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DEVELOPING A DYNAMIC MODEL OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS FOR RISK ASSESSMENT OF ENDOCRINE DISRUPTING CHEMICALS

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By

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Doctor of Medicine (M.D.)

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[2012]

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ABSTRACT

Background: Thyroid hormones serve a host of functions including metabolism, growth, and development. Endocrine disrupting chemicals (EDCs) can cause perturbations to thyroid hormone homeostasis, leading to adverse health effects. Risk assessment of thyroid disruptors requires an approach mechanistically linking toxicological and epidemiological data across multiple scales. Dynamic models can serve the integrating role.

Objective: (1) Construct a dynamic model of the hypothalamus-pituitary-thyroid (HPT) axis, (2) use the model to establish a reference human thyroid population model, and (3) use the population model to predict thyroid-disrupting mechanisms of EDCs.

Methods: An ordinary differential equation (ODE)-based deterministic model of the HPT axis was constructed to capture the feedback regulation between T3, T4, and TSH, their synthesis, metabolism, and plasma buffering. The initial model representing an average euthyroid condition was then optimized by using the NHANES thyroid profile data to establish a reference thyroid population model. Pearson correlation and weighted multiple linear regression of the thyroid profile data and/or optimized model parameters to urinary EDCs including sodium iodide symporter (NIS) inhibitors, environmental phenols, and perfluorinated chemicals were then performed. Hierarchical clustering of EDCs based on thyroid hormone profile and/or optimized model parameters was performed.

Results: The deterministic model recapitulated the mean levels of free T3, free T4, TSH, total T3 and total T4 of a general human population. The model can simulate primary or secondary hyper- or hypothyroid conditions. Using the NHANES dataset, a virtual thyroid population was established with optimized parameter distributions. Correlation analysis (1) confirmed the thyroid-disrupting mechanisms of well-characterized EDCs such as perchlorate and (2) made predictions for novel thyroid-disrupting mechanisms of a number of chemicals such as thiocyanate. Multiple linear regression demonstrated the negative association of thyroid hormones with a number of EDCs however the associations with TSH varied, suggesting different thyroid-disrupting mechanisms. Hierarchical clustering demonstrated the usefulness of optimized model parameters as additional features to help refine chemical grouping.

Conclusions: A dynamic model of the HPT axis can be used to infer novel mechanistic information of thyroid EDCs and it can become an important tool in risk assessment of EDCs by incorporating future *in vitro* testing data.

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ABBREVIATIONS

BP3= Benzphenone-3

BPA= Bisphenol-A

CDC= Centers for Disease Control and Prevention

EDC= Endocrine Disrupting Chemical

HPT= Hypothalamus-Pituitary-Thyroid

NCHS= National Center for Health Statistic

NHANES= National Health and Nutrition Examination Survey

PFCs= Perfluorinated Chemicals

PFDE= Perfluorodecanoic acid

PFOA= Perfluorooctanoic acid

PFOS= Perfluorooctanesulfonic acid

T3= Thyronine

T4= Thyroxine

TG-AB= Thyroglobulin Antibody

TPO-AB= Thyroperoxidase Antibody

TRH=Thyrotrophic Releasing Hormone

TSH=Thyroid Stimulating Hormone

INTRODUCTION

Anatomy and Physiology of the Hypothalamus-Pituitary-Thyroid (HPT) Axis

The hypothalamus occupies the anterior portion of the diencephalon, and is comprised of numerous small nuclei and tracts located on the body of the third ventricle (Lechan et al., 2013). Anteriorly, the hypothalamus spans from the periaqueductal gray matter of the midbrain, and proceeding posteriorly; the mammillary bodies, interpeduncular fossa and cerebral peduncles. Posteriorly, the hypothalamus spans from the region of the anterior commissure, lamina terminalis and the optic chiasm. The hypothalamus controls the hormonal activity of the anterior portion of the pituitary gland, and produces hormones (oxytocin and anti-diuretic hormone) which are stored in the posterior portion of the pituitary gland (Reichlin, 1967). The hypothalamus exerts control of the hormonal activity of the anterior pituitary by producing "trophic" hormones such as corticotropin-releasing hormone (CRH), gonadotropin-releasing hormone (GRH), growth hormone-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH), and somatostatin (Barrett et al., 2010).

The pituitary gland commonly called the "master gland", is a pea shaped organ located at the base of the brain within the "sella turcica" (Sheng et al., 1999). It is anatomically, physiologically and embryologically divided into 2 distinct structures, the adenohypophysis (anterior pituitary) and the neurohypophysis (posterior pituitary) (Takuma et al., 1998). The adenohypophysis produces several hormones including adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), growth hormone (GH), luteinizing hormone (LH), and prolactin. (Barrett et al., 2010). Anti-diuretic hormone (ADH) and oxytocin which are both produced in the hypothalamus and transported via neurophysins are stored in the neurohypophysis.

The thyroid gland is in the anterior aspect of the neck, and it is a bi-lobed butterfly shaped organ connected via a structure called the isthmus, which in a subset of patient is the site for an additional

lobe 'the pyramidal lobe' (Khatawkar et al., 2015). The primary function of the thyroid gland is the production of thyroid hormones which are iodine-containing amino acids; thyroxine (T4) and triiodothyronine (T3), and the natural occurring isomers are the L-isomers (levo-Isomers) (Khatawkar et al., 2015). T4 is also converted to T3 in the periphery via 5' deiodinase (Barrett et al., 2010, Khatawkar et al., 2015).

The thyroid gland is made up of follicular cells, which contains a substance called "colloid" and this is where thyroid hormonal synthesis occurs (Barrett et al., 2010). The thyroglobulin complex within the colloid contains bound thyroid hormones, and for this complex to be synthesized, a series of important steps are required; first, inorganic iodide, a univalent anion, needs to be transported into the thyroid gland via the sodium-iodide symporter (NIS), then the inorganic iodide is converted to iodine via an oxidative process (Barrett et al., 2010, Khatawkar et al., 2015). Within the thyroid gland, thyroperoxidase (TPO) binds iodine to tyrosine forming iodotyrosines, and coupling of mono-iodotyrosines and di-iodotyrosines form thyroid hormones (T3 & T4) (Khatawkar et al., 2015).

Upon requirement of thyroid hormone, TRH from the hypothalamus is produced which stimulated the pituitary gland to produce TSH, then TSH stimulates the thyroid gland which causes the cleavage of formed thyroid hormones coupled on thyroglobulin by proteases present within the lysosomes of the thyroid follicular cells, and this results in the release of thyroid hormones thyronine (T3) and thyroxine (T4) into circulation (Barrett et al., 2010, Khatawkar et al., 2015). T4 is the predominantly produced thyroid hormones with about 80% being thyroxine, and 20% thyronine, however the active form of thyroid hormone is T3. Within the periphery, T4 is being converted to T3 by 5' deiodinase and within the CNS type II 5' deiodinase is responsible for the conversion of T4 to T3 needed for thyroid hormonal homeostasis and function (Lakshmy et al., 1999). This system is referred to as the hypothalamic-pituitary-thyroid (HPT) axis, and this is essential in ensuring thyroid hormone homeostasis (Barrett et al., 2010). These released thyroid hormones are bound by albumin, thyroid

binding prealbumin (or transthyretin), and thyroid binding globulin (TBG) which are all serum proteins, hence only a very tiny fraction (<0.1%) of the released hormones are available in free form (De Escobar et al., 2004, Khatawkar et al., 2015). T3, the active form of thyroid hormone, has a rapid onset of action (within a few hours), and T4 has a much slower onset of action ranging between 4-14 days (Khatawkar et al., 2015). Thyroid hormones (T3 and T4) are metabolized mainly in the liver and excreted into bile via the cytochrome P450 system (CYP450s) (Ye et al., 2017).

Thyroid hormones are required for several physiological processes such as metabolism, appetite, bone growth, menstrual cycle regulation, body weight, central and peripheral nervous system function, heart rate, lipid/cholesterol oxidation and thermogenesis (Witkowska-Sędek et al., 2017). In-utero, thyroid hormone is also associated with growth, development, differentiation, migration and gene expression in tissues and specialized organs including cerebral growth and development (Bernal et al., 1995). Therefore, thyroid hormones are crucial in ensuring timely progression of several important developmental processes through effects on the rate of gene expression and cell differentiation. The receptors for all the T3 isoforms are expressed in the brain, and their "spatial and temporal patterns of expression" is suggestive of a variation in function of the expressed isoforms.

Pathology of the HPT Axis and Prevalence of Thyroid Disease

Thyroid disease states are generally classified into hyperthyroidism and hypothyroidism (Barrett et al., 2010). These states are further classified into primary, secondary and tertiary causes with primary originating from the thyroid gland, secondary from the anterior portion of the pituitary gland, and tertiary from the hypothalamus. In primary disease states, commonly due to thyroid nodules or cancer, there is an increase or decrease in thyroid hormones (T3/T4) produced by the thyroid gland, which have an inverse relationship with TSH, and ultimately TRH (Barrett et al., 2010). In secondary disease states, commonly due to dysfunctional pituitary adenomas secreting TSH, there is a direct positive

relationship between TSH levels and thyroid hormones with an inverse relationship on TRH, while in tertiary disease states there is a direct positive relationship between TRH, TSH and thyroid hormones.

During pregnancy, thyroid hormones required by the developing fetus for a host of functions including growth and differentiation of tissues and organs, and neurodevelopment, are supplied to the fetus from the mother (Aker et al., 2016). T3, T4 and iodine are detected in fetal circulation in the first trimester prior to fetal production of thyroid hormones, however only T4 is transported via the placenta to the fetus and local conversion to T3 occurs in the fetus. Therefore, to meet this increased T4 demand in pregnancy, mothers need to increase production of thyroid hormones, making the balance of thyroid hormones crucial within pregnancy (Aker et al., 2016).

Thyroid disease is prevalent worldwide with 12% of the world's population projected to develop thyroid disease in their lifetime, and are consequent of perturbations in the HPT axis (Bjoro et al. 2000). The prevalence of thyroid disease in different populations is dependent on factors such as race, sex and geographic location. Among US adults, the prevalence of thyroid abnormalities ranges between 1 to 10 percent (Stone et al, 2003). Poor iodine intake is the most common cause of thyroid disease globally with an increased prevalence in developing countries with poor dietary iodine fortification (Bjoro et al. 2000). In developed countries such as the United States with iodine fortification, causes of thyroid disease in the population are resultant of autoimmune disease states such as Hashimoto's disease/thyroiditis, and Grave's disease. Pregnancy substantially affects thyroid glandular function with a 10% increase in gland size in "iodine-replete" regions, and a 20-40 % increase in gland size in iodine deficient regions (Stagnaro-Green et al. 2011). During pregnancy, there is an increase production of thyroid hormones (T3 and T4) by 50% and to meet this increase need, the requirement for iodine which is essential in thyroid hormone synthesis increases by 50%, hence pregnancy has been described as a "stress test for the thyroid".

Endocrine Disrupting Chemicals and their effect on the HPT Axis

Endocrine disrupting chemicals (EDCs) are compounds that can affect components/organs of the endocrine system, lead to perturbations in hormone production and subsequently cause deleterious health outcomes (Andrady, 2015). EDCs have been demonstrated to perturb hormone production by imitating, preventing the production, release, metabolism, disrupting transport and by binding or elimination of naturally produced hormones (Caserta et al., 2008). Conventionally, EDCs were perceived to exert their action exclusively by interacting with nuclear hormone receptors including thyroid receptors (TRs), estrogen receptors (ERs), androgen receptors (ARs) amongst a host of others, however, they have been demonstrated to have other mechanisms of actions (Schug et al., (2011). EDCs also act on nonsteroid receptors, enzymatic pathways involved in steroid synthesis/metabolism, as well as transcriptional coactivators involved in the endocrine and reproductive systems. Other mechanisms of actions of EDCs include effect on genes and epigenetics which have several implications on programming in early development, diseases through the life course, and potentially transgenerational inheritance of disease. EDCs have also been demonstrated to be carcinogenic with substances such as diethylstilbestrol exposure linked to formation of breast cancer in women, as well as malformation of the female genital tract given *in utero* exposure (Soto et al., 2010).

Greater than 800 compounds are either identified or speculated to be EDCs, and the human interaction with the environment can be a significant source of exposure to EDCs (Andrady, 2015). EDCs have a low-dose effect described by the National Toxicology Program as an effect of a chemical on humans noted at a level lower than stipulated and used in traditional toxicological studies (Vandenberg et al., (2012). Therefore, even though they may only be present in small quantities in the environment, they may still lead to deleterious outcomes especially when multiple chemicals affect the same target organ. (Andrady, 2015). To place further emphasis on this point, most EDCs are under the class of compounds referred to as persistent organic pollutants (POPs) (Ngwa et al., 2015). POPs are compounds that are retained in the environment because of their resistance to degradation via

chemical, biological and photolytic processes, and due to this they bioaccumulate in human and animal tissues up the food chains, conferring the capacity of long-range transport (Ngwa et al., 2015). Coupling this fact that EDCs persist in the environment to the low-dose effect, EDCs are compounds of significant concern (Ngwa et al., 2015, Vandenberg et al., 2012). Several chemicals (compounds) have been researched and identified as EDC on the HPT axis, leading to disruption of thyroid hormone synthesis and ultimately causing disease states of hyperthyroidism or hypothyroidism.

Environmental Phenols

Phenols are compounds with a hydroxyl group bonded with an aromatic (cyclical) hydrocarbon group (Amorati et al., 2012). They are classified as simple phenols or polyphenols based on the number of phenol groups coupled on a molecule. Compounds that are phenol based have a characteristic sweet smell and are utilized in a wide array of manufacturing purposes, such as parabens and their use in cosmetics, benzophenone-3 and its use in sunscreens, triclosan and its use as an antibacterial or antifungal agent in soaps, and bisphenol A and its use in soap/plastics (Wu et al., 2016). These compounds are referred to as environmental phenols because they are not readily degraded in the environment, therefore making them persistent organic pollutants.

