Associations between environmental exposures and health outcomes in Yuma County, Arizona

Jenna Honan

November 2022

**PROJECT GOALS**

I will evaluate the potential health risks of exposure to certain environmental contaminants, including toxic metals and perchlorate. The combination of naturally occurring and anthropogenic sources of metals can magnify environmental pollution.1, 2 This research focuses on the historical and current use of metal-containing pesticides, as our project area, Yuma County in southwestern Arizona, is an intensive agricultural area.1, 3, 4 Other artificial sources, such as the Marine Corps Air Station, the U.S. Army Proving Ground, a growing commercial/maquiladoras sector, and heavy winter tourism, may play a role in contamination5, 6. We are also interested in perchlorate as an environmental contaminant due to the long-term pollution of Lake Mead and the Colorado River, the main source of drinking and irrigation water in Yuma.4, 7 Elevated levels of perchlorate in the water would signify a concomitant increase in risk of human ingestion and dermal exposures. Using exposure science, epidemiology, and statistical modeling techniques, we will assess relationships between contaminant exposures and health outcomes, including thyroid dysfunction, cardiovascular disease, metabolic disorders, and obesity, in a sample of 323 volunteer participants. Ultimately, the results from these analyses will be presented to the community members to help them make informed autonomous decisions regarding their own health and wellbeing, with the opportunity to discuss potential interventions.

**PROJECT BACKGROUND**

Elevated metals found in the area include cadmium (Cd), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), and uranium (U). These metals originate from the high volume of pesticide applications and other sources, such as local industry and naturally occurring reservoirs. 1, 8-10 Metal exposures can lead to cardiovascular diseases and metabolic disorders.11-17 Diabetes and heart disease are among the leading causes of mortality in Yuma County for 2017.18 Exposure to multiple contaminants simultaneously, as is likely the case for Yuma residents, can complicate the results and interpretation of modeled associations. A study by Preston et al.19 illustrated the possibility of synergistic effects due to exposure to a mixture of metals. Although the mechanisms of toxicity for each of the metals may be unique, considering the impacts of exposure independently for each metal may lead to errors in association estimates and interpretations of the results.20A correlation analysis of the metal concentrations in our participants’ hair samples shows moderate to strong positive correlation for most of the metals. A principal component analysis (PCA) helps to minimize the effects of autocorrelated concentrations and to increase the statistical power by lowering the number of model inputs.

Perchlorate has accumulated to concentrations of approximately 1.5−8.0 μg/L in the Colorado River, which is the source of both irrigation and drinking water in Yuma County.4 Previous samples of tap water taken from Yuma homes between 1994 and 2004 measured perchlorate at around 6 μg/L,21 and groundwater samples from the aquifer ranged from 4-11 μg/L.22 As of 2022, no regulations exist to define acceptable levels of perchlorate in drinking water.23 Perchlorate is a known thyroid disruptor, as it outcompetes iodide uptake at the sodium-iodide symporter of the thyroid gland, impacting thyroid hormone synthesis.24, 25 By inhibiting iodide uptake, the thyroid hormones thyroxine (T4) and triiodothyronine (T3) are also inhibited, causing the pituitary gland to activate the production of thyroid stimulating hormone (TSH).26, 27 Perchlorate exposure can thereby induce hypothyroidism, which in turn causes numerous detrimental health effects, such as dysregulated metabolism and obesity.25, 28-33 TSH levels in the blood samples above 4.0 mIU/mL may indicate presence of hypothyroidism, while levels below 0.4 mIU/mL could indicate hyperthyroidism.34 Recent studies suggest that the upper range of what may be considered “normal” for TSH should be lowered to 2.5 mIU/mL to better incorporate subclinical hypothyroidism.35-37 In Yuma, abnormal thyroid function associated with perchlorate exposures developed in Yuma neonates.21 Additionally, laboratory animal work demonstrates that perchlorate can induce additional problems during development, such as altered reproductive development.38

