST (dominant hand), total number of contacts† [A,H]	-0.028 (-0.079, 0.022)	0.274	0.043 (-0.157, 0.242)	0.675	0.014 (-0.187, 0.216)	0.889
	-0.001 (-0.045, 0.043)	0.967	0.015 (-0.191, 0.222)	0.884		
	0.013 (-0.022, 0.048)	0.459	0.001 (-0.200, 0.202)	0.990		
	0.004 (-0.030, 0.037)	0.832	0.011 (-0.194, 0.215)	0.918		
SMST (dominant hand), total time touching† [A,H]	-0.024 (-0.066, 0.017)	0.254	0.044 (-0.104, 0.192)	0.561	0.02 (-0.131, 0.171)	0.796
	0.000 (-0.032, 0.033)	0.978	0.019 (-0.135, 0.174)	0.805		
	0.009 (-0.015, 0.033)	0.478	0.011 (-0.139, 0.162)	0.885		
	0.000 (-0.025, 0.025)	0.991	0.02 (-0.133, 0.173)	0.800		
SMST (non-dominant hand), total number of contacts† [A,H]	-0.027 (-0.081, 0.026)	0.313	-0.033 (-0.271, 0.204)	0.782	-0.061 (-0.298, 0.176)	0.614
	-0.012 (-0.050, 0.026)	0.537	-0.049 (-0.287, 0.189)	0.688		
	0.003 (-0.017, 0.022)	0.794	-0.064 (-0.300, 0.173)	0.598		
	-0.009 (-0.041, 0.022)	0.555	-0.051 (-0.288, 0.185)	0.67		
SMST (non-dominant hand), total time touching† [A,H]	-0.032 (-0.099, 0.036)	0.358	-0.040 (-0.360, 0.280)	0.806	-0.072 (-0.390, 0.247)	0.660
	-0.013 (-0.063, 0.036)	0.602	-0.058 (-0.380, 0.263)	0.721		

Table C-2: Thyroid, non-thyroid, and total effects* of PFOA on the neuropsychological tests (n=87) using delta method

Neuropsychological Tests	Thyroid Effect	p	Non-thyroid Effect	p	Total Effect	p
	0.006 (-0.039, 0.052)	0.782	-0.078 (-0.394, 0.238)	0.628		
	-0.012 (-0.054, 0.029)	0.561	-0.059 (-0.378, 0.260)	0.716		

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; WCST, Wisconsin Card Sorting Test; BDI, Beck Depression Inventory; STAI, State Trait Anxiety Inventory; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test, SMST, Static Motor Steadiness Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, and serum total PCB (lipid basis); †Log-natural transformed, H: High score=Impairment, L: Low Score=Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

Mediation Analysis Complete Results: Standard Error Estimation Using Bootstrapping

Table C- 3: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
Memory and Learning				
CVLT, t-score [A,L]	M1	0.369 (-0.233, 1.954)	0.935 (-3.187, 4.808)	1.359 (-2.703, 5.079)
	M2	-0.455 (-1.547, 0.239)	1.251 (-3.013, 5.800)	
	M3	0.108 (-1.596, 1.472)	1.788 (-2.341, 5.577)	
	M4	0.235 (-0.480, 1.220)	1.153 (-2.824, 4.897)	
CVLT, trial 1 score [A,L]	M1	0.069 (-0.058, 0.343)	-0.236 (-0.825, 0.292)	-0.166 (-0.764, 0.368)
	M2	-0.059 (-0.223, 0.044)	-0.149 (-0.754, 0.400)	
	M3	-0.017 (-0.233, 0.198)	-0.107 (-0.714, 0.460)	
	M4	0.029 (-0.039, 0.170)	-0.195 (-0.775, 0.358)	
CVLT, short delay free recall [A,L]	M1	0.107 (-0.058, 0.499)	0.033 (-0.913, 0.934)	0.141 (-0.787, 1.027)
	M2	-0.223 (-0.585, 0.015)	0.145 (-0.901, 1.315)	
	M3	-0.005 (-0.469, 0.329)	0.364 (-0.655, 1.336)	
	M4	0.032 (-0.123, 0.237)	0.109 (-0.840, 1.049)	
CVLT, long delay free recall [A,L]	M1	0.082 (-0.048, 0.430)	0.020 (-1.078, 1.109)	0.102 (-1.003, 1.173)
	M2	-0.223 (-0.585, 0.009)	0.105 (-1.082, 1.367)	
	M3	-0.002 (-0.409, 0.293)	0.325 (-0.791, 1.386)	
	M4	0.013 (-0.161, 0.158)	0.090 (-1.008, 1.192)	
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	M1	0.471 (-1.086, 2.762)	-3.010 (-11.435, 5.203)	-2.539 (-10.778, 5.18)
	M2	-0.318 (-2.215, 1.374)	-1.836 (-10.745, 6.705)	
	M3	-0.703 (-4.322, 2.538)	-2.221 (-10.654, 5.624)	
	M4	-0.396 (-2.802, 0.850)	-2.143 (-10.686, 5.660)	
CVLT, semantic cluster ratio [A,L]	M1	0.005 (-0.043, 0.074)	0.129 (-0.121, 0.348)	0.177 (-0.043, 0.401)
	M2	-0.021 (-0.091, 0.039)	0.209 (-0.011, 0.447)	
	M3	-0.032 (-0.151, 0.058)	0.198 (-0.015, 0.422)	

Table C- 3: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M4	0.000 (-0.041, 0.041)	0.177 (-0.047, 0.404)	
CVLT, learning slope [A,L]	M1	-0.015 (-0.073, 0.023)	0.001 (-0.172, 0.169)	0.001 (-0.168, 0.178)
	M2	-0.018 (-0.074, 0.019)	-0.004 (-0.185, 0.202)	
	M3	0.004 (-0.082, 0.070)	0.019 (-0.150, 0.191)	
	M4	-0.006 (-0.048, 0.019)	0.007 (-0.164, 0.192)	
CVLT, perseverations† [A,H]	M1	0.010 (-0.039, 0.084)	-0.181 (-0.459, 0.085)	-0.171 (-0.457, 0.110)
	M2	-0.019 (-0.102, 0.043)	-0.134 (-0.424, 0.144)	
	M3	-0.037 (-0.180, 0.058)	-0.153 (-0.441, 0.126)	
	M4	0.012 (-0.030, 0.072)	-0.183 (-0.465, 0.084)	
WMS, logical memory delayed recall score [A,L]	M1	-0.006 (-0.275, 0.248)	-0.200 (-1.611, 1.200)	-0.206 (-1.583, 1.154)
	M2	-0.103 (-0.547, 0.223)	-0.210 (-1.665, 1.372)	
	M3	0.004 (-0.586, 0.591)	-0.102 (-1.454, 1.358)	
	M4	-0.007 (-0.264, 0.202)	-0.199 (-1.539, 1.234)	
WMS, logical memory immediate recall score [A,L]	M1	0.055 (-0.165, 0.431)	-1.211 (-2.583, 0.173)	-1.155 (-2.530, 0.264)
	M2	-0.146 (-0.587, 0.192)	-1.156 (-2.550, 0.363)	
	M3	0.001 (-0.647, 0.590)	-1.009 (-2.384, 0.497)	
	M4	-0.015 (-0.327, 0.243)	-1.140 (-2.514, 0.281)	
WMS, visual reproduction delayed recall score [A,L]	M1	0.003 (-0.198, 0.217)	0.620 (-0.286, 1.383)	0.623 (-0.252, 1.402)
	M2	-0.035 (-0.277, 0.181)	0.544 (-0.458, 1.411)	
	M3	0.079 (-0.265, 0.504)	0.658 (-0.287, 1.436)	
	M4	-0.050 (-0.274, 0.095)	0.672 (-0.239, 1.510)	
WMS, visual reproduction immediate recall score [A,L]	M1	-0.006 (-0.193, 0.199)	0.389 (-0.338, 1.039)	0.383 (-0.302, 1.041)
	M2	0.005 (-0.240, 0.243)	0.229 (-0.586, 1.042)	
	M3	0.154 (-0.193, 0.566)	0.378 (-0.397, 1.061)	