- Bisphenol-A

Bisphenol-A (BPA), a compound that is a constituent of plastics, food packaging, and receipts amongst a host of other things, has been shown to be hazardous to human health secondary to its EDC properties (Rochester et al., 2015). BPA has been of concern amongst the scientific community because it is readily available in an extensive array of consumer products, causing widespread exposure across the population coupled with the fact that it is a POP (Vandenberg et al., 2010). In response to this, a shift from using BPA to BPA analogues such as BPS and BPF has been made by several industries that utilize BPA in production of their goods in lieu to provide safer alternatives. However, these analogues have the same effect on human health with demonstrated estrogenic, antiestrogenic, androgenic, and

antiandrogenic properties (Rochester et al., 2015). Analogues used as substitute chemicals to BPA such as BPM, BPS, or BPF are structurally like BPA hence carry the same potential to interact with hormonal receptors, hence leading to adverse health outcomes like their parent compound. BPA has been demonstrated to cause perturbations within organs derived from endodermal origin such as the thyroid, pancreas and prostate glands, and has been implicated in disease states such as diabetes secondary to pancreatic disruption, and thyroid disease states secondary to thyroid disruption (Porreca et al., 2017).

BPA has been demonstrated to exert its effect at the level of the pituitary and the thyroid gland leading to thyroid hormonal production perturbations (Lee et al., 2017). BPA has been demonstrated to show a positive association between TSH and urinary BPA levels, suggestive of a resultant hypothyroid state from exposure (Andrianou et al., 2016). BPA and its analogues such as BPS have antagonistic effects at the level of thyroid hormone receptors, which potentially leads to a resultant hypothyroid state (Skledar et al., 2016). Mammalian studies using rats demonstrated that maternal exposure to BPA increases the expression of "neurogranin" in the hippocampus of the brain, leading to postulations that BPA may have antagonistic actions on thyroid hormonal synthesis through TRβ (Boas et al., 2006).

TPO is an enzyme glycoprotein that catalyzes the transfer and coupling of iodine to thyroglobulin during thyroid hormonal synthesis of T3 or T4 (Song et al., 2012). BPS, compared to methimazole, a drug used to treat hyperthyroidism as a TPO inhibitor, shows effect of TPO inhibition at a much lower dose. BPA has also been implicated as a cause of thyroid autoimmune disease evidenced by a positive correlation between BPA levels and TPO antibody positivity, which is suggestive of a resultant thyroid autoimmune diseases state (Chailurkit et al., 2016). *In utero* exposure to BPA has different consequences by sex, with no effect noted in thyroid function parameters prior to 16 weeks' gestation which is suggestive of a window of susceptibility (Romano et al., 2015). Female neonates show a decrease in TSH levels with each 10-fold increase in BPA concentrations in maternal blood, however no change

is noted in male neonates. This inverse relationship between BPA and TSH is indicative of increase in the amount of thyroid hormone (T3/T4), causing a resultant hyperthyroid state in female neonates, which is the opposite of the effect of BPA on thyroid function in adults (Andrianou et al., 2016, Romano et al., 2015).

- Parabens

Parabens are esters of *para*-hydroxybenzoic acid such as methyl paraben, isopropyl paraben, butyl paraben and propyl paraben, commonly used in pharmaceuticals and in the food industry as a preservative, and in cosmetics such as soaps, shampoos, facial and skin cleansers, and lotions (Koeppe et al., 2013, Darbre et al., 2004). Parabens are readily absorbed in the skin as well as in the gastrointestinal tract, and this raises concern as these compounds have been defined as EDCs, and their applications are in tandem with their route of entry into the bodies of mammalian species (Soni et al., 2002).

Parabens have been demonstrated to have a negative correlation with thyroid hormone species (T3/T4) in an analysis using the cross-sectional data from the NHANES 2007-2008 database, which is indicative of thyroid hormonal disruption by these compounds (Koeppe et al., 2013). Methyl paraben has been demonstrated to show a weak anti-thyroid activity *in vitro* by inhibiting iodine organification in the thyroid gland in a dose-dependent fashion (Boas et al., 2006). Butyl paraben however has been demonstrated to show increased growth of GH3 cells in T-Screen assay, therefore, butyl paraben was described as a thyroid hormone receptor agonist (Taxvig et al., 2008).

Considering the discordance noted between these compounds that are under the same family of chemicals, it is imperative that further research should be conducted to define these chemicals, and their roles as thyroid disrupting chemicals (Taxvig et al., 2008).

- Triclosan

Triclosan (5-chloro-2-(2,4-dichloropheoxy) phenol) is a manufactured chlorinated phenol, utilized for its antimicrobial and preservative properties in many cosmetics and household products. (Schnitzler et al., 2016). Triclosan is measured and detected in various environment, including several rivers in the United States, therefore it is one of the most common exposures with regards to POPs. Triclosan present in cosmetic and personal care products washed down the drain can be effectively removed via sewage treatment, however the presence within the U.S waters exposed aquatic creatures as well as land animals to triclosan leading to accumulation within biologic species that humans may consume, therefore serving as a means of human exposure to triclosan (Mihaich et al., 2017). Triclosan is structurally like thyroid hormone, therefore has been postulated to carry thyroid hormonal disruption properties (Schnitzler et al., 2016).

About 80% of thyroid hormones are bound to transport proteins which increases the half-life of thyroid hormones, as well as transport the thyroid hormones to their site of action (Witkowska-Sędek et al., 2017). Triclosan has been demonstrated to be correlated with a decrease in thyroxine (T4) concentration pre-natally and post-natally among mammalian species (rats) (Johnson et al., 2016). This decrease in T4 concentration has been attributed to triclosan binding with transthyretin (TTR) decreasing the binding sites for thyroid hormones, therefore leading to a reduction in the measureable concentration of thyroid hormone in plasma (Weiss et al., 2015). Triclosan is a non-competitive NIS inhibitor as demonstrated by a dose-dependent decrease in iodide uptake by FRTL-5 cells when grown in the presence of triclosan, triclocarban, BPA or BDE-47 (Wu et. al, 2016). Triclosan inhibits TPO activity in a dose-dependent manner, therefore it inhibits the organification of iodine and ultimately thyroid hormonal synthesis.

Despite studies demonstrating these results, the data proving the relationship between triclosan and thyroid hormonal disruption is still elusive, and further studies showing these associations need to be conducted (Witorsch, 2014).

Sodium-Iodide Symporter (NIS) Inhibitors

- Nitrate, Perchlorate and Thiocyanate

Nitrate are a common contaminant in the drinking water supplies within agricultural communities (Aschebrook-Kilfoy et; al., 2016). Sources of nitrogen contamination in the environment include leaky septic tanks, animal excrement, and nitrogen based fertilizers which since their use in the 1950's have increased nitrogen contamination of the environment (Ward e. al., 2010). Perchlorate is conventionally used as an oxidizing agent in rocket and missile fuel, however it can occur naturally in some soils and within the atmosphere which deposits in soil after precipitation and becomes a source of human exposure (Abt et al., 2016). Perchlorate is ubiquitous in food because of its presence and persistence in soil, source water, irrigation and processing water utilized for agricultural purposes. This is because it is hydrophilic and hence, detectable in snow, rain, ground water, fertilizers (Jugan et al, 2010). Perchlorates have been measured in the U.S drinking water system in levels between (4 – 200) μg/L. Thiocyanate is a detoxification by-product of cyanide and can be found in cigarette smoke as well as plant foods such as cassava, cabbage, turnips, Brussel sprouts, and cauliflower (Erdoğan, 2003).
Perchlorate, thiocyanate and nitrate are univalent anions like iodide which is up-taken by NIS of the thyroid gland for thyroid hormone synthesis, therefore they can compete with iodide for NIS leading to an iodine deficient state and ultimately a hypothyroid state (Aschebrook-Kilfoy et al., 2016).

Thiocyanates in addition to this also upregulates the activity of type II 5' deiodinase which converts T4 to T3 in the CNS and within peripheral tissue, as well as the number of binding sites for the enzyme as an adaptive measure secondary to persistent thiocyanate exposure (Lakshmy et al., 1999). This leads to depletion of T4 with normal levels of T3. Following exposure to these chemicals, TSH levels rise in

response to the low T4/T3 levels and can lead to the formation of goiters. (Aschebrook-Kilfoy et al., 2016).

Current Movement in Chemical Toxicity Testing

Traditional chemical toxicity testing requires exposures of laboratory animals for 14 days, 90 days, and 2 years depending on the apical endpoints of toxicological concerns. Several forces including regulatory, commercial, scientific and animal welfare considerations, are converging to promote a change. Thus, toxicity testing for man-made chemicals is undergoing a strategic transformation from the traditional animal-based approaches with an aim of achieving a more efficient, less expensive, scientifically accurate, and humane way of conducting chemical health risk assessment.

The first consideration comes from the cost and efficiency issue of animal testing. In the United States, greater than 85,000 chemicals are currently manufactured for commercial purposes and 600 are introduced in addition to this pool annually (Trasande, 2016). The law regulating the production and use of these chemicals in the United States is the Toxic Substances Control Act (TSCA), which was passed on October 11, 1976 by President Ford in response to several counts of chemical damage to the environment and health (EPA History, 2016). The prerogative for passing this law was to curb environmental pollution from chemical exposure by ensuring adequate pre-distribution and commercial chemical use testing, require manufacturers and distributors to demonstrate chemicals are not toxic to health and the environment, and confer authority on the Environmental Protection Agency (EPA) to regulate toxic substances. At the time the TSCA was passed, approximately 60,000 chemicals in use then were "grandfathered-in", hence not subjected to adequate toxicity testing and evaluation (Trasande, 2016). The Frank R. Lautenberg Chemical Safety for the 21st Century Act was passed as an amendment to the TSCA to address its inefficiencies (Frank R. Lautenberg, Chemical Safety for the 21st Century Act. 2017). This amendment broadened the reach of the act by mandating the evaluation of existing and "grandfathered-in" chemicals prior to the passing of the act, enabling the EPA to

develop standards that are "risk-based", require manufacturers to disclose chemical information, and create provision allowing the federal government provide funding to the EPA to deliver its responsibilities.

This translates to increased accountability by manufactures of chemicals to their consumers, and the EPA has a huge backlog to address with regards to toxicity assessment of these existing chemicals to inform population safety. Meanwhile, new chemicals are coming online looking for regulatory approval at a fast rate. Therefore, an undue burden is placed on conventional toxicity testing which is primarily animal-based *in vivo* assays. How to reduce the cost of animal testing and accelerate the speed of testing to keep up with the demand of commercial chemicals has become a practical issue which cannot be adequately addressed if we continue the traditional animal testing approaches (Burden et al, 2015).

A second consideration for a new testing approach comes from scientific concerns (Burden et al, 2015). Traditional animal testing is normally conducted in a way that animals are exposed to a chemical at high doses such that some apical endpoint changes are likely to be observed, such as liver toxicity and cancers. Then the responses observed at high doses are extrapolated, in most cases linearly, back to the origin where zero dose is located. In doing so, the responses at low doses can be predicted, after adjusting for uncertainty factors for exposure route difference, inter-species difference, and inter-individual variability. In some cases, a few more dose points may be conducted to derive for no observed adverse effect levels (NOAELs) or lowest observed adverse effect levels (LOAELs), which can be considered as the point of departure (POD) or the point from which linear extrapolation is conducted. Many years of this high-dose extrapolation practice based on animal data has proven that this approach informs little, if any, human health risk for environmental chemicals humans are exposed. In other words, high-dose animal studies bear little relevance to human health risk assessment in most cases and the results are inaccurate and scientifically unsound.

Moreover, the traditional animal studies are still based on top of the gross knowledge of animal anatomy, pathology and physiology dated back 50 years ago when these methods were first established. In the intervening years, biology has advanced dramatically both in our understanding of how biological systems work at molecular and cellular levels, but also the methodologies to interrogate biological systems at these levels. Unfortunately, the traditional animal testing utilizes little of these achievement and modern biology. Therefore, these scientific concerns are one of the driving forces behind the change in animal testing.

A third consideration for a change in chemical testing is about animal right. Increasingly animal welfare becomes a humane concern. Animal right activities of how to replace, refine, and reduce (3Rs) animal usage in biomedical research for human benefits is taking place around the globe, especially in the Europe. For instance, legislature has been passed in the European Union that starting from 2013, cosmetics with ingredients tested in animals are no longer legal in the EU market (EC, 2009).

Taken together, multiple forces are coming together to push for an animal alternative approach to chemical toxicity testing. These alternative approaches would be economical, efficient, humane, and informative to real-world human exposures through making best use of modern science. In 2007, the National Academy of Sciences (NAS) published a report: Toxicity Testing the in 21st Century: a Vision and a Strategy (NRC, 2007). This highly publicized report envisaged that "In a not-so-distant future, virtually all routine toxicity testing would be conducted in human cells or cell lines *in vitro* by evaluating perturbations of cellular responses in a suite of assays anchored on toxicity pathways." Toxicity pathways are defined based on modern biology, which refer to any existing biochemical circuit in the cell that, when sufficiently perturbed, is expected to result in an adverse health outcome.

One potential challenge for human risk assessment by using the advocated cell-based approach is how to interpret these *in vitro* assays results in the *in vivo* context and inform safety assessment at the human

population level. To this end, computational modeling will play an indispensable role. Computational modeling is necessary not only for correlating the *in vitro* derived point of departure chemical concentrations to *in vivo* exposure dose through realistic routes, but also for relating the *in vitro* cell effects to organism-level health effects. This is a situation particularly relevant for EDCs.