The Children’s Exposure to Pesticide Survey (CPS) previously conducted a health survey and collected urine samples in the Yuma area. This project considered pesticide exposures for children younger than 7 years old, with an objective of identifying children with elevated pesticide levels.39 The researchers collected urine samples from 154 children, and the parents of the children completed the Children’s Exposure to Pesticide Survey (CPS). Urine samples collected for this study have been maintained frozen (-20ο C) since 2000, other than during transport from the sampling location to storage and while shipping to and from the analyzing laboratory where freeze-thaw may have occurred. Perchlorate is stable in frozen urine samples.40, 41 We will analyze these samples for perchlorate concentrations to provide a snapshot of exposures prior to the perchlorate remediation efforts at Lake Meade.7 O’Rourke measured creatinine levels in these urine samples when initially collected.39 Variations of urinary creatinine excretion in children are poorly understood,39, 42 and therefore direct comparison between children and adults is not appropriate.43, 44 However, this analysis can provide important historical information about the presence of perchlorate in Yuma and shed light on the impact that long-term exposures can have on a community. The Centers for Disease Control and Prevention (CDC) provides the raw data for perchlorate levels (raw and corrected for creatinine) in children ages 3-5, 6-11, and 12-19 years, and adults 20+ years old from 2000 to 2018 through the National Health and Nutrition Examination Survey (NHANES). We will use this information to compare the national geometric mean and percentiles to the levels in both the von Hippel and O’Rourke studies.

This project will use data collected at two time points, separated by about two decades, for residents and/or workers in Yuma County. Details of the recruitment and sampling efforts for the CPS study can be found in O’Rourke et al., 2000.39 For the most recent sampling event (n=323), the research team completed a health questionnaire with adult participants recruited from the Yuma Regional Medical Center (YMRC), the Regional Center for Border Health (RCBH), and the farmworker advocacy group Campesinos Sin Fronteras (CSF). Using a case-control study design, recruitment attempted to enroll participants at a 1:1 case-to-control ratio, with a case defined as a participant who had previously been diagnosed with at least one thyroid disease or disorder (hyperthyroidism, hypothyroidism, thyroid cancer, or goiter). Of the 320 participants that responded to the thyroid disease or disorder question, 142 (44.4%) self-reported a history of thyroid disease or disorder. From the EMR data, 252 participants had recorded information on presence or absence thyroid problems, with 31.7% (80/252) having a medical chart that indicated presence of a thyroid issue. We will use the same participants for both the perchlorate and the metals analyses, as each participant supplied hair, urine and blood samples, biometrics, and survey responses. Analyses may compare farmworkers (46/323) as a subpopulation compared to non-farmworkers (277/323) to determine if differences exist in concentrations of contaminants and if farmworkers have increased odds of the adverse health outcomes assessed in the models.

**SPECIFIC AIMS**

**Aim 1:** *Determine if associations exist in the sample population between perchlorate exposure concentration and adverse health outcomes, such as thyroid dysfunction and obesity.*

**Aim 1A:** *Model associations between concentrations of perchlorate in urine, after correcting for creatinine, and presence of thyroid disruption, defined here as anyone indicating diagnosis of hypothyroidism, hypothyroidism, hyperthyroidism, goiter, or thyroid cancer, using logistic regression. A second logistic model will be run for creatinine-adjusted urinary perchlorate and only hypothyroidism. Presence of outcomes (binary yes/no) will be based on participant responses to a health survey and electronic medical records. Survey responses for presence or absence of adverse thyroid outcomes will be compared to measured hormone levels in blood samples.*

**Hypothesis:** Participants with higher levels of perchlorate in their urine will have increased odds of self-reported thyroid disruption. After correcting for age, gender, ethnicity, smoking status, and thyroid medications, participants who indicate presence of thyroid dysfunction will experience higher odds of having TSH levels outside (above or below) the reference range than those who have not been diagnosed with a thyroid disease or disorder.34

**Sub-Aim 1A:** *Use the four-parameter logistic regression model (the Hill Equation) to examine only those participants who indicated that they are not taking medications to regulate their thyroid to determine if a dose-response relationship exists between urinary perchlorate concentration and levels of measured hormones of the thyroid axis (TSH, T4, T3).*

**Hypothesis:** Participants not taking medications for thyroid diseases will show a stimulatory association between urinary perchlorate concentration and TSH hormone levels, and an inhibitory association with T4 and withT3. Those using medications will have less prevalent hormonal imbalance via hypothyroidism.