Table C- 3: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M4	-0.051 (-0.269, 0.108)	0.435 (-0.305, 1.108)	
Measures of Attention				
Trail Making Test Part A-time to complete† [A,H]	M1	-0.010 (-0.033, 0.006)	0.030 (-0.042, 0.101)	0.003 (-0.079, 0.081)
	M2	0.006 (-0.015, 0.032)	-0.013 (-0.104, 0.069)	
	M3	0.016 (-0.014, 0.053)	-0.003 (-0.096, 0.079)	
	M4	0.001 (-0.014, 0.019)	0.002 (-0.081, 0.083)	
Trail Making Test Part B-time to complete† [A,H]	M1	-0.004 (-0.025, 0.010)	0.062 (-0.031, 0.166)	0.059 (-0.034, 0.167)
	M2	0.002 (-0.020, 0.030)	0.033 (-0.065, 0.145)	
	M3	0.026 (-0.015, 0.072)	0.057 (-0.041, 0.164)	
	M4	-0.004 (-0.027, 0.011)	0.063 (-0.032, 0.173)	
Executive Function				
Stroop Color Word Test, t-score [A,L]	M1	-0.044 (-0.554, 0.408)	-1.635 (-4.582, 1.043)	-1.679 (-4.577, 1.030)
	M2	0.417 (-0.057, 1.276)	-2.407 (-5.280, 0.417)	
	M3	0.728 (-0.092, 1.814)	-2.095 (-4.918, 0.491)	
	M4	0.155 (-0.310, 0.756)	-1.834 (-4.593, 0.782)	
WCST, perseverative errors† [A,H]	M1	-0.013 (-0.067, 0.024)	-0.237 (-0.455, -0.006)	-0.248 (-0.476, -0.011)
	M2	-0.005 (-0.061, 0.050)	-0.239 (-0.483, 0.020)	
	M3	-0.009 (-0.085, 0.065)	-0.243 (-0.478, 0.008)	
	M4	0.009 (-0.032, 0.064)	-0.254 (-0.485, -0.010)	
WCST, perseverative responses† [A,H]	M1	-0.009 (-0.062, 0.032)	-0.275 (-0.512, -0.031)	-0.283 (-0.529, -0.029)
	M2	-0.004 (-0.066, 0.055)	-0.277 (-0.547, -0.006)	
	M3	-0.006 (-0.087, 0.077)	-0.280 (-0.533, -0.018)	
	M4	0.009 (-0.037, 0.067)	-0.289 (-0.540, -0.033)	
Visual and Spatial Function				
Block Design Subtest, total score [A,L]	M1	0.162 (-0.164, 0.968)	2.514 (-0.492, 5.673)	2.676 (0.021, 5.736)
	M2	0.906 (-0.014, 2.239)	1.242 (-1.829, 4.303)	
	M3	1.434 (0.240, 2.959)	1.770 (-1.057, 4.682)	

Table C- 3: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M4	0.098 (-0.363, 0.771)	2.578 (-0.237, 5.568)	
Digit Symbol Coding, total score [A,L]	M1	-0.119 (-0.819, 0.413)	0.457 (-2.526, 3.826)	-0.526 (-3.22, 2.163)
	M2	0.254 (-0.334, 0.972)	-0.538 (-3.301, 2.662)	
	M3	0.012 (-1.047, 0.819)	0.084 (-2.842, 3.322)	
	M4	0.153 (-0.247, 0.835)	0.185 (-2.616, 3.314)	
Reaction Time				
Reaction time (dominant hand)† [A,H]	M1	0.003 (-0.005, 0.018)	0.012 (-0.042, 0.076)	0.015 (-0.041, 0.076)
	M2	-0.002 (-0.015, 0.011)	0.029 (-0.031, 0.091)	
	M3	-0.014 (-0.040, 0.010)	0.018 (-0.038, 0.079)	
	M4	-0.005 (-0.022, 0.011)	0.021 (-0.033, 0.080)	
Affective State				
BDI, total score [A,H]	M1	-0.046 (-0.294, 0.126)	0.906 (-0.237, 1.878)	0.860 (-0.285, 1.818)
	M2	0.143 (-0.121, 0.555)	0.519 (-0.719, 1.59)	
	M3	0.341 (-0.080, 0.926)	0.718 (-0.451, 1.697)	
	M4	0.050 (-0.131, 0.315)	0.81 (-0.353, 1.797)	
STAI, state anxiety t-score [A,H]	M1	-0.213 (-1.039, 0.216)	1.682 (-1.432, 4.46)	1.469 (-1.636, 4.195)
	M2	-0.003 (-0.723, 0.707)	1.450 (-1.910, 4.700)	
	M3	0.019 (-1.194, 1.056)	1.472 (-1.66, 4.397)	
	M4	0.007 (-0.521, 0.438)	1.462 (-1.695, 4.336)	
STAI, trait anxiety t-score† [A,H]	M1	-0.001 (-0.013, 0.006)	0.018 (-0.037, 0.071)	0.017 (-0.037, 0.072)
	M2	0.001 (-0.012, 0.015)	0.022 (-0.040, 0.083)	
	M3	-0.006 (-0.031, 0.015)	0.022 (-0.035, 0.077)	
	M4	-0.001 (-0.013, 0.009)	0.023 (-0.033, 0.075)	
Motor Function				
FTT (dominant hand), average score [A,L]	M1	0.290 (-0.201, 1.256)	-1.085 (-2.93, 0.813)	-0.789 (-2.771, 1.330)
	M2	-0.372 (-1.108, 0.073)	-0.532 (-2.725, 1.810)	
	M3	-0.257 (-1.214, 0.552)	-0.416 (-2.450, 1.709)	

Table C- 3: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M4	-0.084 (-0.561, 0.188)	-0.708 (-2.696, 1.414)	
FTT (non-dominant hand), average score [A,L]	M1	0.137 (-0.146, 0.657)	-0.339 (-1.785, 1.016)	-0.193 (-1.546, 1.198)
	M2	-0.382 (-1.021, 0.028)	0.253 (-1.193, 1.798)	
	M3	-0.446 (-1.273, 0.103)	0.187 (-1.228, 1.575)	
	M4	-0.118 (-0.553, 0.113)	-0.081 (-1.452, 1.346)	
GPT (dominant hand), time to completion† [A,H]	M1	0.000 (-0.010, 0.008)	-0.032 (-0.086, 0.009)	-0.033 (-0.085, 0.005)
	M2	-0.005 (-0.020, 0.005)	-0.025 (-0.083, 0.017)	
	M3	-0.008 (-0.033, 0.015)	-0.021 (-0.064, 0.015)	
	M4	0.001 (-0.007, 0.010)	-0.033 (-0.089, 0.006)	
GPT (non-dominant hand), time to completion† [A,H]	M1	-0.001 (-0.017, 0.008)	-0.041 (-0.106, 0.007)	-0.033 (-0.08, 0.006)
	M2	0.007 (-0.006, 0.026)	-0.037 (-0.086, 0.008)	
	M3	0.005 (-0.020, 0.030)	-0.040 (-0.087, 0.000)	
	M4	-0.001 (-0.014, 0.010)	-0.041 (-0.104, 0.003)	
SMST (dominant hand), total number of contacts† [A,H]	M1	-0.025 (-0.121, 0.029)	0.008 (-0.275, 0.247)	-0.017 (-0.287, 0.212)
	M2	0.033 (-0.016, 0.104)	-0.021 (-0.304, 0.209)	
	M3	0.004 (-0.095, 0.118)	-0.050 (-0.317, 0.177)	
	M4	0.007 (-0.034, 0.064)	-0.024 (-0.304, 0.202)	
SMST (dominant hand), total time touching† [A,H]	M1	-0.019 (-0.085, 0.028)	-0.045 (-0.261, 0.142)	-0.064 (-0.272, 0.107)
	M2	0.025 (-0.011, 0.081)	-0.076 (-0.286, 0.099)	
	M3	0.011 (-0.070, 0.102)	-0.089 (-0.299, 0.089)	
	M4	0.004 (-0.025, 0.042)	-0.068 (-0.271, 0.103)	
SMST (non-dominant hand), total number of contacts† [A,H]	M1	-0.019 (-0.086, 0.025)	0.045 (-0.241, 0.342)	0.026 (-0.265, 0.328)
	M2	0.021 (-0.029, 0.082)	0.047 (-0.252, 0.336)	

Table C- 3: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M3	-0.022 (-0.118, 0.071)	0.004 (-0.289, 0.293)	
	M4	-0.001 (-0.043, 0.044)	0.027 (-0.260, 0.316)	
SMST (non-dominant hand), total time touching† [A,H]	M1	-0.021 (-0.101, 0.033)	-0.005 (-0.363, 0.355)	-0.025 (-0.376, 0.327)
	M2	0.048 (-0.016, 0.129)	-0.008 (-0.368, 0.345)	
	M3	-0.017 (-0.156, 0.100)	-0.073 (-0.428, 0.269)	
	M4	-0.004 (-0.058, 0.048)	-0.021 (-0.371, 0.328)	