Role of Dynamic Modeling of in Risk Assessment of EDCs

Dynamic models are products of the application of computational toxicology, which involves using mathematical and computational models to aid in evaluating the impact of the hazards that chemicals pose on health and the environment (Kavlock et al., 2010). Developing robust dynamic models require the use of informatics, high-throughput screening (HTS) technologies, and systems biology. Therefore, this is a research area that merges advancement in chemistry and molecular biology with modelling and computational science to improve and advance research in the field of toxicology (Kavlock et al., 2008). Dynamic models improve the effectiveness and efficiency of assessing the harm posed by environmental toxicants, yielding information that pertinent in the protection of human health and the environment. The advances made by computational toxicology has enabled an increase in the scale of chemicals studied, and determining the potential impact on health while decreasing the number of animals needed for conventional toxicology research.

USEPA being the agency within the United States mandated on chemical testing and evaluation, utilizes dynamic modeling to develop "computational tools" for application in risk assessment of chemical produced within various environmental matrixes including air, water, soil and dump sites (Kavlock et al., 2010). The Food and Drug Administration also uses dynamic models for risk assessment of medication side effects during drug development and testing, and to achieve this they merge the strengths of *in vitro*, *in vivo*, and *in silico* testing and build it into their dynamic modeling (Merlot, 2010). They utilize the strengths of dynamic modeling in determining the toxicity of their compounds prior to pre-testing and pharmacokinetic optimization (Merlot, 2010). Dynamic modeling enables the FDA

in determining the mechanism of action of medications, and they can help in determining adverse effects of medication, predicting "off-target" sites of these medications therefore. Dynamic modeling can also be used to support animal testing by bridging the gap between *in vivo* and *in vitro* testing, particularly pertaining to biomarker detection and evaluation as instances arise in *in vivo* testing where the correct biomarker is not being evaluated, and in *in vitro* testing where the effect of the biomarker is not demonstrated in a physiologic system under homeostasis (Merlot, 2010).

Dynamic models have also been shown to be optimal prediction and risk assessment tools. Dynamic models have been utilized to develop aquatic life (fish) physiological based models of the hypothalamic-pituitary-gonadal (HPG) axis, for assessment of the effect of estrogen exposure on plasma steroid hormone and vitellogenin concentrations (Watanabe et al., 2009). Dynamic models have also been used for descriptive purposes for marine life also on the HPG axis to determine the reproductive activity of salmon (Kim et al., 2006). To apply dynamic modeling in human research, adequate knowledge on the anatomy, physiology, and pathology of these systems are needed to construct the dynamic model.

As far as risk assessment of EDCs is concerned, dynamic modeling can facilitate the translation of in *in vitro* assays results to *in vitro* situations. Because endocrine systems are high homeostatic systems, where the negative feedback regulation through the hypothalamus and pituitary plays an extremely important role in maintaining the hormone levels within a narrow physiological range against any internal or external perturbations. Therefore, quantitative EDC testing results observed *in vitro* with isolated endocrine cells cannot be a true representation of the quantitative consequence *in vivo* when the hypothalamic-pituitary-endocrine organ axis is fully operating. A dynamic model of the axis will be able to bridge the gap between cell-based assay results and *in vivo* hormonal changes. Such a model will also allow derivation of mode of action of EDCs or at least the main perturbed physiological processes, based on the hormonal profile under exposure to an EDC. A dynamical model of the endocrine system can also allow incorporation of population variability into the various physiological processes such that

a reference virtual human population may be established to be used for risk assessment based on cell assay data. Finally, it has become clear that many EDCs can have nontraditional, nonmonotonic dose response effects (Vandenberg et al., 2012) but the underlying mechanisms are still unclear. A dynamic model approach of the endocrine system will allow opportunities to explore the biochemical rationales behind these nonmonotonic effects.

Objectives of the Thesis Study

Given the current issues concerning risk assessment of EDCs, as discussed above, including extrapolating increasingly available *in vitro* toxicity testing data to *in vivo* scenarios, taking into consideration of inter-individual variability in human populations, and the frequent nonmonotonic dose responses of EDCs, it is imperative to develop mechanistically-based tools to address these issues. For this thesis study, we chose the HPT axis as the endocrine system to develop a dynamic model to facilitate the risk assessment research on environmental thyroid disruptors. The following aims were set:

(1) Develop a minimal, deterministic model of the HPT axis representing the thyroid hormone regulation in an average human individual.

(2) By using the National Health and Nutrition Examination Survey (NHANES) 2007 – 2012 thyroid profile dataset, explore the parameter space of the HPT model to establish a reference human population model recapitulating inter-individual variability in the thyroid system.

(3) By using the NHANES 2007-2012 urine environmental chemical concentration dataset and the population model, confirm and/or predict HPT processes that can be potentially disrupted leading to thyroid effects by EDCs, including environmental phenols (BPA, benzophenone-3, methyl paraben, ethyl paraben, butyl paraben and propyl paraben), NIS inhibitors (nitrate, perchlorate and thiocyanate), and polyfluorochemicals (PFCs) including perfluorodecanoic acid (PFDE), perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS).

(4) Perform multiple linear regression (MLR) with NHANES thyroid profile data as outcome against urine EDCs in similar categories, sex, age, race, smoking, body mass index (BMI), and urine iodine.

METHODS

Construction of the Computational Model of the Human HPT Axis

Deterministic HPT Model

A deterministic model of the human HPT axis was developed as a coupled ordinary differential equations (ODEs) system. The model contains the following state variables: thyroid-stimulating hormone (TSH), free T4, free T3, thyroxine-binding globulin (TBG)-bound T4, and TBG-bound T3. Thyrotropin-releasing hormone (TRH) is excluded from the model for simplicity and because it is produced in the hypothalamus and thus less prone to chemical perturbations. The HPT processes modeled include: TSH-stimulated T4/T3 production, peripheral T4 conversion into T3, T4/T3 binding with TBG, T4/T3 metabolism, inhibition of TSH production by T4/T3, and TSH degradation (Fig. 1). The resulting ODEs are as follows:

$$dTSH/dt = k3*Km^n/(Km^n+(T3+T4)^n) - k4*TSH, \quad (1)$$

$$dT4/dt = k1*a*TSH - k2*T4 – k6/Kd1*T4*(TBGtot - T4_TBG - T3_TBG) + k6*T4_TBG - k8*T4, \quad (2)$$

$$dT4\_TBG/dt = k6/Kd1*T4*(TBGtot - T4\_TBG - T3\_TBG) - k6*T4\_TBG, \quad (3)$$

$$dT3/dt = k1*(1-a)*TSH + k8*T4 - k9*T3 - k11/Kd2*T3*(TBGtot - T4\_TBG - T3\_TBG) + k11*T3\_TBG, \quad (4)$$

$$dT3\_TBG/dt = k11/Kd2*T3*(TBGtot - T4\_TBG - T3\_TBG) - k11*T3\_TBG. \quad (5)$$

The default parameter values were determined based on those reported in the literature and are listed in Table 1. The resulting ODE model produces steady-state hormone levels representing the means of

respective hormones in a human population (Hoermann, et al., 2015) are listed in Table 2. The model was initially constructed in the numerical simulation program Berkley Madonna and optimized using the built-in Stiff ODE solver. The model was then manually imported into MatLab to explore the multi-parameter space to obtain the population model based on the NHANES thyroid dataset.

Population HPT Model

To establish an HPT model representing the US population thyroid profile, years 2007-2012 NHANES dataset was used. An assumption was made that the thyroid hormone levels in the individuals of the NHANES data were at steady state at the time of blood sample collection. The deterministic model constructed above was optimized by varying a subset of model parameters such that the thyroid hormone levels of the steady state model output match the thyroid data of each individual in the NHANES dataset. For this thesis research, the following parameters, which uniquely determine the hormone steady-state values in the deterministic model, were chosen for optimization: k1, k3, k8, Kd2, and TBGtot. In MatLab, an objective cost function was formulated which is equal to the sum of the squares of the percentage differences between model output and NHANES data. The "fmincon( )" function in MatLab was used to search the parameter subspace for optimization through minimizing the cost function. The search range was 100-fold above and below the default values. The optimized parameter values for each NHANES individual excluding those taking thyroid medications and diagnosed thyroid cancers were recorded and as a result a reference US population model of the HPT axis was obtained, from which a random set of parameters can be chosen to reproduce the thyroid profile representing a thyroid-wise health US population.

Statistical Methods

Study Design and Population

The datasets analyzed consisted of three 2-year cycles of data (2007 - 2012), retrieved from the National Health and Nutrition Examination Survey (NHANES) database conducted by the National Center for

Health Statistics (NCHS), under the Centers for Disease Control and Prevention (CDC). The design of the NHANES database is cross-sectional and the samples within the study represent the general population within the United States, with oversampling of certain demographics such as blacks and Hispanics Americans to produce statistically relevant results pertaining to these listed population groups. Demographic information, medical history, and race/ethnicity were collected from interview, while urine and blood samples were collected via physical examination/specimen collection and then analyzed in a laboratory. The analysis was conducted on the entire represented population of 12 – 80 years of age. Participants taking thyroid medication and/or diagnosed with thyroid cancer (n=36) were excluded from the analysis. A total of 10241 individuals were included in conducting our final analysis.

Study Variables

The primary outcome variables are thyroid profile hormones, which could indicate hyperthyroidism or hypothyroidism. Data pertaining to age, gender, race and ethnicity, sex, income, pregnancy status and BMI were retrieved from the questionnaire and examination data in NHANES included in the analysis. These variables have been associated with variations in thyroid profile (Andrady et al, 2015). Blood samples were obtained to measure the serum concentration of thyroid profile variables such as free T4, free T3, TSH, total T4, total T3, thyroperoxidase antibody (TPO-AB) and thyroglobulin antibody (TG-AB). Smoking was also included in the analysis by including serum cotinine which is a biomarker of tobacco use and has been implicated in causing thyroid hormone disruption (Murphy et al., 2017). Urine samples were obtained and analyzed to determine the concentration of EDCs, and we focused our interest in environmental phenols (BPA, BP3, butyl paraben, methyl paraben, propyl paraben and ethyl paraben) and the NIS inhibitors (nitrate, thiocyanate, and perchlorate), and for HTP model analysis also perfluorinated chemicals (PFCs) including PFDE, PFOA and PFOS for this thesis. Urinary creatinine measures were used to adjust for the urinary concentration of the identified endocrine disruptor chemicals across individuals by age, sex and race. Previous studies shown associations between these chemicals and thyroid hormone disruption, however controversies are still

present linking some of these associations, and some of these chemicals have unknown mechanism of action as stated above. By using the dynamic HPT model, our analysis sets out to develop and propose an alternative method in toxicological risk assessment to predict some of the mechanisms of action of these chemicals. Individuals without thyroid profile measurements listed above were excluded from the analysis. Analysis was performed with the EDCs, and log-transformed thyroid profile variables listed above and the optimized HPT model parameter sets. Correlation analysis were performed between individual thyroid profile variables, HPT model parameters, and EDCs. Multiple linear regression models were run with log transformed thyroid profile variables as the dependent variables of interest, and log transformed EDCs, BMI as well as age, sex, race, cotinine, creatinine to control for urinary measures of the identified endocrine disruptors.

Statistical Analysis

The statistical analysis conducted for this study was done primarily in SAS 9.4. The PROC SURVEYMEANS and PROC FREQ procedures were used to derive descriptive statistics for age, sex, BMI and race in SAS (Table 3). The PROC SURVEYMEANS procedure was then used to explore the descriptive statistics for the thyroid profile variables for the population (Table 4A), and by racial categories (whites, blacks, and Hispanics) (Table 4B). The PROC SURVEYMEANS procedure was then used to explore the descriptive statistics for iodine, cotinine and EDCs for the population (Table 5A), and by racial categories (whites, blacks, and Hispanics) (Table 5B). The PROC TTEST procedure was used to compare the difference in the means between males and females for thyroid panel variables (Table 6). The impetus for this was to deduce if there is a difference in thyroid panel variables between males and females.

The PROC CORR procedure was used to evaluate Pearson correlation between log-transformed thyroid profile variables and log-transformed, creatinine-corrected EDCs (Table 7). The EDCs were corrected with creatinine prior to running the correlation analysis to give a more accurate measure of

the urinary concentrations of the EDCs, hence more accurate correlation analysis. The PROC SURVEYREG procedure was used to conduct several multiple linear regression models with log-transformed thyroid profile variables as dependent variables of interest and with independent variables including log-transformed EDCs classified into two groups (NIS inhibitors and the environmental phenols), BMI, age, race, sex, log-transformed creatinine and log-transformed cotinine as independent variables (Table 8 and 9). Log transformation of endocrine disrupting chemical variables as well as thyroid profile variables was done as the transformed data conform more to normal distributions and also lessen the effect of extremely high measurement values on the analysis. The PROC SURVEYMEANS and PROC SURVEYREG procedures were used to account for survey weights, thereby making the analysis representative of the US population. Hierarchical clustering of EDCs based on thyroid profile data and/or HPT model parameters was performed by using the pdist( ), squareform( ), linkage( ), and dendrogram( ) functions in MatLab. Specifically, if a chemical is significantly positively correlated with a hormone or parameter, +1 is assigned to qualitatively describe the relationship between this hormone or parameter and this particular chemical; if a chemical is significantly negatively correlated with a hormone or parameter, -1 is assigned; if a chemical is not correlated with a hormone or parameter, 0 is assigned. At last, Euclidian distance was used to calculate the similarity between chemicals.