**Aim 1B:** *Analyze the associations between perchlorate levels in urine and body mass index (BMI) as a continuous outcome and obesity as a binary outcome.*

**Hypothesis:** Analyses will be completed separately for cases (those with hypothyroidism) and controls (those without hypothyroidism). After correcting for sample dilution caused by differences in individual hydration using urinary creatinine, the cases will show a positive association between perchlorate concentrations and both BMI and obesity. For controls, a correlation is still expected to be present, but with a smaller magnitude, as hormonal imbalance via hypothyroidism will be less prevalent.

**Aim 2:** *Model associations between metal concentrations present in the hair of participants using results of a robust PCA analysis as a surrogate for general metal exposure and adverse health outcomes that have been shown previously to correlate with metal exposures.*

**Aim 2A:** *Use logistic regressions to assess statistical relationships between concentrations of metals mixtures (Cd, Cu, Hg, Mn, Pb, and U) measured in hair samples using results of a robust PCA and all-type (grouped) cardiovascular disorders, which will include coronary heart disease (CHD), hypertension, stroke, angina, congestive heart failure (CHF), heart attack, and abnormal cholesterol levels.* *PCA results will act as an indicator for generalized exposure to the metals included in the analysis. The number of vectors included will be based on how well each explains the data variance.*

**Hypothesis:** We expect that the higher concentrations of metal exposures will be associated with increased log odds of cardiovascular disease and related outcomes.

**Aim 2B:** *Compare results of the robust PCA with metabolic health outcomes, such as diabetes mellitus (Types 1 and 2 were not differentiated in the survey) and obesity, in the sample population using logistic or linear regressions.*

**Hypothesis:** Those with higher concentrations of metals in the hair samples will have elevated prevalence of the metabolic disorders and obesity.

**Aim 3:** *Complete a retrospective exposure analysis of perchlorate exposures in young children living in Yuma, Arizona by modeling associations between urinary perchlorate and obesity.*

**Aim 3A:** *Measure concentrations of perchlorate in urine samples collected from 1998-2003 from children in Yuma County. Logistic regression will be used to determine if associations exist between resulting creatinine-adjusted perchlorate levels and obesity outcomes reported by parents in the Childhood Pesticide Study (CPS).*

**Hypothesis:** Because the previous samples were collected from children, and because remediation efforts for perchlorate have been implemented at the source of contamination, past urine samples will most likely indicate higher concentrations of perchlorate than the current samples. Health outcomes have not had as much time to manifest in children as in adults; however, a study by Polhamus et al.45 in 2009 found that about 13% of Arizona children under the age of 5 were overweight or obese. We hypothesize that logistic regression will show an increased odds of obesity (BMI > 30) for those with higher perchlorate concentrations. Distributions of concentration levels in these urine samples can be compared to those more recently collected for Aim 1.

**PROJECT APPROACHES AND METHODOLOGIES**

Hair samples collected from each participant were analyzed for the concentration of these six metals, similar to other studies.46-49 We will analyze blood concentrations of free and total TSH, T3, and T4 as tests for thyroid function in Yuma residents, as has been commonly done in other studies.50-52 Additionally, we will examine blood concentrations of the stress hormone cortisol. An imbalance of these hormones can lead to numerous symptoms, including obesity, tremors, altered metabolism, etc.53 Finally, we will examine urinary creatinine levels as a covariate to correct for individual variations that may impact perchlorate concentration.41, 54, 55 We will use creatinine rather than specific gravity as a measure of urinary dilution to be comparable to similar studies.39, 41

We will explore quantitative associations between environmental pollutants and prevalence of negative health outcomes using well-established epidemiological and biostatistical techniques to determine if exposures associated with increased risk of the outlined health concerns. Using descriptive statistics, we will summarize the sample data generally. We will estimate levels of exposure for these environmental contaminants based on concentrations in biological samples. Odds ratios will provide information about the association between health effects and exposures. We will run multivariable linear or logistic regressions based on the input and outcome variable types (e.g., continuous, categorical, or binary). Most of the analyses pertaining to metal exposures will use a robust PCA, although some analyses may consider direct associations between outcomes and individual metal concentrations. We will use parametric statistical models when assumptions have been met. Concentration data distributions will be examined for normality using Shapiro Wilk test. Hormone level distributions are expected to be right-skewed due to the inclusion of individuals with thyroid dysfunction. Due to successive random dilutions commonplace in the environment, the data for both contaminant concentrations and for hormone levels are expected to require lognormal transformation.56 During model development, we will use a backwards stepwise elimination of covariates, explained in more detail below. We will assess data for possible effect modifiers, discussed below, and adjust models if necessary. Where appropriate, we will determine if significant differences between groups exist, such as by gender, age, socioeconomic status, estimated exposure levels, and other relevant variables, using appropriate biostatistical tests. We will compare the data collected from the EMR records to the self-report data collected from the surveys/interviews. Although we expect high congruence between self-reported outcomes and EMR data, barriers such as lack of access to healthcare, stigma or apprehension in seeking care, misinterpretation of symptoms due to non-local doctors being unfamiliar with local health concerns, or language barriers, can cause dissimilarities between the sources.