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; WCST, Wisconsin Card Sorting Test; BDI, Beck Depression Inventory; STAI, State Trait Anxiety Inventory; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test, SMST, Static Motor Steadiness Test; CI, 95 Percentile Confidence Intervals; PFOS, Perfluorooctane Sulfonate; *Adjusted for age, sex, education, cigarette, and serum total PCB (lipid basis); †Log-natural transformed, H: High score=Impairment, L: Low Score=Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

Table C-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
Memory and Learning				
CVLT, t-score [A,L]	M1	0.322 (-0.149, 1.493)	2.172 (-0.665, 4.869)	2.504 (-0.413, 5.516)
	M2	0.066 (-0.576, 0.864)	2.438 (-0.545, 5.492)	
	M3	-0.146 (-0.764, 0.497)	2.639 (-0.296, 5.464)	
	M4	0.275 (-0.27, 1.024)	2.222 (-0.729, 5.259)	
CVLT, trial 1 score [A,L]	M1	0.060 (-0.031, 0.237)	0.241 (-0.169, 0.783)	0.302 (-0.14, 0.899)
	M2	-0.026 (-0.129, 0.067)	0.328 (-0.111, 0.913)	
	M3	-0.024 (-0.115, 0.06)	0.326 (-0.112, 0.897)	
	M4	0.024 (-0.047, 0.129)	0.278 (-0.162, 0.866)	
CVLT, short delay free recall [A,L]	M1	0.089 (-0.032, 0.391)	0.794 (0.080, 1.520)	0.883 (0.110, 1.651)
	M2	-0.017 (-0.204, 0.158)	0.900 (0.112, 1.673)	
	M3	-0.078 (-0.321, 0.212)	0.960 (0.228, 1.628)	
	M4	0.036 (-0.091, 0.202)	0.847 (0.086, 1.608)	
CVLT, long delay free recall [A,L]	M1	0.068 (-0.044, 0.346)	0.557 (-0.366, 1.261)	0.626 (-0.260, 1.303)
	M2	-0.013 (-0.160, 0.139)	0.639 (-0.288, 1.352)	
	M3	-0.073 (-0.305, 0.195)	0.698 (-0.188, 1.326)	
	M4	0.013 (-0.120, 0.149)	0.612 (-0.293, 1.340)	
CVLT, proactive interference (list B adjusted for trial 1) [A,L]	M1	0.949 (-0.851, 3.729)	-7.607 (-13.820, -0.646)	-6.657 (-12.916, 0.342)
	M2	-0.384 (-2.489, 0.875)	-6.273 (-12.854, 0.564)	
	M3	-0.046 (-1.104, 1.004)	-6.611 (-12.872, 0.040)	
	M4	-0.489 (-2.648, 0.678)	-6.169 (-12.859, 0.665)	
CVLT, semantic cluster ratio [A,L]	M1	-0.003 (-0.049, 0.049)	0.150 (-0.094, 0.345)	0.176 (-0.031, 0.352)
	M2	-0.010 (-0.063, 0.039)	0.186 (-0.030, 0.366)	
	M3	-0.003 (-0.042, 0.038)	0.179 (-0.027, 0.353)	
	M4	-0.002 (-0.043, 0.036)	0.178 (-0.026, 0.356)	
CVLT, learning slope [A,L]	M1	-0.016 (-0.068, 0.015)	0.053 (-0.067, 0.208)	0.045 (-0.056, 0.179)

Table C-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M2	0.002 (-0.036, 0.040)	0.043 (-0.059, 0.180)	
	M3	-0.006 (-0.039, 0.022)	0.051 (-0.052, 0.181)	
	M4	-0.002 (-0.033, 0.029)	0.048 (-0.054, 0.178)	
CVLT, perseverations† [A,H]	M1	0.008 (-0.034, 0.067)	0.07 (-0.142, 0.324)	0.078 (-0.128, 0.341)
	M2	-0.026 (-0.101, 0.028)	0.104 (-0.116, 0.381)	
	M3	-0.011 (-0.067, 0.027)	0.089 (-0.127, 0.345)	
	M4	0.014 (-0.025, 0.069)	0.065 (-0.154, 0.329)	
WMS, logical memory delayed recall score [A,L]	M1	-0.004 (-0.254, 0.219)	0.174 (-1.275, 1.494)	0.17 (-1.321, 1.495)
	M2	-0.003 (-0.308, 0.331)	0.173 (-1.444, 1.592)	
	M3	-0.025 (-0.246, 0.189)	0.195 (-1.345, 1.459)	
	M4	-0.010 (-0.274, 0.196)	0.180 (-1.320, 1.580)	
WMS, logical memory immediate recall score [A,L]	M1	0.044 (-0.176, 0.356)	0.135 (-1.156, 1.495)	0.178 (-1.107, 1.585)
	M2	-0.051 (-0.363, 0.321)	0.229 (-1.128, 1.611)	
	M3	-0.044 (-0.281, 0.223)	0.222 (-1.113, 1.568)	
	M4	-0.033 (-0.340, 0.242)	0.211 (-1.143, 1.624)	
WMS, visual reproduction delayed recall score [A,L]	M1	0.007 (-0.183, 0.201)	0.070 (-0.773, 0.708)	0.077 (-0.748, 0.697)
	M2	0.062 (-0.102, 0.272)	0.015 (-0.802, 0.699)	
	M3	-0.009 (-0.136, 0.091)	0.087 (-0.750, 0.735)	
	M4	-0.063 (-0.289, 0.067)	0.141 (-0.699, 0.770)	
WMS, visual reproduction immediate recall score [A,L]	M1	-0.017 (-0.210, 0.150)	-0.082 (-0.719, 0.485)	-0.099 (-0.722, 0.44)
	M2	0.097 (-0.073, 0.315)	-0.196 (-0.838, 0.345)	
	M3	0.012 (-0.093, 0.142)	-0.111 (-0.724, 0.420)	
	M4	-0.065 (-0.278, 0.081)	-0.034 (-0.671, 0.491)	
Measures of Attention				

Table C-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
Trail Making Test Part A-time to complete† [A,H]	M1	-0.007 (-0.027, 0.006)	0.049 (-0.025, 0.128)	0.046 (-0.027, 0.129)
	M2	0.006 (-0.007, 0.026)	0.04 (-0.036, 0.124)	
	M3	0.001 (-0.009, 0.015)	0.045 (-0.027, 0.130)	
	M4	0.001 (-0.012, 0.017)	0.045 (-0.03, 0.128)	
Trail Making Test Part B-time to complete† [A,H]	M1	-0.001 (-0.020, 0.014)	0.017 (-0.058, 0.112)	0.017 (-0.057, 0.113)
	M2	0.014 (-0.008, 0.040)	0.004 (-0.074, 0.099)	
	M3	0.000 (-0.014, 0.015)	0.016 (-0.059, 0.115)	
	M4	-0.005 (-0.028, 0.013)	0.021 (-0.054, 0.115)	
Executive Function				
Stroop Color Word Test, t-score [A,L]	M1	-0.076 (-0.614, 0.284)	-1.386 (-3.824, 0.902)	-1.462 (-3.902, 0.863)
	M2	0.310 (-0.098, 0.969)	-1.772 (-4.148, 0.641)	
	M3	0.123 (-0.239, 0.563)	-1.585 (-3.960, 0.704)	
	M4	0.248 (-0.174, 0.753)	-1.710 (-4.132, 0.652)	
WCST, perseverative errors† [A,H]	M1	-0.022 (-0.083, 0.015)	-0.066 (-0.291, 0.156)	-0.088 (-0.311, 0.136)
	M2	-0.011 (-0.055, 0.026)	-0.077 (-0.306, 0.163)	
	M3	-0.001 (-0.035, 0.031)	-0.087 (-0.310, 0.145)	
	M4	0.016 (-0.021, 0.072)	-0.101 (-0.316, 0.134)	
WCST, perseverative responses† [A,H]	M1	-0.017 (-0.078, 0.020)	-0.086 (-0.325, 0.158)	-0.103 (-0.346, 0.142)
	M2	-0.011 (-0.058, 0.027)	-0.092 (-0.341, 0.167)	
	M3	-0.002 (-0.036, 0.032)	-0.101 (-0.341, 0.146)	
	M4	0.017 (-0.022, 0.080)	-0.117 (-0.349, 0.136)	
Visual and Spatial Function				
Block Design Subtest, total score [A,L]	M1	0.332 (-0.126, 1.088)	-0.462 (-2.522, 2.041)	-0.226 (-2.083, 1.978)
	M2	0.805 (-0.105, 1.899)	-1.031 (-2.870, 1.195)	
	M3	0.364 (-0.789, 1.354)	-0.590 (-2.432, 1.830)	