RESULTS

Deterministic HPT Model

The constructed deterministic HPT model produces steady-state levels of free T4, free T3, TSH, total T4 and total T3 at concentrations indicated in Table 2, which are comparable to the mean values in a general human population (Hoermann et al., 2015). To test the capability of the dynamic model of simulating altered thyroid conditions, parameters k1, which governs the synthesis rate of thyroid hormones, and k3, which governs the synthesis rate of TSH, were varied separately from the default values in the model. Figure 2 shows the model output. By increasing k1 by 10-fold, both free T3 and free T4 increase by about 50%, whereas TSH decreases by nearly 10-fold, indicating a primary hyperthyroid condition (Figure 2A). In contrast, by decreasing k1 by 10-fold, both free T3 and free T4 decrease by about 30%, whereas TSH increases by about 6-fold, indicating a primary hypothyroid condition (Figure 2B). By increasing k3 by 10-fold, both free T3 and free T4 increase by about 50%, and TSH also increases by about 50%, indicating a secondary hyperthyroid condition (Figure 3C). By decreasing k3 by 10-fold, both free T3 and free T4 decrease by about 40%, and TSH also decreases by about 40%, indicating a secondary hypothyroid condition (Figure 3D). These simulation results demonstrated that the deterministic HPT model is capable of reproducing the four major non-cancer thyroid clinical conditions.

Population HPT Model

Using the deterministic HPT model, we next set out to obtain a population HPT model by using the NHAMES thyroid profile data excluding those individuals taking thyroid medications and having thyroid cancers. After parameter optimization, which minimizes the differences between the NHANES data and model hormone output by searching the parameter space, distributions of the following parameters, which can uniquely determine the hormone steady-state levels, were obtained (Figure 3): k1: TSH-stimulated T3/T4 production rate constant; k3: TSH production rate constant; k8: T4-to-T3 conversion rate constant; Kd2: dissociation constant for binding between free T3 and TBG;

TBGtot: total TBG. These parameter distributions allow the HPT model to output hormone distributions (Figure 4) comparable to the US population in the 2007-2012 NHANES dataset with similar statistics (results not shown).

Statistical Summary of NHANES Data

The demographic variables age, sex, BMI and race are summarized in Table 3. The mean age in the population is 43.9 with a standard deviation of 20.71. The minimum and maximum age among study participants is 12 and 80 respectively. There is an almost even distribution among sexes with 50.01% of the population male, and 49.9% female. The mean BMI of the study participants was 28.07 kg/m^2 with a standard deviation of 6.77. There were 121 individuals with unmeasured BMI, and the minimum and maximum BMI recorded was 13.18 kg/m^2 and 73.43 kg/m^2 respectively. Whites accounted for the majority among racial divisions with 43.36%, blacks 20.64% and Hispanics 36%.

Thyroid profile variables free T4, free T3, TSH, total T4, total T3, TPO-AB and TG-AB are summarized in Table 4 by the population, as well as by racial categories (white, black and Hispanic). Chemical variables iodine, cotinine, NIS inhibitors (nitrate, perchlorate, thiocyanate) and environmental phenols (BPA, BP3, butyl paraben, methyl paraben, propyl paraben and ethyl paraben) are summarized in Table 5 for the population, as well as by racial categories (white, black and Hispanic).

Table 6 summarizes the difference in the means between males and females for thyroid profile variables. There was a statistically significant difference in thyroid profile variables free T4, free T3, TSH, total T3, total T4, TPO-AB and TG-AB ($p < .0001$). free T4, TPO-AB, total T4, TSH and TG-AB levels were higher and free T3 and total T3 were lower in women. TPO-AB and TG-AB are markers of pathologies in the thyroid gland such as thyroiditis, and autoimmune disease, and the difference in the mean concentration is outstanding with TPO-AB (Males: 10.75; Females: 25.69), and TG-AB (Males: 7.15; Females: 11.57).

Correlation Analysis

Pearson correlation analysis results of thyroid profile variables and NIS inhibitors are summarized in Table 7A. Perchlorate has a negative correlation with free T4, free T3, total T3 and total T4 and positive correlation with TSH, conforming to its NIS inhibitory role produces primary hypothyroid hormone profile. k1, which governs the thyroid hormone synthesis rate, is the only model parameters significantly correlated with perchlorate, thus further confirming the mode of action of perchlorate (Figure 5). Perchlorate however demonstrated a positive correlation with TPO-AB and TG-AB (<.0001). This is indicative that perchlorate exposure increases the likelihood of developing thyroiditis, or autoimmune diseases of the thyroid. Thiocyanate demonstrates negative correlation with free T4, TSH, and total T4, and no statistical significant correlation with free T3, and total T3. With model parameters, thiocyanate shows the most significantly negative correlation with k3 and positive correlation with k8 (Figure 5). This result suggests novel action sites of thiocyanate where it may also act centrally on the pituitary gland as well as on T4-to-T3 conversion. Thiocyanate is negatively correlated with TG-AB and TPO-AB. Nitrate demonstrates a negative correlation with total T4 ($p<.0001$), however there is no statistical significant correlation with other hormones.

Correlation analysis results of thyroid profile variables and environmental phenols are summarized in Table 7B. BPA demonstrates a negative correlation with free T3 and free T4 respectively, however no statistical significant correlation was noted with total T3, total T4, TSH, TPO-AB, and TG-AB. Interestingly, k3 is the only model parameter significant correlated (negatively) with BPA, suggesting BPA may cause hypothyroidism through inhibiting TSH secretion. BP3 demonstrates a negative correlation with free T3, free T4 and total T4, with no statistically significant correlations for the other thyroid panel variables. k1 is the only model parameter that is significantly correlated with BP3, suggesting an inhibitory role of BP3 on thyroid secretion. Triclosan demonstrated significantly negative correlation with free T3, free T4, total T3, k1, and positive correlation with TSH (Figure 5), suggesting

a similar mechanism as BP3. The paraben family, including methyl, ethyl, propyl, and butyl paraben, shows strong negative correlation with free T3 ($p < .0001$) and total T3. k3 and k8 appear to be the primary model parameters that are affected by these compounds. Ethyl paraben is slightly positive correlated with TPO-AB ($p=0.036$), propyl paraben is negatively correlated with free T4 ($p=0.01$).

With respect to the PFC family, which includes PFOA, PFOS, and PFDE, they are negatively correlated free T3 and total T3 and positively correlated with TSH (Table 7C). k1 and Kd2 are the most significantly correlated (negative) model parameters, suggesting an inhibitory action on the thyroid gland by these chemicals and interference with T3 binding to TBG. PFDE and PFOS (Figure 5) also seem to disrupt k8, while PFDE and PFOS affect total TBG.

Hierarchical Clustering

Hierarchical clustering using the qualitative correlation between a chemical and thyroid hormone and optimized model parameters was performed. If the clustering only used hormone profiles, only a crude clustering was obtained (Figure 6, top panel). However, when optimized model parameters were added as additional dimensions, much more refined clustering results were obtained (Figure 6, lower panel).

Multiple Linear Regression

Multiple linear regression procedures for NIS inhibitors are summarized in Table 8. Perchlorate and thiocyanate have negative effects on free T4 and total T4 ($p < .0001$) respectively. Nitrate, perchlorate and thiocyanate all effect TG-AB levels with nitrate and perchlorate having a negative effect ($p=0.0147$, 0.0207) respectively, and thiocyanate having a positive effect ($p<0.0001$). free T3, total T3, TSH and TPO-AB are not significantly correlated with the three NIS inhibitors. Interestingly urinary iodine is positively correlated with free T3, total T3 and total T4, but not free T4 and TSH.

Multiple linear regression procedures for environmental phenols are summarized in Table 9. BP3 and butyl paraben have negative effects on free T4 ($p=0.015$, 0.0093) respectively. BPA has a negative effect on TSH ($p=0.0296$). Ethyl paraben has a negative effect on total T3 ($p=0.0039$). BPA. BP3, methyl paraben, propyl paraben, and ethyl paraben have negative effects on total T4 ($p=0.0002$, 0.0086, 0.0287, 0.0245, 0.0061) respectively. BPA showed a positive effect on TG-AB levels ($p=0.0232$), however BP3 demonstrated a negative effect on TG-AB levels ($p=0.0066$). Neither of the chemicals in this family is significantly correlated with free T3 and TPO-AB.

DISCUSSION

To conduct effective risk assessment for EDCs, it becomes increasingly imperative that a mechanistically-based mathematical model of the hypothalamic-pituitary-endocrine axes is constructed such that it can integrate information collected across multiple scales of the endocrine organization, including data concerning molecular, cellular, organism-level perturbations due to exposure to environmental EDCs. Such a model should ideally capture the physiology of the endocrine system to allow integration of the multi-scale data in a mechanistically-coherent manner. Past and ongoing molecular and cellular studies of the normal physiology of the endocrine system allow one to formulate a computational model of a hypothalamic-pituitary-endocrine axis around its operating set-point and simulate its robust capability of resisting perturbations exerted by internal or external variations. As far as EDCs are concerned, large amount of data exists and are being generated at both the molecular and cellular levels as an effort to understand their mode of action of toxicity and screen for toxicity using animal-alternative methods, as exemplified in the EPA ToxCast and multi-agency Tox21 efforts (Judson et al., 2009; Shukla et al., 2010). How to effectively utilize these data beyond their screening purpose has become a challenging issue. On the other hand, there are many cross-sectional epidemiological surveys and measurements interrogating the endocrine health and internal EDCs levels in large human populations, as exemplified by the NHANES study. How to utilize these population data beyond their primary association study purpose is another challenging issue.

The present thesis study was aimed to formulate an HPT axis model that is based on the feedback regulation between TSH and T3 and T4. Although not obtainable in the present theses, our ultimate goal with future versions of this model is to be able to incorporate multi-scale data into the model and make health risk predictions for thyroid disruptors. While the HPT model captures the main thrust of the feedback regulation for maintaining thyroid hormone homeostasis, the HPT model was kept sufficiently simple by having a minimal set of parameters at the moment to minimize overfitting issues due to small number of dimensions of the human data used to optimize the model.

TSH and thyroid hormones (T3 and T4) form a negative feedback loop where TSH stimulated thyroid hormone secretion and T3/T4 inhibits TSH secretion. To achieve robust homeostasis, i.e., keeping the controlled variables (T3 and T4 in the HPT model) within a narrow-preset range, there must be a reasonably high signal amplification (gain) within the feedback loop (Zhang et. al, 2007). Normal T3 and T4 serum levels vary in a very narrow range in human population (about 2-fold) while TSH can vary by a much larger extent (about 10-fold) (Figure. 4 and Table 4). And also, there appears to be an inverse relationship between TSH and T3/T4, indicating that the primary variation must originate in T3/T4, which through inhibiting TSH, leads to a negative correlation between TSH and T3/T4 (Hoermann et al., 2015). If the original variation comes from TSH, then a positive correlation would be expected. More importantly, the larger fold-change in TSH indicates that the signaling gain of the feedback loop must be situated in the arm of T3/T4 inhibition of TSH, rather than the arm of TSH stimulation of T3/T4 of the feedback loop. As a result, in the dynamic model a Hill function, which was used to amplify biochemical signal, was implemented as an inhibition term in the ODE of TSH (Equation 1) to describe the feedback regulation. This implementation allows the model to simulate clinical non-cancer thyroid conditions in a quantitative way. As shown in Figure 2A and 2B, varying parameter k1 to alter T3/T4 production leads to relatively smaller fold change in T3/T4 but opposite, much larger fold change in TSH, a hormonal profile conforming to clinical primary hyper- or hypothyroidism. This is a design principle by nature where the range control of TSH is sacrificed for a tight control of the T3/T4 range, which is much more physiologically important. In contrast as shown in Figure 2C and 2D, varying k3 to alter TSH production leads to similar fold changes in both TSH and T3/T4 of same direction, a hormonal profile conforming to clinical secondary hyper- or hypothyroidism. The steady-state output of this model produces hormone levels that are comparable to human population means (Table 2).

To make the model useful for population risk assessment, it is necessary to parametrize it so that it can reproduce population distributions of these thyroid profile hormones. The NHANES 2007-2012 thyroid profile data provide a very useful resource to calibrate the model for a reference population after excluding those individuals taking thyroid-related medications or having thyroid cancers. The thyroid profile data measured 5 hormones, free T3, free T4, TSH, total T3 and total T4. The HPT model also outputs 5 hormones, free T3, free T4, TSH, T3-TBG and T4-TBG. Because the bound forms of T3 and T4 are primarily with TBG, an assumption was made here that T3-TBG and T4-TBG are equivalent to total T3 and total T4, respectively. The computational HPT model has 12 parameters (Table 1). Clearly if all the parameters are used to optimize the model against the NHANES thyroid profile data, it will result in highly correlated parameter distributions because multiple combinations of parameter values can give rise to same hormone levels. To alleviate this problem, a subset of parameters, which can uniquely determine the hormone levels and are likely to be disrupted by EDCs, were chosen: k1, k3, k8, Kd2, and TBGtot. Optimization against the NHANES data led to distributions of these parameters as shown in Figure 3. Most of these parameters are distributed in a log-normal fashion within 2 orders of magnitude. The distributions of these parameters likely reflect variabilities of genetic, epigenetic, and environmental factors among individuals in the NHANES survey. Randomly selecting parameter combinations from these distributions establishes a reference thyroid population as shown in Figure 4. In future, relevant *in vitro* data which measure the effects of a particular chemical on a parameter can be applied to this virtual thyroid population to predict the population effects of the chemical at a particular concentration. In addition, chemical mixer effects can also be predicted based on the corresponding parameters disrupted by the mixed chemicals.

Many of the individuals in the NHANES dataset were also measured for their environmental chemical concentrations in their urine. Such data provide an opportunity to examine the association between chemical exposure and thyroid effects. With such an epidemiological study alone, it is hard to reveal insights into the thyroid disrupting mechanisms of the chemicals. The population model and the

optimized parameter distributions provide a chance to link chemical exposure to alteration of a particular model parameter for a mechanistic insight. Also, because biochemical processes, which are captured by model parameters, are among the initiating event of EDCs, it may be more sensitive to detect significant changes in these parameters in situations where there are no significant alterations in the hormone profile because of the feedback regulation. In this thesis study examining correlations between model parameters and urine EDCs of the NHANES data confirmed some of the known mode of actions and revealed potentially novel ones.