*Data Collected During Most Recent Study Visit*

Sociodemographic information included age, gender, education, residency status, occupation, race/ethnicity, self-reported height and weight, self-reported smoking status, and self-reported medical conditions (e.g., thyroid cancer, hypertension, polycystic ovarian syndrome, diabetes mellitus). Research staff measured BMI by dividing the participant’s weight (kg) by their squared height (m). They collected approximately 150 mg of hair, clipped with shears at the nape of the neck and stored at room temperature in paper envelopes. The clippings were later analyzed for metal concentrations, specifically Cd, Cu, Hg, Mn, Pb, and U. Participants provided roughly 5-10 mL of blood via venipuncture into heparinized (anti-coagulating) vacuum syringes, later centrifuged into plasma and other cellular fractions. Plasma was frozen at -80oC until processed and analyzed for hormones (TSH, T4, T3, and cortisol). Finally, each participant provided a urine sample at a single time point, which was stored at -20oC until analyzed for perchlorate and creatinine concentrations.8

*Independent (Predictor) Variables*

1. We will consider urinary perchlorate as an independent variable. The concentration data are continuous and lognormally distributed. Because the concentration of perchlorate in urine will be more diluted for participants who are more hydrated, we will correct perchlorate levels based on creatinine levels to minimize external individual variations. We will do this by adjusting for log creatinine in the regression models.
2. A robust PCA of the metal concentrations showed that two groupings were most responsible for the variation in the data. Therefore, we will use PC1 and PC2 as surrogate independent variables rather than the individual metals in the regression modeling, focusing on general instead of individual toxicity. Based on the results from using the PCA and on input from the literature, we may consider individual metals for analysis as well.

*Dependent (Outcome) Variables*

The analysis will consider selected health indicators as outcome variables based on community input and agreement.

1. We will evaluate blood concentration levels of free and total TSH, T3, T4, and cortisol to determine if over- or under-production of these hormones is present. We will also compare our sample to the national averages. We will analyze the data to determine if a dose-response relationship exists with perchlorate.
2. Binary (presence/absence) health outcome values include thyroid disorders (goiter, thyroid disease, hypothyroidism, hyperthyroidism, thyroid cancer) and metabolic disorders (diabetes, gout, obesity).
3. Continuous health outcome data are available for obesity via BMI measurements and for hypertension via blood pressure measurements. We may also create categorical (based on thresholds) or binary (yes/no) variables for obesity and hypertension.

*Covariates*

Variables that may be important covariates include age, gender, occupation, SES (as income), residency status (year-round vs “snowbird”), race/ethnicity (and/or birth country?), and smoking status. Because we are testing a number of dependent outcome variables with limited power, we have *a priori* selected age, gender, income, ethnicity, and drinking water source (tap, private well, bottled, or other) to be included in the models and will test smoking as a modifier variable using likelihood ratio tests, with an interaction threshold set at p < 0.05. Explanatory variables included in the model will be tested for correlations with one another and grouped using variation inflation factor analysis, and if highly correlated, only one of those that are related will be chosen for model inclusion.57

**Statistical Analysis**

We will model associations between independent variables and health outcomes using linear or logistic regression based on whether the outcome variables are binary or continuous. We will choose model covariates *a priori* and use backwards stepwise elimination to exclude any variables that do not change the beta coefficient on the independent variable by >15%. This will provide our team with the most economical model in terms of simplicity and accuracy. For outcomes with low case numbers (less than 5), we will emphasize that the analysis was underpowered during our interpretations. We can use results of underpowered analyses when directing future research efforts.