Table C-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M4	0.137 (-0.370, 0.846)	-0.363 (-2.350, 1.900)	
Digit Symbol Coding, total score [A,L]	M1	-0.227 (-0.987, 0.187)	0.794 (-1.194, 3.362)	0.547 (-1.28, 2.873)
	M2	0.000 (-0.425, 0.554)	0.547 (-1.363, 2.894)	
	M3	0.114 (-0.243, 0.645)	0.453 (-1.525, 2.907)	
	M4	0.083 (-0.253, 0.474)	0.464 (-1.361, 2.926)	
Reaction Time				
Reaction time (dominant hand)† [A,H]	M1	0.004 (-0.003, 0.021)	-0.013 (-0.059, 0.044)	-0.008 (-0.055, 0.048)
	M2	-0.006 (-0.024, 0.004)	-0.002 (-0.046, 0.051)	
	M3	-0.001 (-0.008, 0.006)	-0.008 (-0.052, 0.050)	
	M4	-0.008 (-0.027, 0.004)	0.000 (-0.043, 0.051)	
Affective State				
BDI, total score [A,H]	M1	-0.044 (-0.238, 0.105)	0.403 (-0.690, 1.193)	0.359 (-0.712, 1.101)
	M2	0.169 (-0.083, 0.451)	0.190 (-0.837, 1.030)	
	M3	0.049 (-0.182, 0.263)	0.310 (-0.734, 1.105)	
	M4	0.065 (-0.119, 0.310)	0.294 (-0.785, 1.101)	
STAI, state anxiety t-score [A,H]	M1	-0.202 (-0.829, 0.187)	1.112 (-1.738, 3.192)	0.909 (-1.898, 3.041)
	M2	0.075 (-0.509, 0.683)	0.834 (-2.035, 3.016)	
	M3	-0.006 (-0.411, 0.317)	0.915 (-1.939, 3.011)	
	M4	0.124 (-0.275, 0.690)	0.785 (-2.087, 2.929)	
STAI, trait anxiety t-score† [A,H]	M1	0.000 (-0.009, 0.009)	0.001 (-0.049, 0.035)	0.007 (-0.038, 0.038)
	M2	-0.002 (-0.016, 0.009)	0.009 (-0.036, 0.041)	
	M3	0.000 (-0.007, 0.009)	0.001 (-0.047, 0.036)	
	M4	-0.002 (-0.015, 0.007)	0.009 (-0.035, 0.041)	
Motor Function				
FTT (dominant hand), average score [A,L]	M1	0.315 (-0.047, 1.154)	-0.594 (-2.071, 1.245)	-0.279 (-1.77, 1.829)
	M2	-0.129 (-0.584, 0.247)	-0.150 (-1.722, 1.907)	
	M3	-0.143 (-0.662, 0.260)	-0.138 (-1.699, 1.844)	

Table C-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M4	-0.049 (-0.433, 0.283)	-0.228 (-1.706, 1.855)	
FTT (non-dominant hand), average score [A,L]	M1	0.150 (-0.055, 0.620)	0.357 (-0.862, 1.506)	0.502 (-0.741, 1.734)
	M2	-0.218 (-0.625, 0.080)	0.720 (-0.582, 1.900)	
	M3	-0.140 (-0.575, 0.289)	0.643 (-0.557, 1.737)	
	M4	-0.140 (-0.486, 0.114)	0.645 (-0.661, 1.916)	
GPT (dominant hand), time to completion† [A,H]	M1	-0.001 (-0.008, 0.007)	-0.004 (-0.048, 0.032)	-0.004 (-0.047, 0.029)
	M2	-0.005 (-0.018, 0.007)	0.001 (-0.041, 0.033)	
	M3	-0.002 (-0.010, 0.005)	-0.002 (-0.041, 0.029)	
	M4	0.000 (-0.009, 0.009)	-0.005 (-0.048, 0.029)	
GPT (non-dominant hand), time to completion† [A,H]	M1	0.000 (-0.012, 0.012)	-0.009 (-0.060, 0.025)	-0.009 (-0.058, 0.025)
	M2	0.001 (-0.012, 0.016)	-0.010 (-0.057, 0.024)	
	M3	0.003 (-0.007, 0.017)	-0.012 (-0.058, 0.023)	
	M4	-0.003 (-0.016, 0.007)	-0.006 (-0.055, 0.029)	
SMST (dominant hand), total number of contacts† [A,H]	M1	-0.027 (-0.102, 0.017)	0.026 (-0.224, 0.196)	-0.002 (-0.249, 0.173)
	M2	0.000 (-0.052, 0.054)	-0.001 (-0.245, 0.176)	
	M3	0.012 (-0.025, 0.062)	-0.014 (-0.250, 0.159)	
	M4	0.005 (-0.045, 0.060)	-0.007 (-0.270, 0.167)	
SMST (dominant hand), total time touching† [A,H]	M1	-0.023 (-0.077, 0.013)	0.032 (-0.137, 0.157)	0.009 (-0.159, 0.132)
	M2	0.001 (-0.040, 0.048)	0.008 (-0.163, 0.143)	
	M3	0.009 (-0.019, 0.046)	0.000 (-0.165, 0.132)	
	M4	0.001 (-0.034, 0.035)	0.008 (-0.161, 0.133)	
SMST (non-dominant hand), total number of contacts† [A,H]	M1	-0.030 (-0.118, 0.015)	-0.037 (-0.305, 0.229)	-0.067 (-0.329, 0.168)
	M2	-0.011 (-0.067, 0.034)	-0.057 (-0.321, 0.181)	

Table C-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests (n=87) using bootstrapping

Neuropsychological Tests		Thyroid Effect	Non-thyroid Effect	Total Effect
	M3	0.002 (-0.033, 0.040)	-0.069 (-0.338, 0.176)	
	M4	-0.009 (-0.058, 0.024)	-0.058 (-0.316, 0.172)	
SMST (non-dominant hand), total time touching† [A,H]	M1	-0.036 (-0.144, 0.022)	-0.040 (-0.400, 0.315)	-0.075 (-0.414, 0.271)
	M2	-0.010 (-0.079, 0.054)	-0.066 (-0.426, 0.281)	
	M3	0.007 (-0.054, 0.078)	-0.082 (-0.434, 0.278)	
	M4	-0.010 (-0.069, 0.038)	-0.065 (-0.411, 0.282)	

Abbreviations: CVLT, California Verbal Learning Test; WMS, Wechsler Memory Scale; WCST, Wisconsin Card Sorting Test; BDI, Beck Depression Inventory; STAI, State Trait Anxiety Inventory; FTT, Finger Tapping Test; GPT, Grooved Pegboard Test, SMST, Static Motor Steadiness Test; CI, 95 Percentile Confidence Intervals; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, and serum total PCB (lipid basis); †Log-natural transformed, H: High score=Impairment, L: Low Score=Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

Mediation Analysis: Allowing Interaction between PFCs and Thyroid Markers (Using Delta Method)

Table C-5: Thyroid, non-thyroid, and total effects* of PFCs on the selected neuropsychological tests allowing interaction between thyroid markers and PFCs (n=87)

Neuropsychological Tests		Non-thyroid Effect (Controlled)	p	Non-thyroid effect (Natural)#	p	Thyroid Effect	p	Total effect	p
PFOS (ng/mL)†									
Executive Function									
WCST, perseverative errors†[H]	M1	-0.260 (-0.493, -0.028)	0.028	-0.245 (-0.483, -0.008)	0.043	-0.025 (-0.086, 0.036)	0.421	-0.270 (-0.506, -0.035)	0.024
	M2	-0.258 (-0.524, 0.008)	0.057	-0.264 (-0.562, 0.034)	0.082	-0.002 (-0.117, 0.112)	0.970	-0.266 (-0.516, -0.017)	0.036
	M3	-0.288 (-0.526, -0.050)	0.018	-0.314 (-0.568, -0.061)	0.015	0.031 (-0.042, 0.104)	0.400	-0.283 (-0.522, -0.045)	0.020
	M4	-0.266 (-0.502, -0.029)	0.028	-0.266 (-0.503, -0.029)	0.028	0.006 (-0.038, 0.049)	0.789	-0.260 (-0.499, -0.021)	0.033
WCST, perseverative responses†[H]	M1	-0.300 (-0.552, -0.047)	0.020	-0.283 (-0.541, -0.024)	0.032	-0.024 (-0.083, 0.036)	0.436	-0.306 (-0.562, -0.051)	0.019
	M2	-0.290 (-0.578, -0.002)	0.048	-0.294 (-0.616, 0.029)	0.074	-0.004 (-0.128, 0.12)	0.952	-0.298 (-0.568, -0.028)	0.031
	M3	-0.324 (-0.583, -0.065)	0.014	-0.350 (-0.624, -0.075)	0.012	0.032 (-0.046, 0.11)	0.417	-0.317 (-0.576, -0.059)	0.016
	M4	-0.301 (-0.557, -0.045)	0.021	-0.301 (-0.558, -0.045)	0.021	0.006 (-0.04, 0.053)	0.789	-0.295 (-0.554, -0.036)	0.025
Visuospatial Function									
Block Design Subtest, total score[L]	M1	1.938 (-0.812, 4.687)	0.167	1.690 (-1.163, 4.543)	0.246	0.815 (-0.32, 1.949)	0.159	2.504 (-0.303, 5.312)	0.080
	M2	1.023 (-1.774, 3.821)	0.473	0.483 (-2.495, 3.46)	0.751	2.053 (0.286, 3.82)	0.023	2.536 (-0.292, 5.364)	0.079
	M3	1.729 (-0.95, 4.407)	0.206	1.660 (-1.062, 4.382)	0.232	0.987 (-0.32, 2.293)	0.139	2.647 (-0.168, 5.462)	0.065