Perchlorate is a typical NIS inhibitor, which blocks iodide uptake by the thyroid gland leading to reduction in thyroid hormone secretion in the form of primary hypothyroidism. The NHANES data is clearly consistent with this mode of action, where both T3 and T4 were significantly correlated negatively with urinary perchlorate and TSH was significantly correlated positively with urinary perchlorate (Figure 5 and Table 7A). Reassuringly, among the model parameters optimized, only k1, which is the rate constant for thyroid hormone production, is significantly correlated negatively with perchlorate, thus confirming the mode of action of perchlorate. In contrast, thiocyanate, which is also believed to be an NIS inhibitor, is associated with a very different thyroid profile where both T4 and TSH are significantly correlated negatively with thiocyanate, a hormone profile consistent with secondary hypothyroidism (Figure 5 and Table 7A). The insignificant changes in T3 makes it hard to pinpoint the exact mechanism of thiocyanate here. However, the model parameter distribution predicted that with thiocyanate exposure, k3 which governs the production of TSH and k8 which governs the T4-to-T3 conversion, are significantly correlated negatively and positively with thiocyanate, respectively. Absent other confounding chemical exposures, such results would suggest that thiocyanate may promote the activity of deiodinase converting T4 to T3. Centrally in the pituitary, such increased conversion will lead to increased local T3, thus increased inhibition of TSH secretion, which is consistent with decreasing k3 values. Decreased TSH will lead to decreased T4 and T3, but

peripherally increased deiodinase activity will convert more T4 to T3, which further decreases T4 while compensating for T3, resulting in a thyroid hormone profile like the NHANES data.

As reviewed in Introduction, multiple thyroid disrupting mechanisms have been proposed for environmental phenols. The NHANES data shows clearly that T3 is significantly correlated negatively with the four parabens measured with mixed result on T4 and TSH (Table 7B). Mechanistically, the model parameter distributions showed negative correlation of k3 and k8 with parabens. Such a result suggests that parabens may inhibit T4-to-T3 conversion and acting centrally to inhibit TSH secretion. The latter is consistent with the finding that butyl paraben may function as a thyroid hormone receptor agonist (Taxvig et al., 2008). Triclosan exposure is significantly correlated negatively with T3 and T4 and positively with TSH, consistent with a primary hypothyroidism profile (Figure 5 and Table 7B). Model parameter distributions show that k1 is the only significantly altered parameters by triclosan. This is consistent with the proposed mechanism for triclosan which may inhibit NIS non-competitively and TPO, thus inhibiting thyroid hormone secretion (Wu et al., 2016). BPA shows significant negative correlation with free T3 and T4 and model parameter k3, suggesting a central role of BPA inhibiting TSH. BPA has been demonstrated to exert its effect at the level of the pituitary in addition to the thyroid gland leading to thyroid hormonal production perturbations (Lee et al., 2017).

A far as PFCs are concerned, all PFDE, PFOA and PFOS show significant negative correlation with free T3, positive correlation with TSH, and positive correlation with k1, consisting with a mode of action of inhibiting thyroid hormone secretion at the level of thyroid gland (Table 7C). In addition, Kd2 and TBGtot appear also to be significantly correlated with the chemicals, suggesting their possible role in interfering with TBG binding. For PFDE and PFOS, k8 is also negatively correlated, suggesting inhibition of deiodinase, which will help keep T4 level unchanged in the presence of significant increases in TSH. In comparison, PFOA is also negatively correlated with free T4 without the negative correlation with k8.

Chemical toxicants can be classified based on several features, including their structures, physio-chemical properties, biological targets, hazards, and at a higher level, endpoint outcomes. The grouping of EDCs can be based on chemical structures, disrupted thyroid hormone profile, or known mechanisms. However, many of the EDCs have unknown thyroid disrupting mechanisms, thus endpoint alterations, such as thyroid profile changes, can only provide limited reliable information for chemical clustering or classification, especially due to the negative feedback regulation often making changes in T3 and T4 too small to produce statistically significant results in a variable population. Hierarchical clustering based on the Pearson correlation results demonstrated that only crude results can be obtained if using thyroid hormone profile as the only input features (Figure 6). In comparison, when the optimized model parameters are used as additional features, a much more refined clustering resulted (Figure 6). While parabens are grouped together and are close to BPA and 4-tert-octylphenol as environmental phenol family, triclosan is in the same group as perchlorate, iodine, and PFOS, although chemically they belong to very different families. Interestingly thiocyanate is in a group of its own, keeping with the surprising result regarding its potential novel mechanisms presented above.

In addition to dynamic modeling, the present thesis also conducted epidemiological studies of the thyroid profiles and EDCs with respect to sex, age, race, and smoking etc. The results of this study demonstrate that females are differentially affected by EDCs compared to their male counterpart. The mean concentration for the EDCs analyzed in this paper are significantly higher in females in comparisons to males, in all except for nitrate. This can be secondary to the lipophilicity with respect to the environmental phenols, leading to a higher bioavailability in females compared to males (Schmidt et al., 2002). Nitrate, perchlorate and thiocyanate are all univalent anions that are metabolized by enteric bacteria prior to entering circulation, and are metabolized and excreted by the kidneys afterwards (Schultz et al., 1985). Renal function has been demonstrated to be different between males and females, and this can therefore be responsible the difference in the concentration of the univalent anions

concentrations (perchlorate and thiocyanate) between males and females (Sabolić et al., 2007). The correlation analysis revealed the points of perturbations among the thyroid panel variables, and offered a preliminary description of the mechanism of action of these chemicals. The EDCs analyzed in this paper demonstrate mainly an increased likelihood to cause hypothyroid states as evidenced by the negative correlation with the thyroid hormones. However, the correlation with TSH is chemical-dependent, suggesting different hypothyroid mechanisms of the EDCs or co-exposures to other EDCs of different thyroid-disrupting mechanisms. The MLR models provided a more comprehensive analysis of the association between EDCs and thyroid panel variables. Perchlorate and thiocyanate demonstrate a negative association with free T4 and total T4, which is indicative of hypothyroidism in keeping with its definition in literature as a blocker of NIS. Nitrate, perchlorate and thiocyanate also demonstrate associations with TG-AB with nitrate and perchlorate demonstrating negative associations and thiocyanate demonstrating a positive association. Thyroperoxidase antibodies and thyroglobulin antibodies have been linked to the development of Hashimoto's thyroiditis which is a cause of hypothyroidism, as well as thyroid carcinoma posing additional reasons for concern in individuals exposed to thiocyanate (Ott et al., 1987).

The environmental phenols were also demonstrated to have negative associations with thyroid hormone variables which lead to hypothyroidism. BPA also show statistically significant positive associations with TG-AB, which can potentially lead to a hypothyroid state, with the worse endpoint potentially being thyroid carcinoma. The elevated levels of TG-AB noted with exposure to thiocyanate and BPA, which are suggestive of a casual association for autoimmune thyroid disease, may be resultant of underlying thyroiditis caused by exposure, or can be related to a direct consequence of exposure, hence further research to determine this relationship need to be conducted.

The effect of the NIS inhibitors and environmental phenols on thyroid panel variables differentially affect women in comparison to men. Women of reproductive age furthermore will be differentially

affected by this because of the increase requirement for thyroid hormones during pregnancy, and particularly with the profile generated by these chemicals showing a negative association with free T4 and total T4. This raises issues of more concern because T4 is the only form of thyroid hormone capable of crossing the placenta during the early gestational periods when the fetus does not autonomously produce thyroid hormones. As discussed above this can lead to potential harm with regards to fetal development, and therefore is imperative that it should be addressed.

LIMITATIONS AND FUTURE RESEARCH

Tease out co-exposure effect.

The population data used to optimize the dynamic model is cross-sectional in nature, and steady state hormone levels were assumed which may not be the case in certain individuals. Cross-sectional data provides descriptive information about the population that the samples were drawn from. Therefore, this poses challenges in the cause-effect pathway. Another limitation of the thesis study is the uncertainty of the confounding effects of co-exposed chemicals, which would bias the mechanistic insight into the EDC of interest. To address these issues, future research will need to focus on using human cell or organoid models to determine the mechanistic action of these EDCs which will provide more relevant results for the optimization of the dynamic model. These *in vitro* data can also be utilized by the population model to make quantitative *in vivo* predictions. In addition to the dynamic model used in this paper, future research will expand this model to contain more biochemical details such as thyroid hormone receptor signal and TSH signaling which can be targets of thyroid-disrupting EDCs.

FIGURES

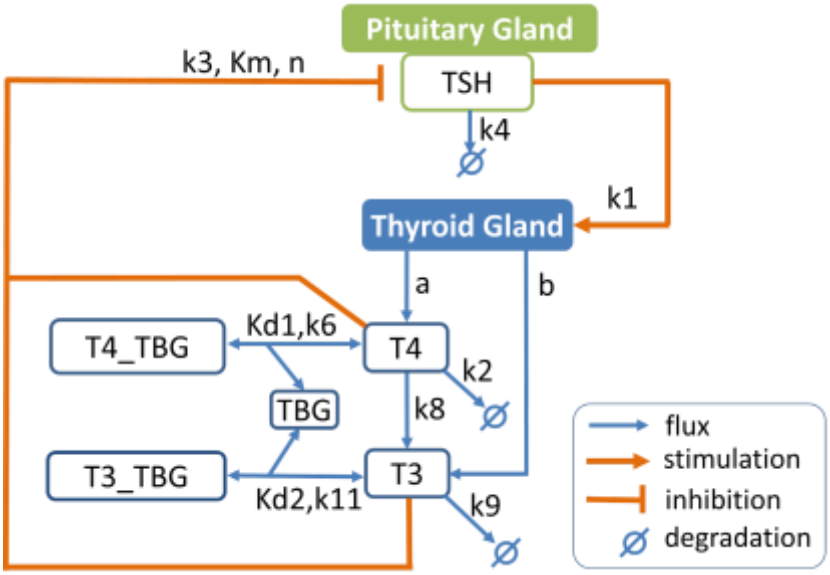

Figure 1. Deterministic model structure capturing main steps of thyroid hormone homeostasis and regulation: TSH-stimulated T4/T3 production, peripheral T4 conversion into T3, T4/T3 binding with TBG, T4/T3 metabolism and T4/T3 inhibition of TSH production. For simplicity, TRH in the hypothalamus was omitted. ODEs describing these physiological processes are provided below. Blue lines denote mass fluxes; orange lines denote regulatory events.

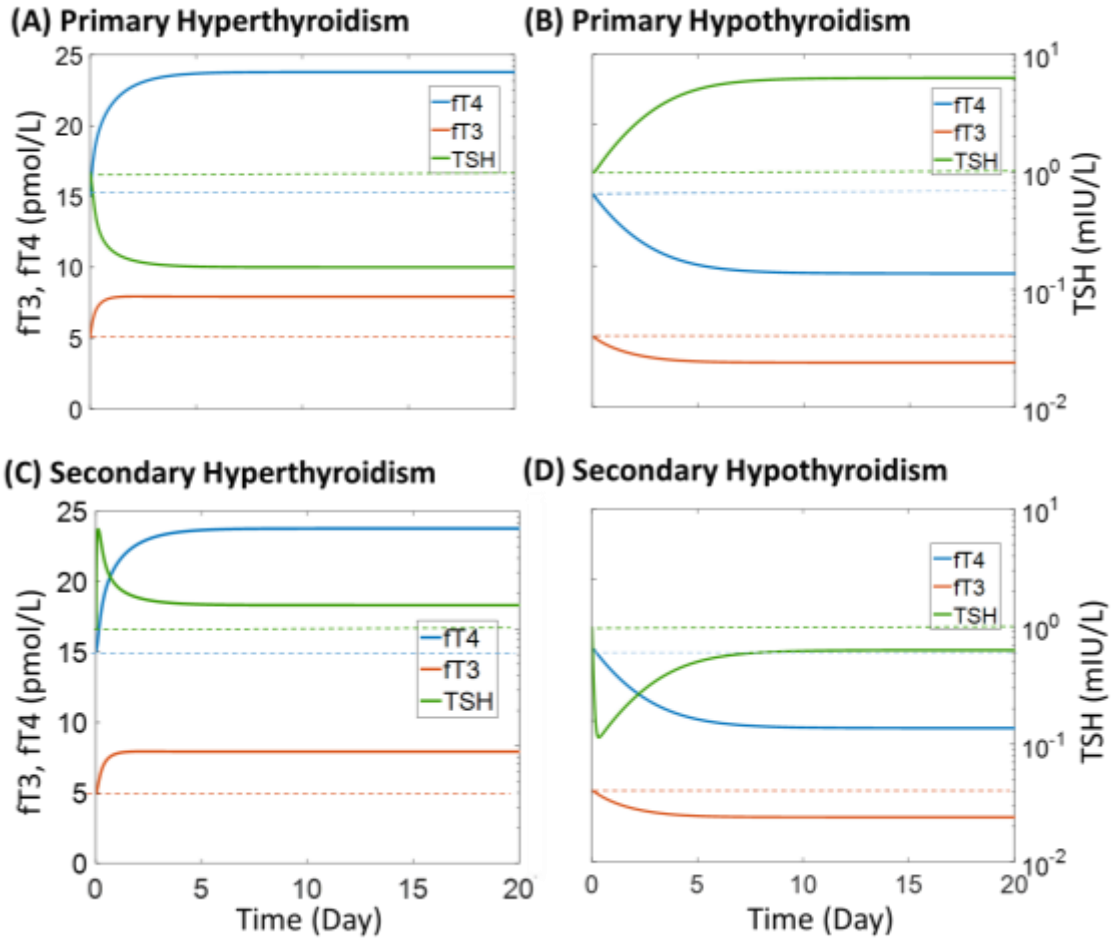

Figure 2. Model Simulation of primary/secondary hyper/hypothyroidism by increasing or decreasing parameters k1 by 10-fold and k3 by 10-fold, respectively. k1 controls the production rate of thyroid hormones; k3 controls the production rate of TSH.