We will treat data from our biometric collection efforts preferentially, followed by EMR data and self-report data, respectively. We will use Pearson or Spearman tests to compare data collected from the EMR records to the self-report data collected from the surveys/interviews. We will replace concentration data below the limit of detection (LOD) with the LOD / or imputation via the maximum likelihood estimation (MLE) for parametric data. 58-60 For data that are highly left-censored (less than 35% detection frequency), particularly for the PCA, exclusion from the analyses will be considered.60

Perchlorate will be used directly as an independent variable in the thyroid analyses. For the metal hair concentration data, a robust principal component analysis (PCA) revealed that two component groupings were responsible for approximately 75% of the variance. We will evaluate the normality of each continuous predictive parameter prior to model development by graphical visualization, comparison of median and mean values, and testing with the Shapiro-Wilk test. We will transform skewed data, which are common in biological and environmental samples, in order to use parametric tests. If transforming does not normalize the data, we will use nonparametric approaches. We will develop linear or logistic regression models using concentrations of perchlorate and results from the PCA (PC1, PC2, and PC1+PC2) as predictor variables, depending on the outcome variable types. For combined health outcomes, such as for cardiovascular disease, we do not expect to need adjustment for multiple comparisons to avoid Type 1 errors, because each endpoint was selected before analysis, and each is highly correlated with the others.

Participant demographics and characteristics will be expressed as means ( SD) for quantifiable variables and as percentages for categorical variables. We will calculate descriptive statistics for the concentrations of toxicants in biological samples and compare these to national averages and other similar studies using unpaired t-tests or ANOVA. We will run multiple regressions, adjusted when appropriate, to evaluate the associations between each covariate and the biomarker concentrations. We will complete multivariable regression analyses separately for PC1, PC2, and PC1+PC2. We will set statistical significance at p < 0.05, and we will report 95% confidence intervals. Finally, we will compare our sample population’s concentration levels to relevant National Health and Nutrition Examination Survey (NHANES) databases. Analyses for categorical variables will use chi-square tests, while those for continuous variables will use Pearson correlations and ANOVA or MANOVA tests. We will perform all statistical analyses in RStudio Version 1.2.5033 using R software 4.2.1.

References:

1. Sugeng AJ, Beamer PI, Lutz EA, Rosales CB. Hazard-ranking of agricultural pesticides for chronic health effects in Yuma County, Arizona. *Science of The Total Environment*. 2013;463-464:35-41.

2. Credo J, Chandos A, Checinski C, von Hippel FA, Ingram JC. Sample preparation method for metal(loid) contaminant quantitation in rodent hair collected in Yuma County, Arizona. *Environmental Monitoring and Assessment*. 2021;193(8):522.

3. Jones MC, Credo JM, Ingram JC, Baldwin JA, Trotter Jr. RT, Propper CR. Arsenic concentrations in ground and surface waters across Arizona including Native lands. *Journal of Contemporary Water Research & Education*. 2020;169(1):44-60.

4. Sanchez CA, Krieger RI, Khandaker N, Moore RC, Holts KC, Neidel LL. Accumulation and perchlorate exposure potential of lettuce produced in the Lower Colorado River region. *Journal of Agricultural and Food Chemistry*. 2005;53(13):5479-86.

5. NAVFAC. Record of decision for munitions response program sites 4 and 6 In: Navy USDot, editor. Marine Corps Air Station Yuma, Yuma, Arizona. San Diego, California: Naval Facilities Engineering Command Southwest; 2016.

6. Yuma County Chamber of Commerce. Moving Your Business to Yuma County 2021 [Available from: <https://www.yumachamber.org/moving-your-business-to-yuma-county.html>.

7. NDEP. Perchlorate. In: Protection NDoE, editor. Environmental Clean Up: Black Mountain Industrial (BMI) Complex2020.

8. Trotter II R, Baldwin J, Buck CL, Remiker M, Aguirre A, Milner T, et al. Health impacts of perchlorate and pesticide exposure: Protocol for community-engaged research to evaluate environmental toxicants in a US border community. *JMIR Research Protocols*. 2021;10(8):e15864.

9. Gómez-Alvarez A, Meza-Figueroa D, Villalba-Atondo AI, Valenzuela-García JL, Ramírez-Hernández J, Almendariz-Tapia J. Estimation of potential pollution from mine tailings in the San Pedro River (1993–2005), Mexico–US border. *Environmental Geology*. 2009;57(7):1469-79.