Table C-5: Thyroid, non-thyroid, and total effects* of PFCs on the selected neuropsychological tests allowing interaction between thyroid markers and PFCs (n=87)

Neuropsychological Tests		Non-thyroid Effect (Controlled)	p	Non-thyroid effect (Natural)#	p	Thyroid Effect	p	Total effect	p
	M4	2.588 (-0.183, 5.358)	0.067	2.461 (-0.371, 5.293)	0.089	0.187 (-0.395, 0.769)	0.528	2.648 (-0.154, 5.45)	0.064
PFOA (ng/mL)†									
Memory and Learning									
CVLT, t-score [L]	M1	2.164 (-0.636, 4.964)	0.130	2.180 (-0.589, 4.949)	0.123	0.222 (-0.405, 0.85)	0.487	2.403 (-0.41, 5.215)	0.094
	M2	2.861 (-0.145, 5.867)	0.062	2.760 (-0.176, 5.696)	0.065	0.181 (-0.498, 0.86)	0.601	2.941 (-0.025, 5.907)	0.052
	M3	2.742 (-0.127, 5.611)	0.061	2.727 (-0.108, 5.562)	0.059	-0.165 (-0.646, 0.315)	0.501	2.562 (-0.337, 5.461)	0.083
	M4	2.708 (-0.106, 5.523)	0.059	2.568 (-0.232, 5.369)	0.072	0.409 (-0.309, 1.126)	0.264	2.977 (0.163, 5.791)	0.038
CVLT, short delay free recall [L]	M1	0.753 (0.001, 1.505)	0.050	0.772 (0.026, 1.518)	0.042	0.045 (-0.113, 0.203)	0.577	0.817 (0.06, 1.575)	0.034
	M2	0.973 (0.161, 1.785)	0.019	0.954 (0.161, 1.746)	0.018	0.005 (-0.168, 0.179)	0.951	0.959 (0.161, 1.757)	0.019
	M3	0.927 (0.177, 1.677)	0.015	0.932 (0.191, 1.673)	0.014	-0.102 (-0.337, 0.132)	0.392	0.830 (0.052, 1.608)	0.037
	M4	0.972 (0.207, 1.738)	0.013	0.936 (0.174, 1.697)	0.016	0.067 (-0.088, 0.221)	0.399	1.002 (0.242, 1.763)	0.010
CVLT, long delay free recall [L]	M1	0.591 (-0.187, 1.369)	0.136	0.593 (-0.176, 1.362)	0.131	0.049 (-0.116, 0.214)	0.561	0.642 (-0.137, 1.421)	0.106
	M2	0.645 (-0.19, 1.48)	0.130	0.648 (-0.166, 1.461)	0.119	-0.013 (-0.192, 0.165)	0.885	0.635 (-0.185, 1.454)	0.129
	M3	0.625 (-0.143, 1.393)	0.111	0.641 (-0.122, 1.405)	0.100	-0.115 (-0.375, 0.146)	0.387	0.526 (-0.273, 1.325)	0.197
	M4	0.709 (-0.085, 1.502)	0.080	0.687 (-0.098, 1.471)	0.086	0.04 (-0.102, 0.182)	0.577	0.727 (-0.056, 1.511)	0.069

Table C-5: Thyroid, non-thyroid, and total effects* of PFCs on the selected neuropsychological tests allowing interaction between thyroid markers and PFCs (n=87)

Neuropsychological Tests		Non-thyroid Effect (Controlled)	p	Non-thyroid effect (Natural)#	p	Thyroid Effect	p	Total effect	p
CVLT, proactive interference[L]	M1	-7.868 (-16.478, 0.743)	0.073	-7.995 (-16.554, 0.563)	0.067	1.188 (-1.23, 3.607)	0.335	-6.807 (-15.463, 1.848)	0.123
	M2	-6.081 (-14.756, 2.593)	0.169	-5.793 (-14.646, 3.059)	0.200	-0.641 (-2.347, 1.064)	0.461	-6.435 (-15.069, 2.199)	0.144
	M3	-6.662 (-15.183, 1.858)	0.125	-6.651 (-15.22, 1.918)	0.128	-0.154 (-1.27, 0.961)	0.786	-6.805 (-15.36, 1.749)	0.119
	M4	-7.022 (-15.742, 1.697)	0.114	-7.109 (-15.929, 1.711)	0.114	-0.284 (-1.387, 0.818)	0.613	-7.393 (-16.14, 1.354)	0.098
CVLT, semantic cluster ratio[L]	M1	0.169 (-0.036, 0.374)	0.107	0.163 (-0.041, 0.366)	0.117	0.002 (-0.036, 0.039)	0.934	0.165 (-0.041, 0.37)	0.116
	M2	0.210 (-0.014, 0.434)	0.066	0.205 (-0.013, 0.424)	0.066	-0.004 (-0.052, 0.044)	0.862	0.201 (-0.019, 0.421)	0.074
	M3	0.200 (-0.014, 0.415)	0.067	0.198 (-0.014, 0.411)	0.067	-0.004 (-0.031, 0.022)	0.741	0.194 (-0.022, 0.409)	0.078
	M4	0.214 (0.001, 0.426)	0.049	0.205 (-0.006, 0.416)	0.057	0.007 (-0.03, 0.043)	0.713	0.212 (0.001, 0.422)	0.049

Abbreviations: CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, and serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

Non Thyroid Effect (Controlled) = an average change in test score per IQR increase in ln PFC at the mean TH value

#Non Thyroid Effect (Natural) = an average change in test score per IQR increase in ln PFC but for each individual TH were kept at the level it would have taken when ln PFC were at the first quartile

Thyroid Effect= change in test score for those with ln PFC level at the third quartile, but if TH were changed from the level it would take if ln PFC were at the first quartile to the level it would take if ln PFC were at the third quartile

Total Effect= average change in test per IQR increase in a PFC

Mediation Analysis: Using Lipid Adjustment (Delta Method)

Table C-6: Thyroid, non-thyroid, and total effects* of PFCs on the selected neuropsychological tests (n = 87)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
PFOS (ng/mL)†							
Executive Function							
WCST, perseverative errors†[H]	M1	-0.014 (-0.053, 0.025)	0.490	-0.248 (-0.483, -0.013)	0.038	-0.260 (-0.496, -0.024)	0.031
	M2	-0.006 (-0.081, 0.069)	0.878	-0.254 (-0.501, -0.007)	0.044		
	M3	-0.005 (-0.046, 0.036)	0.809	-0.255 (-0.494, -0.016)	0.036		
	M4	0.006 (-0.033, 0.045)	0.771	-0.263 (-0.496, -0.030)	0.027		
WCST, perseverative responses†[H]	M1	-0.011 (-0.046, 0.024)	0.542	-0.285 (-0.541, -0.030)	0.028	-0.295 (-0.551, -0.039)	0.024
	M2	-0.003 (-0.085, 0.079)	0.942	-0.292 (-0.559, -0.025)	0.032		
	M3	-0.003 (-0.047, 0.041)	0.885	-0.292 (-0.550, -0.033)	0.027		
	M4	0.006 (-0.035, 0.047)	0.772	-0.298 (-0.551, -0.046)	0.021		
Visuospatial Function							
Block Design Subtest, total score[L]	M1	0.412 (-0.301, 1.126)	0.257	1.781 (-1.036, 4.597)	0.215	2.559 (-0.252, 5.37)	0.074
	M2	1.265 (0.038, 2.493)	0.043	1.294 (-1.543, 4.130)	0.371		
	M3	0.808 (-0.278, 1.893)	0.145	1.751 (-0.929, 4.432)	0.200		
	M4	0.039 (-0.193, 0.272)	0.741	2.520 (-0.297, 5.336)	0.080		
PFOA (ng/mL)†							
Memory and Learning							
CVLT, t-score[L]	M1	0.267 (-0.311, 0.846)	0.365	2.282 (-0.560, 5.123)	0.116	2.560 (-0.272, 5.392)	0.076
	M2	0.063 (-0.405, 0.531)	0.791	2.497 (-0.368, 5.362)	0.088		
	M3	-0.077 (-0.506, 0.351)	0.723	2.627 (-0.175, 5.429)	0.066		
	M4	0.258 (-0.308, 0.823)	0.372	2.292 (-0.538, 5.121)	0.112		