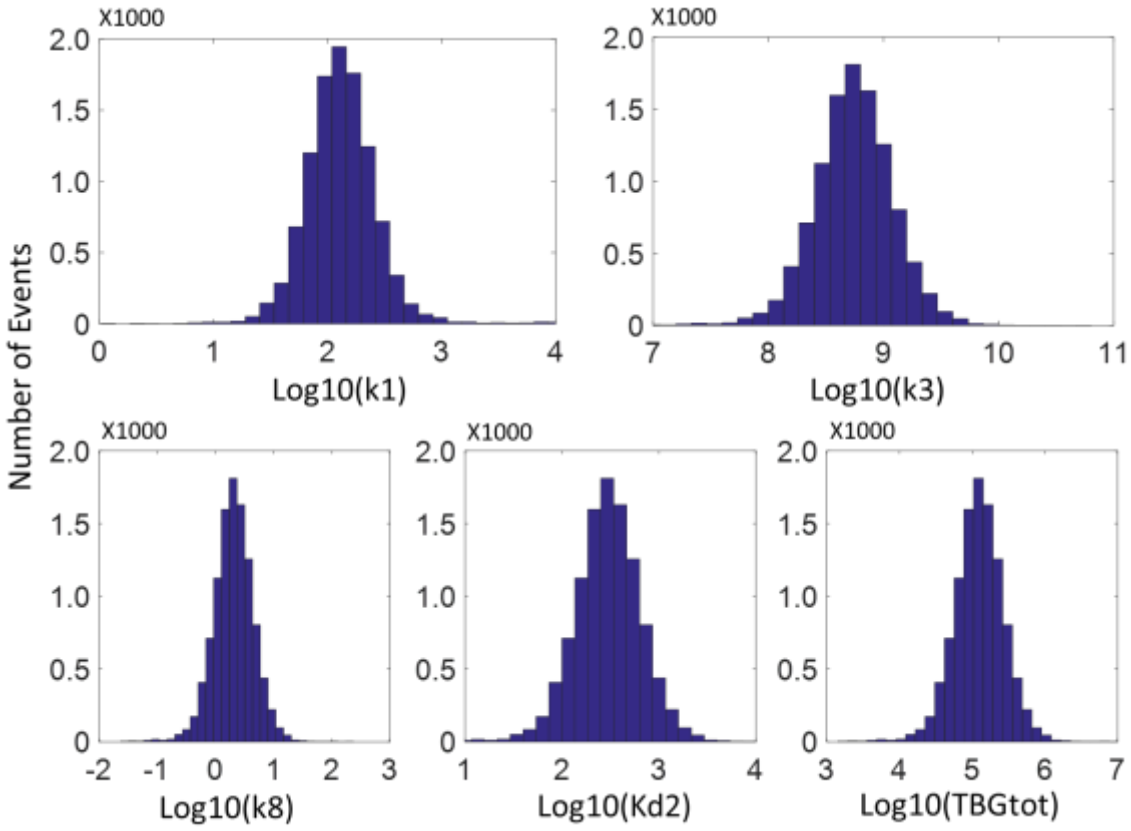

Figure 3. Distributions of model parameters optimized to NHANES thyroid profile data.

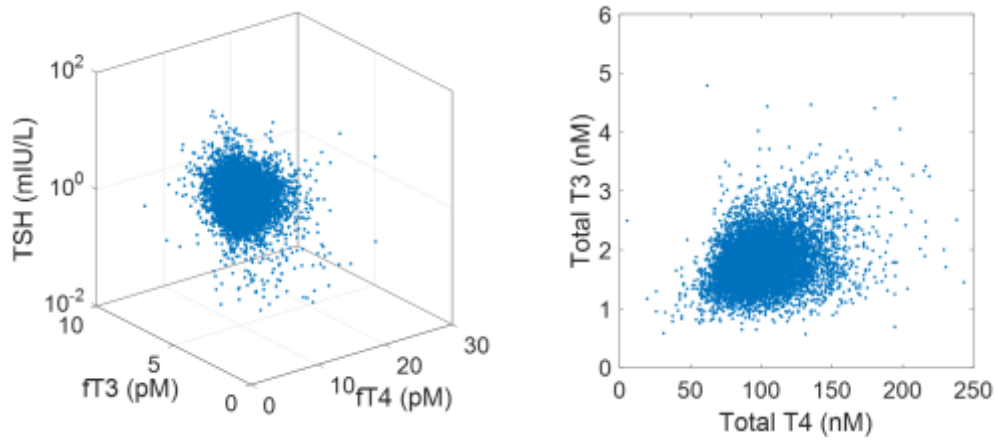

Figure 4. Statistical distributions of the population HPT model hormone output (10548 data points).

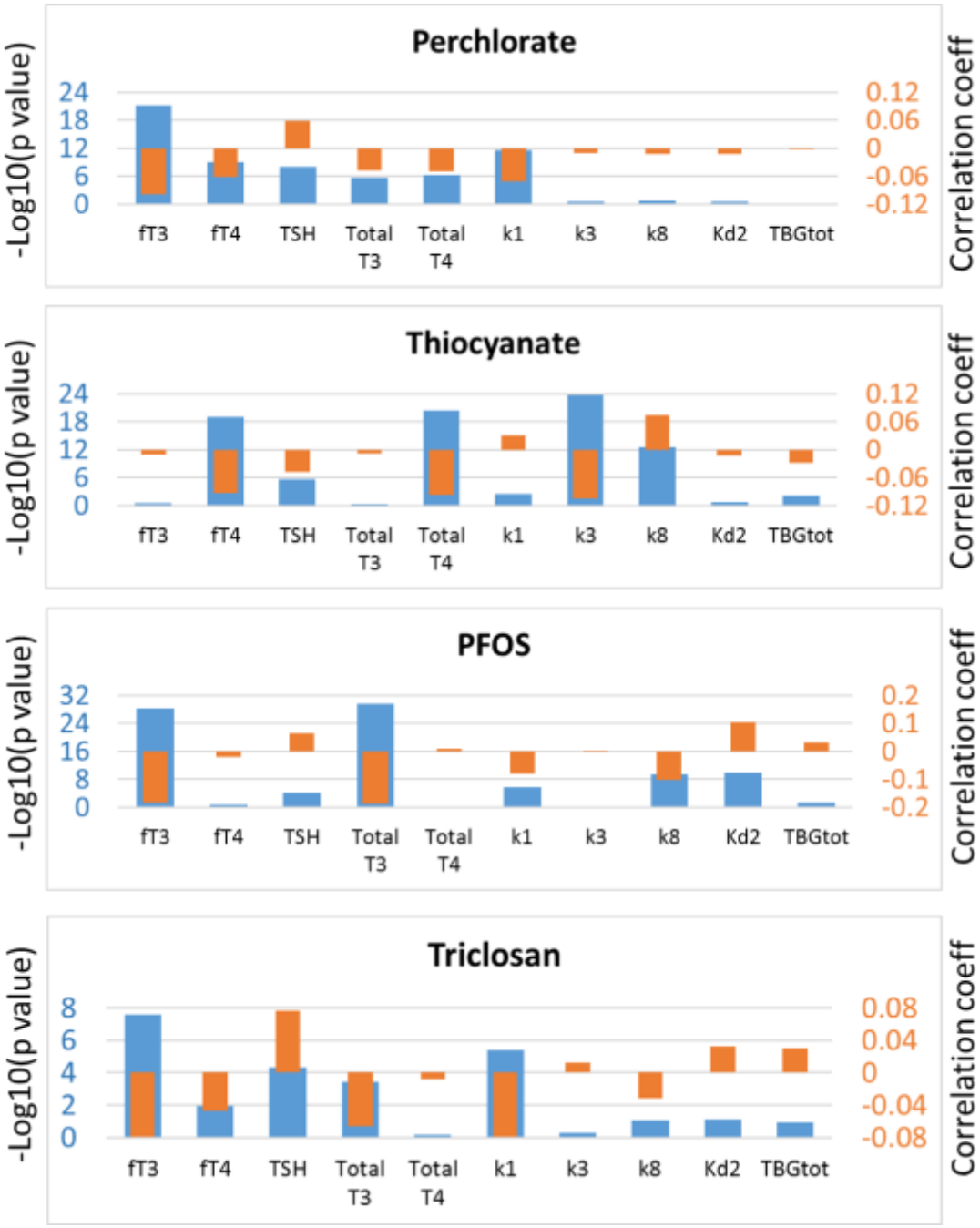

Figure 5. Pearson correlation analysis between thyroid data, optimized model parameters and perchlorate, thiocyanate, triclosan and PFOS.

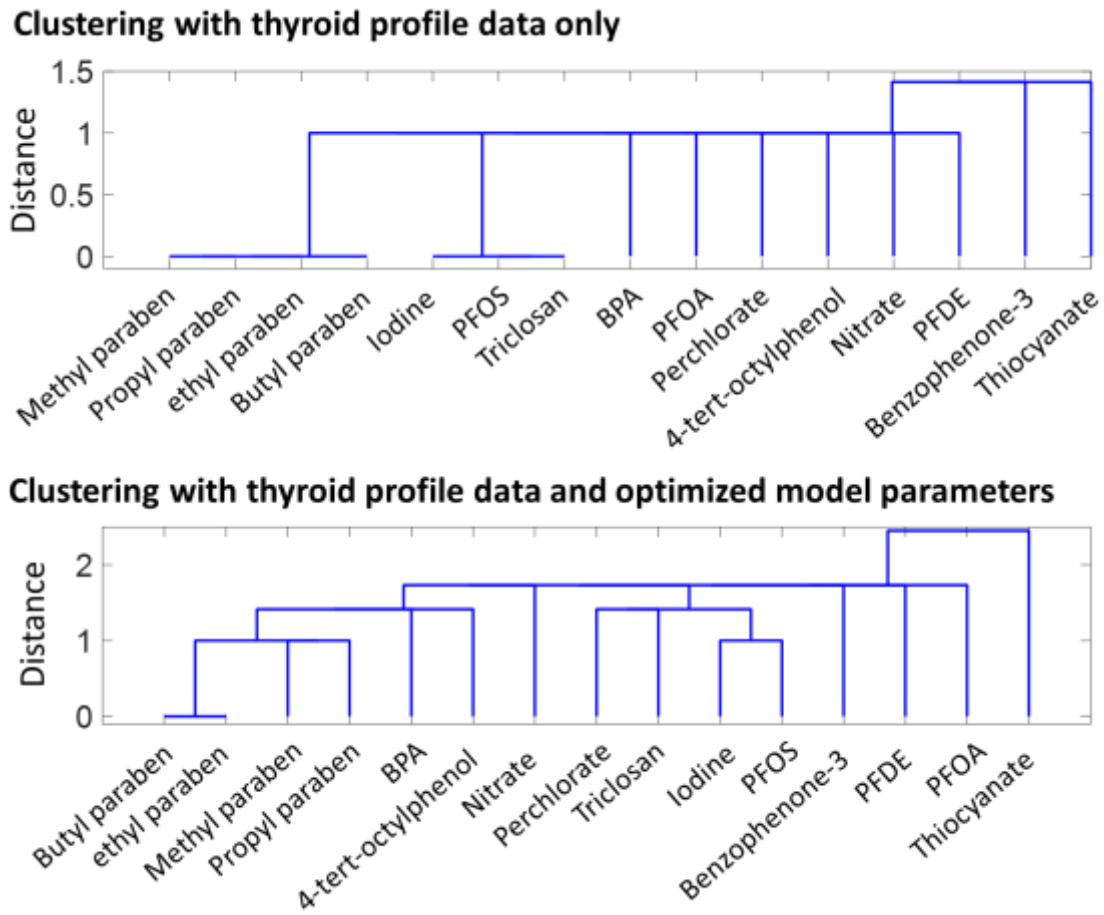

Figure. 6. Chemical clustering for thyroid effects using thyroid profile data only (top panel0 or using thyroid profile data and optimized model parameters (lower panel).