Table C-6: Thyroid, non-thyroid, and total effects* of PFCs on the selected neuropsychological tests (n = 87)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
CVLT, short delay free recall [L]	M1	0.082 (-0.083, 0.247)	0.331	0.710 (-0.052, 1.472)	0.068	0.792 (0.03, 1.554)	0.042
	M2	-0.024 (-0.150, 0.103)	0.714	0.816 (0.044, 1.587)	0.038		
	M3	-0.044 (-0.282, 0.193)	0.715	0.836 (0.111, 1.561)	0.024		
	M4	0.028 (-0.086, 0.142)	0.631	0.764 (-0.004, 1.532)	0.051		
CVLT, long delay free recall [L]	M1	0.054 (-0.090, 0.198)	0.462	0.588 (-0.204, 1.379)	0.146	0.641 (-0.144, 1.427)	0.109
	M2	-0.009 (-0.135, 0.118)	0.893	0.650 (-0.145, 1.446)	0.109		
	M3	-0.040 (-0.254, 0.175)	0.716	0.681 (-0.075, 1.438)	0.078		
	M4	0.019 (-0.093, 0.132)	0.736	0.622 (-0.170, 1.414)	0.124		
CVLT, proactive interference[L]	M1	0.934 (-0.938, 2.805)	0.328	-7.360 (-15.876, 1.156)	0.09	-6.427 (-14.921, 2.067)	0.138
	M2	-0.142 (-1.229, 0.945)	0.798	-6.285 (-14.842, 2.272)	0.150		
	M3	-0.012 (-0.274, 0.251)	0.929	-6.415 (-14.907, 2.077)	0.139		
	M4	-0.287 (-1.328, 0.754)	0.589	-6.140 (-14.641, 2.361)	0.157		
CVLT, semantic cluster ratio[L]	M1	-0.006 (-0.038, 0.026)	0.707	0.137 (-0.070, 0.344)	0.195	0.154 (-0.056, 0.363)	0.150
	M2	-0.012 (-0.049, 0.025)	0.541	0.165 (-0.046, 0.377)	0.126		
	M3	-0.004 (-0.029, 0.021)	0.730	0.158 (-0.050, 0.366)	0.137		
	M4	-0.002 (-0.031, 0.027)	0.887	0.156 (-0.056, 0.367)	0.148		

Abbreviations: CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, serum total PCB (wet basis), and total lipid; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator)

6.4 Appendix D: Supplementary Results for Chapter 5

Table D-1: Thyroid, non-thyroid, and total effects* of PFOS on selected neuropsychological tests among those ≤ median age of 63 years using delta method (n = 45)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
CVLT, t-score [L]	M1	0.302 (-0.778, 1.381)	0.584	-3.234 (-8.079, 1.610)	0.191	-2.991 (-7.912, 1.929)	0.233
	M2	-0.491 (-1.752, 0.770)	0.445	-2.500 (-7.397, 2.396)	0.317		
	M3	-0.469 (-1.892, 0.954)	0.518	-2.410 (-7.144, 2.323)	0.318		
	M4	-0.270 (-1.191, 0.652)	0.567	-2.474 (-7.440, 2.492)	0.329		
CVLT, long delay free recall [L]	M1	0.083 (-0.214, 0.380)	0.584	-0.61 (-1.916, 0.696)	0.360	-0.527 (-1.854, 0.801)	0.437
	M2	-0.099 (-0.407, 0.209)	0.529	-0.428 (-1.773, 0.917)	0.533		
	M3	-0.180 (-0.705, 0.346)	0.503	-0.347 (-1.579, 0.885)	0.581		
	M4	-0.096 (-0.379, 0.188)	0.508	-0.431 (-1.755, 0.893)	0.523		
CVLT, semantic cluster ratio [L]	M1	-0.003 (-0.038, 0.032)	0.849	0.010 (-0.328, 0.348)	0.954	0.007 (-0.330, 0.343)	0.969
	M2	-0.043 (-0.137, 0.052)	0.378	0.049 (-0.287, 0.386)	0.775		
	M3	-0.026 (-0.107, 0.056)	0.537	0.032 (-0.299, 0.363)	0.849		
	M4	-0.004 (-0.054, 0.045)	0.867	0.011 (-0.329, 0.351)	0.950		
CVLT, learning slope [A,L]	M1	-0.005 (-0.031, 0.021)	0.712	-0.104 (-0.309, 0.101)	0.320	-0.090 (-0.302, 0.123)	0.408
	M2	0.001 (-0.042, 0.044)	0.962	-0.091 (-0.307, 0.126)	0.412		
	M3	-0.021 (-0.085, 0.043)	0.517	-0.068 (-0.273, 0.136)	0.512		
	M4	-0.022 (-0.079, 0.035)	0.447	-0.067 (-0.276, 0.141)	0.526		
WMS, visual reproduction delayed recall score [A,L]	M1	0.058 (-0.173, 0.289)	0.623	0.179 (-1.204, 1.561)	0.800	0.237 (-1.152, 1.625)	0.739
	M2	0.077 (-0.227, 0.380)	0.621	0.160 (-1.251, 1.571)	0.824		
	M3	-0.010 (-0.169, 0.149)	0.903	0.246 (-1.151, 1.644)	0.730		
	M4	0.094 (-0.192, 0.380)	0.521	0.143 (-1.245, 1.530)	0.840		
Stroop Color Word Test, t score [A,L]	M1	-0.138 (-0.666, 0.390)	0.608	-3.425 (-6.304, -0.545)	0.020	-3.563 (-6.462, -0.664)	0.016
	M2	0.428 (-0.455, 1.310)	0.342	-3.991 (-6.867, -1.115)	0.007		
	M3	0.112 (-0.341, 0.565)	0.629	-3.675 (-6.576, -0.773)	0.013		

Table D-1: Thyroid, non-thyroid, and total effects* of PFOS on selected neuropsychological tests among those ≤ median age of 63 years using delta method (n = 45)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
	M4	-0.165 (-0.717, 0.387)	0.558	-3.398 (-6.306, -0.490)	0.022		
WCST, perseverative errors† [A,H]	M1	-0.020 (-0.092, 0.051)	0.576	-0.212 (-0.497, 0.073)	0.145	-0.232 (-0.523, 0.059)	0.118
	M2	-0.034 (-0.112, 0.045)	0.400	-0.198 (-0.491, 0.094)	0.183		
	M3	-0.014 (-0.065, 0.037)	0.591	-0.218 (-0.508, 0.072)	0.141		
	M4	0.000 (-0.042, 0.042)	0.991	-0.232 (-0.526, 0.063)	0.123		
WCST, perseverative responses† [A,H]	M1	-0.018 (-0.082, 0.047)	0.592	-0.243 (-0.555, 0.069)	0.127	-0.261 (-0.576, 0.055)	0.105
	M2	-0.036 (-0.120, 0.048)	0.405	-0.225 (-0.542, 0.092)	0.164		
	M3	-0.015 (-0.069, 0.040)	0.597	-0.246 (-0.561, 0.069)	0.126		
	M4	-0.001 (-0.046, 0.045)	0.974	-0.260 (-0.579, 0.059)	0.110		
Block Design Subtest, total score [A,L]	M1	0.937 (-0.547, 2.421)	0.216	0.539 (-3.205, 4.283)	0.778	2.189 (-1.668, 6.046)	0.266
	M2	0.236 (-0.622, 1.094)	0.590	1.953 (-1.963, 5.869)	0.328		
	M3	0.274 (-0.609, 1.157)	0.543	1.915 (-1.885, 5.716)	0.323		
	M4	-0.200 (-0.908, 0.507)	0.579	2.390 (-1.482, 6.261)	0.226		

Abbreviations: CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator); p = p-value

Table D-2: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests among those > median age of 63 years using delta method (n = 42)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
CVLT, t-score [L]	M1	0.107 (-0.494, 0.708)	0.727	5.328 (0.838, 9.818)	0.020	5.439 (0.977, 9.901)	0.017
	M2	0.872 (-1.841, 3.585)	0.529	4.567 (-0.620, 9.754)	0.084		
	M3	-0.029 (-1.062, 1.004)	0.956	5.473 (0.892, 10.055)	0.019		
	M4	0.456 (-1.474, 2.386)	0.643	4.984 (0.138, 9.829)	0.044		
CVLT, long delay free recall [L]	M1	-0.017 (-0.179, 0.146)	0.839	1.119 (-0.196, 2.434)	0.095	1.102 (-0.205, 2.409)	0.100
	M2	-0.007 (-0.807, 0.793)	0.986	1.109 (-0.423, 2.642)	0.156		
	M3	-0.097 (-0.431, 0.238)	0.571	1.199 (-0.136, 2.534)	0.078		
	M4	-0.372 (-0.994, 0.249)	0.240	1.474 (0.085, 2.864)	0.038		
CVLT, semantic cluster ratio [L]	M1	-0.006 (-0.046, 0.035)	0.777	0.315 (-0.045, 0.676)	0.087	0.380 (-0.011, 0.772)	0.057
	M2	0.021 (-0.219, 0.261)	0.865	0.359 (-0.100, 0.819)	0.125		
	M3	0.018 (-0.077, 0.113)	0.710	0.362 (-0.039, 0.764)	0.077		
	M4	-0.054 (-0.227, 0.120)	0.545	0.434 (0.009, 0.858)	0.045		
CVLT, learning slope [A,L]	M1	-0.033 (-0.131, 0.064)	0.503	0.196 (-0.062, 0.455)	0.136	0.163 (-0.110, 0.436)	0.242
	M2	-0.069 (-0.239, 0.101)	0.425	0.232 (-0.085, 0.549)	0.152		
	M3	-0.005 (-0.069, 0.060)	0.891	0.167 (-0.113, 0.448)	0.242		
	M4	-0.137 (-0.288, 0.015)	0.076	0.299 (0.025, 0.574)	0.033		
WMS, visual reproduction delayed recall score [A,L]	M1	-0.107 (-0.432, 0.218)	0.519	1.100 (-0.014, 2.213)	0.053	0.993 (-0.153, 2.138)	0.089
	M2	-0.355 (-1.073, 0.362)	0.332	1.348 (0.024, 2.672)	0.046		
	M3	-0.180 (-0.544, 0.185)	0.334	1.172 (0.025, 2.320)	0.045		
	M4	-0.301 (-0.839, 0.237)	0.273	1.294 (0.071, 2.517)	0.038		
Stroop Color Word Test, t score [A,L]	M1	0.043 (-0.426, 0.513)	0.856	0.007 (-3.828, 3.843)	0.997	0.051 (-3.759, 3.860)	0.979
	M2	0.879 (-1.483, 3.241)	0.466	-0.828 (-5.259, 3.604)	0.714		
	M3	0.518 (-0.624, 1.660)	0.374	-0.467 (-4.307, 3.373)	0.812		
	M4	0.796 (-0.945, 2.536)	0.370	-0.745 (-4.843, 3.354)	0.722		
WCST, perseverative errors† [A,H]	M1	0.005 (-0.031, 0.041)	0.796	-0.259 (-0.635, 0.118)	0.178	-0.244 (-0.620, 0.132)	0.203
	M2	0.152 (-0.056, 0.361)	0.153	-0.396 (-0.794, 0.002)	0.051		

Table D-2: Thyroid, non-thyroid, and total effects* of PFOS on the selected neuropsychological tests among those > median age of 63 years using delta method (n = 42)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
	M3	0.031 (-0.058, 0.121)	0.494	-0.279 (-0.652, 0.093)	0.141		
	M4	0.139 (-0.042, 0.320)	0.133	-0.385 (-0.749, -0.021)	0.038		
WCST, perseverative responses† [A,H]	M1	0.007 (-0.038, 0.053)	0.753	-0.316 (-0.727, 0.095)	0.131	-0.298 (-0.709, 0.112)	0.154
	M2	0.172 (-0.058, 0.401)	0.142	-0.47 (-0.904, -0.037)	0.034		
	M3	0.033 (-0.063, 0.128)	0.502	-0.337 (-0.744, 0.071)	0.105		
	M4	0.152 (-0.046, 0.349)	0.133	-0.453 (-0.851, -0.055)	0.026		
Block Design Subtest, total score [A,L]	M1	0.053 (-0.437, 0.542)	0.833	2.966 (-0.986, 6.918)	0.141	3.019 (-0.897, 6.935)	0.130
	M2	3.121 (0.377, 5.865)	0.026	-0.102 (-4.244, 4.041)	0.962		
	M3	1.138 (-0.673, 2.948)	0.218	1.881 (-1.791, 5.552)	0.315		
	M4	0.016 (-1.688, 1.719)	0.986	3.003 (-1.277, 7.284)	0.169		

Abbreviations: CVLT, California Verbal Learning Test; WCST, Wisconsin Card Sorting Test; WMS, Wechsler Memory Scale; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator) p = p-value

Table D-3: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests among those ≤ median age of 63 years using delta method (n = 45)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
CVLT, t-score [L]	M1	0.371 (-0.635, 1.377)	0.470	1.135 (-3.181, 5.451)	0.606	1.506 (-2.806, 5.819)	0.494
	M2	0.171 (-0.660, 1.003)	0.686	1.335 (-2.920, 5.590)	0.539		
	M3	0.552 (-0.789, 1.893)	0.420	0.954 (-3.222, 5.130)	0.654		
	M4	-0.118 (-0.849, 0.614)	0.753	1.623 (-2.635, 5.881)	0.455		
CVLT, trial 1 score [A,L]	M1	0.042 (-0.089, 0.172)	0.532	-0.134 (-0.755, 0.486)	0.672	-0.093 (-0.709, 0.523)	0.768
	M2	0.032 (-0.117, 0.180)	0.675	-0.124 (-0.725, 0.476)	0.685		
	M3	0.046 (-0.088, 0.179)	0.502	-0.138 (-0.753, 0.477)	0.659		
	M4	0.005 (-0.039, 0.049)	0.827	-0.098 (-0.714, 0.519)	0.757		
CVLT, short delay free recall [L]	M1	0.088 (-0.168, 0.344)	0.499	0.793 (-0.369, 1.955)	0.181	0.881 (-0.275, 2.038)	0.135
	M2	0.042 (-0.164, 0.249)	0.688	0.839 (-0.305, 1.982)	0.150		
	M3	0.195 (-0.256, 0.646)	0.396	0.686 (-0.400, 1.772)	0.216		
	M4	-0.028 (-0.207, 0.150)	0.755	0.910 (-0.236, 2.056)	0.120		
CVLT, long delay free recall [L]	M1	0.115 (-0.178, 0.408)	0.440	0.116 (-1.069, 1.301)	0.848	0.232 (-0.956, 1.419)	0.702
	M2	0.036 (-0.145, 0.217)	0.698	0.196 (-0.984, 1.375)	0.745		
	M3	0.212 (-0.274, 0.698)	0.393	0.019 (-1.085, 1.124)	0.972		
	M4	-0.036 (-0.256, 0.184)	0.750	0.267 (-0.902, 1.437)	0.654		
CVLT, proactive interference [L]	M1	1.359 (-1.705, 4.423)	0.385	-3.411 (-13.795, 6.973)	0.520	-2.053 (-12.593, 8.488)	0.703
	M2	0.305 (-1.251, 1.861)	0.701	-2.358 (-12.835, 8.120)	0.659		
	M3	0.652 (-1.433, 2.737)	0.540	-2.705 (-13.263, 7.853)	0.616		
	M4	0.144 (-0.878, 1.166)	0.782	-2.197 (-12.720, 8.326)	0.682		
Stroop Color Word Test, t-score [A,L]	M1	-0.146 (-0.639, 0.348)	0.563	-4.089 (-6.523, -1.654)	0.001	-4.234 (-6.648, -1.82)	0.001
	M2	-0.066 (-0.407, 0.275)	0.705	-4.168 (-6.570, -1.766)	0.001		
	M3	0.014 (-0.342, 0.370)	0.940	-4.248 (-6.688, -1.808)	0.001		
	M4	-0.066 (-0.479, 0.346)	0.752	-4.168 (-6.553, -1.783)	0.001		
Block Design Subtest, total score [A,L]	M1	0.536 (-0.717, 1.790)	0.402	-2.752 (-5.829, 0.324)	0.080	-2.518 (-5.907, 0.871)	0.145
	M2	-0.087 (-0.545, 0.371)	0.709	-2.431 (-5.806, 0.944)	0.158		