TABLES

Table 1. Deterministic HPT model parameters

Parameter Name	Value	Definition	Note
k1	256.14 pM/(mU/L)/h	Rate constant of TSH-stimulated synthesis of T4 and T3	
k2	11.935 h^{-1}	Rate constant of free T4 metabolism	T4 (total or free) in plasma half-life=5-7days (use 6 days here). Since 0.03% T4 is in free form which is the form being degraded, k2 and k8 are parameterized to make the total T4 half-life=6 days.
k3	1.11e9 mU/L/h	Max synthesis rate of TSH	
k4	0.693 h^{-1}	Rate constant for TSH clearance	TSH half-life=1 h
k6	69.3 h^{-1}	Dissociation rate constant for T4_TBG dissociation into free T4 and TBG	Assuming half-life = 36 sec
Kd1	60 pM	Binding affinity between free T4 and TBG	The literature has k6/k5 = 50-300 pM. This value also give percentage of TBG saturation around 25% and together with total TBG it allows free T4 to be 0.03% and TBG-bound T4 99.97%.
k8	4.11 h^{-1}	Rate constant for conversion of T4 into T3 by deiondinase in peripheral tissues	This process accounts for 80% of T3 production; this number also means that about 25% of T4 produced is converted to T3, and the remaining 75% of T4 is metabolized into something else.
k11	69.3 h^{-1}	Dissociation rate constant for T3_TBG dissociation into free T3 and TBG	Assuming half-life = 36 sec
Kd2	600 pM	Binding affinity between free T3 and TBG	This value determines that free T3 is 0.3% and TBG-bound T3 is 99.7%.
n	4	Hill coefficient of the Hill function describing the inhibition of TSH synthesis by T3 and T4	This value needs to be large such that T3 and T4 can be maintained within a narrow range by sacrificing TSH homeostasis, which can range more than 10-fold.

a	0.94	Fraction of T4 production by the thyroid gland.	So, this value makes the ratio of amounts of T4 and T3 produced by thyroid gland around 15.67:1, which accounts for 20% of T3 production. The remaining 80% of T3 production is through T4 conversion.
TBGtot	250 nM	Total TBG in blood	Total TBG concentration in blood is 180-350 nM

Table 2. Deterministic HPT model state variables

Variable Name	Steady-State Value	Definition
TSH	1 mU/L	TSH
T4	15 pM	Free T4
T4_TBG	50 nM	TBG-bound T4
T3	5 pM	Free T3
T3_TBG	1.66 nM	TBG-bound T3

Table 3. Descriptive Statistics on Study Variables (Age, Sex, BMI and Race), N=10241

Variable	Mean (SD)/n (%)	N Missing (%)	Min	Max
Age	43.90 (20.71)	N/A	12	80
Sex				
Male	5131 (50.01)	N/A		
Female	5110 (49.90)			
BMI (kg/m^2)	28.07 (6.77)	121	13.18	73.43
Race				
White	4440 (43.36)	N/A		
Black	2114 (20.64)			
Hispanic	3687 (36.00)			

BMI: Body Mass Index, SD: Standard Deviation

Table 4A. Descriptive Statistics for Concentration of Thyroid Hormones for Study Population

Population Statistics (n=10241)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Free T4 (pmol/L)	10241	10.34	2.18	7.7	13.5
Free T3 (pg/mL)	10241	3.25	0.58	2.6	4
TSH (uIU/mL)	10241	1.93	2.58	0.53	4.15
Total T4 (ug/dL)	10241	7.92	1.64	5.6	10.8
Total T3 (ng/dL)	10241	116.57	26.75	81	160
TPO-AB (IU/mL)	10241	18.21	87.55	0.18	73.8
TG-AB (IU/mL)	10241	9.35	88.37	0.6	7.3

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody, SD: Standard Deviation, CL: Confidence Limit

Table 4B. Descriptive Statistics for Concentration of Thyroid Hormones for Study Population by Race

Statistics for Whites (n=4440)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Free T4 (pmol/L)	4440	10.34	2.11	7.7	14.2
Free T3 (pg/mL)	4440	3.18	0.43	2.56	3.93
TSH (uIU/mL)	4440	2.14	2.96	0.56	4.7
Total T4 (ug/dL)	4440	7.78	1.57	5.6	10.6
Total T3 (ng/dL)	4440	113.72	24.82	78	157
TPO-AB (IU/mL)	4440	20.58	86.43	0.18	119.15
TG-AB (IU/mL)	4440	12.81	107.35	0.6	12.45
Statistics for Blacks (n=2114)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Free T4 (pmol/L)	2114	10.21	2.42	7.7	13
Free T3 (pg/mL)	2114	3.23	0.58	2.6	3.99
TSH (uIU/mL)	2114	1.59	2.3	0.46	3.39
Total T4 (ug/dL)	2114	7.92	1.79	5.5	11
Total T3 (ng/dL)	2114	116.3	28.03	80	161
TPO-AB (IU/mL)	2114	10.8	81.38	0.18	9.9
TG-AB (IU/mL)	2114	3.56	45.12	0.6	2
Statistics for Hispanics (n=3687)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Free T4 (pmol/L)	3687	10.41	2.12	7.7	13.3
Free T3 (pg/mL)	3687	3.34	0.71	2.7	4.1
TSH (uIU/mL)	3687	1.86	2.2	0.56	3.94
Total T4 (ug/dL)	3687	8.1	1.62	5.8	10.9
Total T3 (ng/dL)	3687	120.16	27.81	86	162
TPO-AB (IU/mL)	3687	19.6	91.99	0.18	73.9
TG-AB (IU/mL)	3687	8.52	81.32	0.6	7.6

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody, SD: Standard Deviation, CL: Confidence Limit

Table 5A. Descriptive Stats for Iodine/Cotinine/EDC Chemicals for Study Population

Population Statistics (n=10241)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Iodine (ug/L)	10043	363.32	7840.14	35.1	597.6
Cotinine (ng/mL)	10237	52.23	122.84	0.01	339
Nitrate (ng/mL)	10081	56926.89	52285.71	11100	131000
Perchlorate (ng/mL)	10081	5.53	8.37	0.76	15.7
Thiocyanate (ng/mL)	10081	2346.6	3471.76	177	9020
Bisphenol - A (ng/mL)	3919	3.36	7.03	0.28	10.5
Benzophenone-3 (ng/mL)	3919	268.54	2020.94	0.7	885
Butyl Paraben (ng/mL)	3919	4.01	27.31	0.14	14.4
Methyl Paraben (ng/mL)	3919	18.58	98.83	0.71	69.2
Propyl Paraben (ng/mL)	3919	246.66	542.02	3.5	1070
Ethyl Paraben (ng/mL)	3919	59.21	161.11	0.14	270

SD: Standard Deviation, CL: Confidence Limit

Table 5B. Descriptive Stats for Iodine/Cotinine/EDC Chemicals for Study Population by Race

Statistics for Whites (n=4440)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Iodine (ug/L)	4343	474.09	11628.63	34.5	638.7
Cotinine (ng/mL)	4439	68.04	139.76	0.01	379
Nitrate (ng/mL)	4365	54792.66	50169.94	10900	128000
Perchlorate (ng/mL)	4365	5.52	9.54	0.76	15.2
Thiocyanate (ng/mL)	4365	2656.01	3679.13	205	10000
Bisphenol - A (ng/mL)	1580	3.4	7.37	0.28	10.75
Benzophenone-3 (ng/mL)	1580	338.05	2772.67	0.6	980.5
Butyl Paraben (ng/mL)	1580	3.89	20.32	0.14	14.35
Methyl Paraben (ng/mL)	1580	18.35	114.33	0.71	62.25
Propyl Paraben (ng/mL)	1580	160.75	387.39	2.7	686
Ethyl Paraben (ng/mL)	1580	45.7	138.28	0.14	232
Statistics for Blacks (n=2114)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Iodine (ug/L)	2083	280.95	1635.42	34.7	567
Cotinine (ng/mL)	2113	65.85	138.93	0.01	383
Nitrate (ng/mL)	2094	55712.73	44968.25	10900	126000
Perchlorate (ng/mL)	2094	5.1	6.44	0.73	14.4
Thiocyanate (ng/mL)	2094	3015.81	4301.94	207	11900
Bisphenol - A (ng/mL)	894	3.8	5.95	0.28	11.2
Benzophenone-3 (ng/mL)	894	180.31	1022.85	0.6	591
Butyl Paraben (ng/mL)	894	4.55	45.44	0.14	9.2
Methyl Paraben (ng/mL)	894	25.17	111.66	0.71	90.9
Propyl Paraben (ng/mL)	894	409.02	756.58	6.4	1610
Ethyl Paraben (ng/mL)	894	85.29	202.15	0.5	391
Statistics for Hispanics (n=3687)					
Variable	**N**	**Mean**	**SD**	**95% CL for Mean**	
Iodine (ug/L)	3617	277.76	2600.31	35.9	559.5
Cotinine (ng/mL)	3685	25.36	78.73	0.01	200
Nitrate (ng/mL)	3622	60200.87	58230.92	11400	141000
Perchlorate (ng/mL)	3622	5.79	7.83	0.78	16.7
Thiocyanate (ng/mL)	3622	1586.83	2364.42	150	5530
Bisphenol - A (ng/mL)	1445	3.05	7.25	0.28	9.2
Benzophenone-3 (ng/mL)	1445	247.12	1420.44	0.8	929
Butyl Paraben (ng/mL)	1445	3.82	17.15	0.14	19.9
Methyl Paraben (ng/mL)	1445	14.77	66.75	0.71	61.3
Propyl Paraben (ng/mL)	1445	240.15	504.57	3.8	1080
Ethyl Paraben (ng/mL)	1445	57.84	153.69	0.2	273

SD: Standard Deviation, CL: Confidence Limits

Table 6. T Tests showing the difference in the means between Males and Females for Thyroid Panel Variables

Variable	Mean	95% CL Mean		Comparison by Gender			
Free T4 (pmol/L)				**Equality of Variances (Free T4)**			
Male	10.31	10.26	10.36	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	10.37	10.29	10.43	Folded F	5109	1.71	<.0001
Free T3 (pg/ml)				**Equality of Variances (Free T3)**			
Male	3.34	3.33	3.35	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	3.16	3.14	3.17	Folded F	5109	2.31	<.0001
TPO Antibodies(IU/mL)				**Equality of Variances (TPO-AB)**			
Male	10.75	9.01	12.49	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	25.69	22.79	28.59	Folded F	5109	2.75	<.0001
Total T4 (ug/dL)				**Equality of Variances (Total T4)**			
Male	7.66	2.4	23.1	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	8.18	2	27.6	Folded F	5109	1.28	<.0001
Total T3 (ng/dL)				**Equality of Variances (Total T3)**			
Male	118	117.3	118.6	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	115.2	114.4	116.0	Folded F	5109	5.32	<.0001
TSH (uIU/mL)				**Equality of Variances (TSH)**			
Male	1.87	1.82	1.91	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	1.98	0.002	99.564	Folded F	5109	3.76	<.0001
TG-AB (IU/mL)				**Equality of Variances (TG-AB)**			
Male	7.15	5.01	9.29	**Method**	**Num DF**	**F Value**	**Pr > F**
Female	11.57	8.89	14.25	Folded F	5109	1.55	<.0001

TG-AB: Thyroglobulin antibody, TPO-AB: Thyroperoxidase antibody

Table 7A. Correlation Analysis for NIS Inhibitors

Variable		Nitrate	Perchlorate	Thiocyanate
Free T4 (pmol/L)	rho	-0.01787	-0.0622	-0.09267
	p value	0.081	<.0001	<.0001
	n	9529	9549	9542
Free T3 (pg/mL)	rho	-0.0171	-0.0984	-0.0104
	p value	0.0948	<.0001	0.3082
	n	9529	9549	9542
TSH (uIU/mL)	rho	-0.0005	0.0584	-0.0483
	p value	0.0026	<.0001	<.0001
	n	9529	9549	9542
Total T3 (ng/dL)	rho	0.0145	-0.0486	-0.008
	p value	0.1581	<.0001	0.4326
	n	9529	9549	9542
Total T4 (ug/dL)	rho	-0.043	-0.051	-0.097
	p value	<.0001	<.0001	<.0001
	n	9529	9549	9542
TPO-AB (IU/mL)	rho	0.0299	0.0418	-0.0351
	p value	0.0035	<.0001	0.0006
	n	9529	9549	9542
TG-AB (IU/mL)	rho	0.0201	0.0201	-0.0359
	p value	0.0497	<.0001	0.0004
	n	9529	9549	9542
k1	rho	-0.0038	-0.0717	0.0304
	p value	0.7111	<.0001	0.003
	n	9529	9549	9542
k3	rho	-0.0165	-0.0103	-0.1042
	p value	0.1081	0.3156	<.0001
	n	9529	9549	9542
k8	rho	0.0046	-0.0125	0.0743
	p value	0.6547	0.221	<.0001
	n	9529	9549	9542
kd2	rho	-0.0547	-0.0119	-0.0122
	p value	<.0001	0.244	0.2315
	n	9529	9549	9542
TBGtot	rho	-0.0318	-0.0031	-0.0278
	p value	0.0019	0.7588	0.0066
	n	9529	9549	9542

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody, rho: correlation coefficient.

Table 7B. Correlation Analysis for Environmental Phenols

Variable		BPA	BP3	Butyl Paraben	Ethyl Paraben	Methyl Paraben	Propyl Paraben	Triclosan
Free T4 (pmol/L)	rho	-0.0375	-0.0441	-0.0196	-0.0109	-0.0249	-0.04331	-0.0475
	p value	0.0287	0.0082	0.4679	0.6482	0.13	0.0103	0.0110
	n	3401	3604	1370	1766	3700	3511	2862
Free T3 (pg/mL)	rho	-0.0456	-0.0698	-0.1788	-0.1546	-0.1559	-0.1643	-0.1039
	p value	0.0078	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	n	3401	3604	1370	1766	3700	3511	2862
TSH (uIU/mL)	rho	-0.0262	0.0228	0.0471	0.0052	0.0373	0.0261	0.0761
	p value	0.1272	0.1719	0.0816	0.8247	0.0234	0.122	<.0001
	n	3401	3604	1370	1766	3700	3511	2862
Total T3 (ng/dL)	rho	-0.022	-0.0318	-0.1131	-0.0941	-0.0812	-0.0933	-0.0664
	p value	0.1996	0.0566	<.0001	0.0001	<.0001	<.0001	0.0004
	n	3401	3604	1370	1766	3700	3511	2862
Total T4 (ug/dL)	rho	-0.028	-0.049	-0.009	-0.035	0.031	0.005	-0.0079
	p value	0.107	0.004	0.735	0.147	0.057	0.773	0.6741
	n	3401	3604	1370	1766	3700	3511	2862
TPO-AB (IU/mL)	rho	0.0326	0.0186	0.0262	0.0499	0.0234	0.0124	0.0485
	p value	0.0571	0.2643	0.3332	0.0361	0.1541	0.4625	0.0094
	n	3401	3604	1370	1766	3700	3511	2862
TG-AB (IU/mL)	rho	0.0102	0.0282	0.0628	0.0477	0.0147	0.0114	0.0331
	p value	0.5529	0.0901	0.0201	0.045	0.373	0.4982	0.0763
	n	3401	3604	1370	1766	3700	3511	2862
k1	rho	0.0155	-0.0332	-0.0607	-0.0087	0.0191	0.0051	-0.0862
	p value	0.3662	0.0465	0.0246	0.7133	0.2448	0.7624	<.0001
	n	3401	3604	1370	1766	3700	3511	2862
k3	rho	-0.0585	-0.0249	-0.0152	-0.0492	-0.0889	-0.0933	0.0119
	p value	0.0006	0.1357	0.5743	0.0387	<.0001	<.0001	0.5247
	n	3401	3604	1370	1766	3700	3511	2862
k8	rho	0.0013	-0.0103	-0.1112	-0.1018	-0.0875	-0.0775	-0.0320
	p value	0.9394	0.5352	<.0001	<.0001	<.0001	<.0001	0.0870
	n	3401	3604	1370	1766	3700	3511	2862
kd2	rho	-0.0016	-0.0238	0.0045	-0.0312	0.0319	0.029	0.0332
	p value	0.925	0.1528	0.8681	0.1906	0.0525	0.086	0.0760
	n	3401	3604	1370	1766	3700	3511	2862
TBGtot	rho	0.0033	-0.0167	0.0051	-0.0311	0.055	0.0413	0.0300
	p value	0.8453	0.3174	0.8495	0.1909	0.0008	0.0143	0.1087
	n	3401	3604	1370	1766	3700	3511	2862

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody, BPA: Bisphenol-A, BP3: Benzophenone-3, rho: correlation coefficient.