Table D-3: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests among those ≤ median age of 63 years using delta method (n = 45)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
	M3	-0.297 (-1.105, 0.511)	0.471	-2.221 (-5.583, 1.142)	0.196		
	M4	-0.044 (-0.358, 0.271)	0.786	-2.474 (-5.859, 0.911)	0.152		

Abbreviations: CVLT, California Verbal Learning Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator); p = p-value

Table D-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests among those > median age of 63 years using delta method (n = 42)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
CVLT, t-score [L]	M1	0.093 (-0.713, 0.900)	0.821	4.041 (0.256, 7.825)	0.036	4.140 (0.441, 7.839)	0.028
	M2	0.839 (-1.514, 3.193)	0.485	3.3 (-1.033, 7.633)	0.135		
	M3	-0.186 (-1.521, 1.150)	0.785	4.32 (0.395, 8.245)	0.031		
	M4	0.429 (-1.283, 2.142)	0.623	3.707 (-0.343, 7.758)	0.073		
CVLT, trial 1 score [A,L]	M1	0.135 (-0.123, 0.392)	0.305	0.554 (-0.17, 1.279)	0.134	0.689 (-0.050, 1.428)	0.068
	M2	-0.030 (-0.496, 0.436)	0.900	0.719 (-0.155, 1.592)	0.107		
	M3	-0.132 (-0.420, 0.157)	0.371	0.821 (0.047, 1.595)	0.038		
	M4	0.089 (-0.254, 0.432)	0.610	0.6 (-0.210, 1.409)	0.147		
CVLT, short delay free recall [L]	M1	0.070 (-0.181, 0.322)	0.583	0.989 (-0.092, 2.070)	0.073	1.059 (-0.003, 2.122)	0.051
	M2	0.156 (-0.516, 0.829)	0.648	0.903 (-0.349, 2.155)	0.158		
	M3	-0.197 (-0.614, 0.221)	0.356	1.256 (0.144, 2.368)	0.027		
	M4	-0.108 (-0.599, 0.384)	0.668	1.167 (0.002, 2.332)	0.050		
CVLT, long delay free recall [L]	M1	-0.061 (-0.303, 0.181)	0.623	1.337 (0.274, 2.400)	0.014	1.276 (0.233, 2.320)	0.017
	M2	-0.157 (-0.818, 0.504)	0.641	1.434 (0.204, 2.664)	0.022		
	M3	-0.212 (-0.628, 0.205)	0.319	1.488 (0.399, 2.577)	0.007		
	M4	-0.413 (-0.960, 0.133)	0.138	1.690 (0.591, 2.788)	0.003		
CVLT, proactive interference [L]	M1	0.723 (-2.452, 3.898)	0.655	-18.005 (-33.218, -2.792)	0.020	-17.282 (-32.260, -2.305)	0.024
	M2	3.624 (-4.560, 11.808)	0.385	-20.906 (-37.662, -4.150)	0.014		
	M3	1.752 (-3.033, 6.536)	0.473	-19.034 (-34.498, -3.570)	0.016		
	M4	-0.527 (-5.986, 4.933)	0.850	-16.755 (-32.687, -0.824)	0.039		
Stroop Color Word Test, t-score [A,L]	M1	0.023 (-0.654, 0.701)	0.946	0.926 (-2.295, 4.148)	0.573	0.950 (-2.200, 4.099)	0.554
	M2	0.397 (-1.594, 2.389)	0.696	0.553 (-3.162, 4.267)	0.771		
	M3	0.561 (-0.669, 1.790)	0.371	0.389 (-2.911, 3.689)	0.817		
	M4	0.513 (-0.965, 1.992)	0.496	0.437 (-3.002, 3.875)	0.804		
Block Design Subtest, total score	M1	0.048 (-0.664, 0.759)	0.895	2.062 (-1.307, 5.430)	0.230	2.110 (-1.175, 5.394)	0.208
	M2	2.829 (0.459, 5.199)	0.019	-0.720 (-4.181, 2.742)	0.684		

Table D-4: Thyroid, non-thyroid, and total effects* of PFOA on the selected neuropsychological tests among those > median age of 63 years using delta method (n = 42)

Neuropsychological Tests		Thyroid Effect (β (CI))	p	Non-Thyroid Effect (β (CI))	p	Total Effect (β (CI))	p
[A,L]	M3	1.441 (-0.271, 3.153)	0.099	0.669 (-2.525, 3.862)	0.682		
	M4	0.074 (-1.434, 1.583)	0.923	2.035 (-1.587, 5.658)	0.271		

Abbreviations: CVLT, California Verbal Learning Test; CI, 95% Confidence Intervals; PFOS, Perfluorooctane Sulfonate; PFOA, Perfluorooctanoic Acid; *Adjusted for age, sex, education, cigarette, serum total PCB (lipid basis); †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; #Total effect for M1 (Model with Thyroid Stimulating Hormone as a Mediator), M2 (Model with Total Thyroxine as a Mediator), M3 (Model with Free Thyroxine as a Mediator), and M4 (Model with Total Triiodothyronine as a Mediator); p = p-value

Table D-5: Individual and joint effects* of PFOA† and serum total PCB (lipid basis) † on neuropsychological tests (n = 130)

Neuropsychological Tests	Individual PFOA Effect (β (CI))^{M1}	Individual Total PCB Effect β (CI))^{M2}	Joint Effect (β (CI))^{J}	p^{p}
WCST, perseverative errors† [A,H]	-0.281 (-0.450, -0.112)	-0.063 (-0.238, 0.112)	-0.096 (-0.283, 0.09)	0.013
WCST, perseverative responses† [A,H]	-0.309 (-0.492, -0.126)	-0.082 (-0.272, 0.108)	-0.112 (-0.314, 0.09)	0.010
WCST, number of categories completed [B,L]				
25th quantile	0.973 (-0.517, 2.463)	0.54 (-1.277, 2.356)	-1.73 (-4.245, 0.784)	0.153
50th quantile	0.765 (-0.639, 2.168)	0.703 (-0.703, 2.11)	-2.553 (-4.688, -0.418)	0.018
75th quantile	0.147 (-0.914, 1.209)	-0.267 (-1.395, 0.86)	-0.988 (-3.119, 1.143)	0.338
GPT (dominant hand), time to completion† [A,H]	-0.041 (-0.084, 0.002)	-0.028 (-0.078, 0.021)	-0.017 (-0.068, 0.034)	0.061

Abbreviations: GPT, Grooved Pegboard Test; WCST, Wisconsin Card Sorting Test; CI, 95% Confidence Intervals; *Adjusted for age, sex, and education; †Log-natural transformed, H: High Score = Impairment, L: Low Score = Impairment; A: Linear regression; B: Quantile regression; M1= Individual effect of PFOA (i.e., change in a neuropsychological test score per IQR increase in PFOA among reference PCB group); M2 = Individual effect of serum total PCB (i.e., change in a neuropsychological test score per IQR increase in serum total PCB among reference PFOA group); J = Joint effect of PFOA and serum total PCB (i.e., change in neuropsychological test score per concurrent IQR increment in both PFOA and serum total PCB); p = p-value of a product term between PFOA and PCB

Table D-6: Associations of thyroid markers and PFCs with malingering*

	β	p-value
Total Thyroxine (µg/dL, n=130)[a]	0.371	0.653
Free Thyroxine (ng/dL, n=130)[a]	-0.206	0.817
Total Triiodothyronine (ng/dL, n=130)[a]	0.530	0.398
Thyroid Stimulating Hormone (µIU/mL, n=130)[a†]	-0.002	0.999
Perfluorooctanoic Acid (ng/mL, n=157)[b†]	0.266	0.688
Perfluorooctane Sulfonate (ng/mL, n=157)[b†]	0.423	0.499

*Note: Test of Memory Malingering is a 50-item recognition test with two trials, and any subject with scoring below 45 correct on either trial 1 or 2 was considered to have shown malingering or symptom exaggeration. None scored < 45 in the trial 2, whereas n=6 in thyroid-neuropsychological dataset (i.e., out of 130) and n= 5 in PFC-neuropsychological dataset (i.e., out of 157) scored less than 45 in the trial 1.

a= Logistic regression results adjusting for age, sex, education, and cigarette smoking

b=Logistic regression results adjusting for age, sex, education, and serum total PCB (lipid basis)

Abbreviations: PFCs, Perfluorinated Compounds