Table 7C. Correlation Analysis for PFCs

Variable		PFDE	PFOA	PFOS
Free T4 (pmol/L)	rho	-0.0154	-0.052	-0.0213
	p value	0.416	0.0017	0.1988
	n	2800	3652	3655
Free T3 (pg/mL)	rho	-0.1779	-0.1029	-0.183
	p value	<.0001	<.0001	<.0001
	n	2800	3652	3655
TSH (uIU/mL)	rho	0.0445	0.0666	0.0648
	p value	0.0184	0.0001	0.0001
	n	2800	3652	3655
Total T3 (ng/dL)	rho	-0.1524	-0.1005	-0.1873
	p value	<.0001	<.0001	<.0001
	n	2800	3652	3655
Total T4 (ug/dL)	rho	0.0565	0.0078	0.0108
	p value	0.0028	0.6386	0.5133
	n	2800	3652	3655
TPO-AB (IU/mL)	rho	0.0593	0.0323	0.055
	p value	0.0017	0.0513	0.0009
	n	2800	3652	3655
TG-AB (IU/mL)	rho	0.0616	0.0233	0.0344
	p value	0.0011	0.1595	0.0374
	n	2800	3652	3655
k1	rho	-0.0581	-0.0788	-0.0782
	p value	0.0021	<.0001	<.0001
	n	2800	3652	3655
k3	rho	-0.0114	0.0019	0.001
	p value	0.5482	0.9093	0.9521
	n	2800	3652	3655
k8	rho	-0.1035	-0.0232	-0.1031
	p value	<.0001	0.1602	<.0001
	n	2800	3652	3655
kd2	rho	0.1126	0.0932	0.1058
	p value	<.0001	<.0001	<.0001
	n	2800	3652	3655
TBGtot	rho	0.0759	0.054	0.0312
	p value	0.0001	0.0011	0.0594
	n	2800	3652	3655

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody, PFDE: Perfluorodecanoic acid, PFOA: Perfluorooctanoic acid, PFOS: Perfluorooctanesulfonic acid, rho: correlation coefficient.

Table 8. MLR Models for NIS Inhibitors

Variable	Estimate	SE	p-value
Log Free T4			
Log Nitrate	0.002473	0.00437914	0.5723
Log Perchlorate	-0.0162833	0.00320033	<.0001
Log Thiocyanate	-0.0167883	0.00274054	<.0001
Age	0.00073	0.00012333	<.0001
Race	0.003117	0.00192795	0.106
Gender=F vs. M	-0.0037467	0.00464379	0.4198
Log Cotinine	0.0014748	0.00075597	0.0511
Log Creatinine	0.0102205	0.00503027	0.0422
BMI	-0.0011331	0.00036772	0.0021
Log Iodine	0.0047072	0.00323625	0.1458
Log Free T3			
Log Nitrate	-0.0006498	0.00256114	0.7997
Log Perchlorate	-0.0039044	0.00200133	0.0511
Log Thiocyanate	-0.0020633	0.00162167	0.2033
Age	-0.0031052	0.00007728	<.0001
Race	-0.0095363	0.00131839	<.0001
Gender=F vs. M	-0.0588622	0.00288618	<.0001
Log Cotinine	0.000618	0.0004538	0.1733
Log Creatinine	0.0181555	0.00311953	<.0001
BMI	0.0008659	0.00020948	<.0001
Log Iodine	-0.0084902	0.00201049	<.0001
Log TSH			
Log Nitrate	-0.0031474	0.01866953	0.8661
Log Perchlorate	0.0073422	0.01358789	0.589
Log Thiocyanate	-0.0046002	0.01116941	0.6805
Age	0.0023178	0.00049539	<.0001
Race	-0.020526	0.00750199	0.0062
Gender=F vs. M	-0.0641352	0.0190496	0.0008
Log Cotinine	-0.0117469	0.00296325	<.0001
Log Creatinine	-0.0437171	0.02164975	0.0435
BMI	0.0073302	0.00135687	<.0001
Log Iodine	0.0149726	0.01377772	0.2772
Log total T3			
Log Nitrate	-0.0016021	0.00698187	0.8185
Log Perchlorate	-0.005264	0.0050712	0.2993
Log Thiocyanate	-0.0032478	0.00425825	0.4457
Age	-0.0045384	0.00018644	<.0001
Race	-0.0140411	0.00309477	<.0001
Gender=F vs. M	0.0307073	0.00751687	<.0001

Log Cotinine	0.0015394	0.00118113	0.1925
Log Creatinine	0.0220461	0.00813644	0.0067
BMI	0.0028085	0.00052484	<.0001
Log Iodine	-0.0175224	0.00506386	0.0005
Log total T4			
Log Nitrate	-0.0060358	0.00483477	0.2119
Log Perchlorate	-0.0222433	0.00355197	<.0001
Log Thiocyanate	-0.0194636	0.00294936	<.0001
Age	0.0004653	0.00013129	0.0004
Race	-0.0038432	0.00205381	0.0613
Gender=F vs. M	0.0595461	0.0051933	<.0001
Log Cotinine	0.0020838	0.00083653	0.0128
Log Creatinine	0.020668	0.00557204	0.0002
BMI	0.0032119	0.0003778	<.0001
Log Iodine	0.0079417	0.00346531	0.0219
Log TPO-AB			
Log Nitrate	0.0509534	0.04579957	0.2659
Log Perchlorate	0.0286983	0.03414541	0.4007
Log Thiocyanate	-0.049752	0.02888233	0.085
Age	0.0053352	0.00124333	<.0001
Race	-0.0519131	0.01722412	0.0026
Gender=F vs. M	0.3688847	0.05009926	<.0001
Log Cotinine	-0.0131077	0.00819584	0.1098
Log Creatinine	-0.0458793	0.0541102	0.3965
BMI	-0.0009239	0.00382836	0.8093
Log Iodine	-0.0075825	0.03263152	0.8163
Log TG-AB			
Log Nitrate	-0.0706391	0.02893983	0.0147
Log Perchlorate	-0.0504378	0.02180698	0.0207
Log Thiocyanate	0.0816995	0.01734531	<.0001
Age	-0.0023618	0.00083153	0.0045
Race	0.1031391	0.01153569	<.0001
Gender=F vs. M	0.1092105	0.03218413	0.0007
Log Cotinine	0.0277177	0.00486499	<.0001
Log Creatinine	0.0710846	0.03696665	0.0545
BMI	0.0102335	0.00220114	<.0001
Log Iodine	-0.0547027	0.02158931	0.0113

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody.

Table 9. MLR Models for Environmental Phenols

Variable	Estimate	SE	p-value
Log Free T4			
Log BPA	-0.0054717	0.00352146	0.1203
Log BP3	-0.0102286	0.00420457	0.015
Log Butyl Paraben	-0.0053451	0.00205388	0.0093
Log Methyl Paraben	-0.0073973	0.00471603	0.1168
Log Propyl Paraben	0.005032	0.00358424	0.1604
Log Ethyl Paraben	-0.0008904	0.00208019	0.6686
Log Creatinine	0.0056839	0.00651404	0.383
Log Iodine	-0.0034774	0.0040761	0.3936
Log Cotinine	-0.0017192	0.00084331	0.0416
BMI	-0.0009899	0.00052223	0.0581
Gender=F vs. M	0.0045533	0.00705082	0.5185
Age	0.0003218	0.00015604	0.0392
Race	0.0091836	0.00245042	0.0002
Lbp3*Lmpara	0.0034064	0.00125057	0.0065
Lbp3*Lppara	-0.0026134	0.00102174	0.0106
Log Free T3			
Log BPA	-0.0026325	0.00245095	0.2829
Log BP3	-0.0007225	0.0009502	0.4471
Log Butyl Paraben	0.0004758	0.00126339	0.7065
Log Methyl Paraben	-0.0010782	0.00195954	0.5822
Log Propyl Paraben	-0.0013112	0.00145469	0.3674
Log Ethyl Paraben	-0.0022577	0.0013029	0.0832
Log Creatinine	0.0177303	0.00440157	<.0001
Log Iodine	-0.0091192	0.00250057	0.0003
Log Cotinine	0.0002899	0.00056063	0.6051
BMI	0.0005399	0.00034876	0.1217
Gender=F vs. M	-0.0522801	0.00477118	<.0001
Age	-0.0031918	0.00009878	<.0001
Race	-0.0086544	0.0015852	<.0001
Log TSH			
Log BPA	-0.0292232	0.01342979	0.0296
Log BP3	0.0065338	0.00563521	0.2463
Log Butyl Paraben	0.007454	0.00848703	0.3798
Log Methyl Paraben	-0.0090537	0.01127544	0.422
Log Propyl Paraben	-0.0042203	0.00845127	0.6176
Log Ethyl Paraben	-0.0110879	0.00861581	0.1982
Log Creatinine	-0.0592919	0.02637739	0.0246
Log Iodine	0.0589775	0.0144902	<.0001
Log Cotinine	-0.0189107	0.00348766	<.0001

BMI	0.0056603	0.00198194	0.0043
Gender=F vs. M	-0.0553881	0.02822121	0.0498
Age	0.0025064	0.00059413	<.0001
Race	-0.0342158	0.0090602	0.0002
Log total T3			
Log BPA	-0.0065892	0.00588555	0.263
Log BP3	0.0035187	0.00249175	0.158
Log Butyl Paraben	0.0009529	0.00358676	0.7905
Log Methyl Paraben	0.0067828	0.00478259	0.1562
Log Propyl Paraben	-0.0062905	0.00379399	0.0974
Log Ethyl Paraben	-0.0105421	0.00364874	0.0039
Log Creatinine	0.0146298	0.01020208	0.1517
Log Iodine	-0.0180137	0.00636295	0.0047
Log Cotinine	0.0010673	0.00133562	0.4243
BMI	0.0024269	0.00070208	0.0006
Gender=F vs. M	-0.0196914	0.01125176	0.0802
Age	-0.004591	0.00024725	<.0001
Race	-0.0071155	0.0038172	0.0624
Log total T4			
Log BPA	-0.0150057	0.00405864	0.0002
Log BP3	-0.004336	0.00164828	0.0086
Log Butyl Paraben	-0.0041384	0.00246196	0.0929
Log Methyl Paraben	0.0073132	0.00334274	0.0287
Log Propyl Paraben	-0.005985	0.00265986	0.0245
Log Ethyl Paraben	-0.0066237	0.00241376	0.0061
Log Creatinine	0.0207458	0.00709852	0.0035
Log Iodine	-0.0014733	0.00424085	0.7283
Log Cotinine	-0.0018652	0.00091664	0.0419
BMI	0.0028918	0.00049314	<.0001
Gender=F vs. M	0.067417	0.00770168	<.0001
Age	0.0004619	0.00016767	0.0059
Race	-0.0004522	0.00263616	0.8638
Log TPO-AB			
Log BPA	0.0014043	0.03325362	0.9663
Log BP3	-0.0044942	0.01546161	0.7713
Log Butyl Paraben	0.0346672	0.02194394	0.1142
Log Methyl Paraben	-0.0068996	0.03048617	0.821
Log Propyl Paraben	-0.0289484	0.02378773	0.2237
Log Ethyl Paraben	0.0254773	0.0215674	0.2376
Log Creatinine	-0.0826005	0.0599608	0.1684
Log Iodine	0.00714	0.03730374	0.8482
Log Cotinine	-0.0055357	0.00857327	0.5185

BMI	0.0012931	0.00433367	0.7654
Gender=F vs. M	0.2748754	0.06632261	<.0001
Age	0.0059348	0.00145184	<.0001
Race	-0.0911778	0.02490387	0.0003
Log TG-AB			
Log BPA	0.2325249	0.10237441	0.0232
Log BP3	-0.0275842	0.01014522	0.0066
Log Butyl Paraben	-0.0268221	0.01499917	0.0738
Log Methyl Paraben	0.0287976	0.01891123	0.1279
Log Propyl Paraben	0.0198048	0.01519715	0.1926
Log Ethyl Paraben	-0.014821	0.01397617	0.289
Log Creatinine	-0.0100513	0.03839851	0.7935
Log Iodine	-0.0342128	0.0256051	0.1816
Log Cotinine	0.0379349	0.00508802	<.0001
BMI	0.012696	0.00259128	<.0001
Gender=F vs. M	0.1271931	0.04432021	0.0041
Age	-0.0018563	0.00102926	0.0714
Race	0.1008462	0.01526239	<.0001
Lbpa*Liodine	-0.0442043	0.02000789	0.0272

TPO-AB: Thyroperoxidase antibody, TG-AB: Thyroglobulin antibody, BPA: Bisphenol-A, BP3: Benzophenone-3, F: Female, M: Male, Lbp3: Log Benzophenone-3, Lbpa: Log Bisphenol-A, Lmpara: Log Methyl Paraben, Lppara: Log Propyl Paraben, Liodine: Log iodine.

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