10. Hanson GH. U.S.–Mexico integration and regional economies: evidence from border-city pairs. *Journal of Urban Economics*. 2001;50(2):259-87.

11. Menke A, Guallar E, Cowie CC. Metals in urine and diabetes in U.S. adults. *Diabetes*. 2015;65(1):164-71.

12. Paithankar JG, Saini S, Dwivedi S, Sharma A, Chowdhuri DK. Heavy metal associated health hazards: An interplay of oxidative stress and signal transduction. *Chemosphere*. 2021;262:128350.

13. Bagchi D, Bagchi M. Metal Toxicology Handbook. 1st ed. ed. Bagchi D, Bagchi M, editors: CRC Press; 2020.

14. Carmona A, Roudeau S, Ortega R. Molecular mechanisms of environmental metal neurotoxicity: A focus on the interactions of metals with synapse structure and function. *Toxics*. 2021;9(9):198.

15. Wright RO, Baccarelli A. Metals and Neurotoxicology. *The Journal of Nutrition*. 2007;137(12):2809-13.

16. Chen QY, DesMarais T, Costa M. Metals and mechanisms of carcinogenesis. *Annu Rev Pharmacol Toxicol*. 2019;59:537-54.

17. Kim HS, Kim YJ, Seo YR. An overview of carcinogenic heavy metal: molecular toxicity mechanism and prevention. *J Cancer Prev*. 2015;20(4):232-40.

18. PRC. 2019 Community Health Needs Assessment Report: Yuma County, Arizona. Yuma Regional Medical Center; 2019. Report No.: 2019-0162-02.

19. Preston S, Coad N, Townend J, Killham K, Paton GI. Biosensing the acute toxicity of metal interactions: Are they additive, synergistic, or antagonistic? *Environmental Toxicology and Chemistry*. 2000;19(3):775-80.

20. Billionnet C, Sherrill D, Annesi-Maesano I. Estimating the health effects of exposure to multi-pollutant mixture. *Annals of Epidemiology*. 2012;22(2):126-41.

21. Brechner RJ, Parkhurst GD, Humble WO, Brown MB, Herman WH. Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona. *Journal of Occupational and Environmental Medicine*. 2000:777-82.

22. ADEQ. Perchlorate occurrence study: Sampling and analysis plan. Hydrologic Support and Assessment Section, Division WQ; 2004.

23. Friedman L. E.P.A. Decides Against Limiting Perchlorate in Drinking Water. The New York Times. 2022.

24. Gardell AM, von Hippel FA, Adams EM, Dillon DM, Petersen AM, Postlethwait JH, et al. Exogenous iodide ameliorates perchlorate-induced thyroid phenotypes in threespine stickleback. *General and Comparative Endocrinology*. 2017;243:60-9.

25. Wolff J. Perchlorate and the thyroid gland. *Pharmacological Reviews*. 1998;50(1):89-106.

26. Lawrence JE, Lamm SH, Pino S, Richman K, Braverman LE. The effect of short-term low-dose perchlorate on various aspects of thyroid function. *Thyroid*. 2000;10(8):659-63.

27. JB S, JB W. Effect of perchlorate on the human thyroid gland. *Metabolism: Clinical and Experimental*. 1952;1(6):533-9.

28. Steinmaus CM. Perchlorate in Water Supplies: Sources, Exposures, and Health Effects. *Current Environmental Health Reports*. 2016;3(2):136-43.

29. Niziński P, Błażewicz A, Kończyk J, Michalski R. Perchlorate – properties, toxicity and human health effects: an updated review. *Reviews on Environmental Health*. 2021;36(2):199-222.

30. Pleus RC, Corey LM. Environmental exposure to perchlorate: A review of toxicology and human health. *Toxicology and Applied Pharmacology*. 2018;358:102-9.

31. Maqbool F, Mostafalou S, Bahadar H, Abdollahi M. Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms. *Life Sciences*. 2016;145:265-73.

32. Dodds ED, Kennish JM, von Hippel FA, Bernhardt R, Hines ME. Quantitative analysis of perchlorate in extracts of whole fish homogenates by ion chromatography: comparison of suppressed conductivity detection and electrospray ionization mass spectrometry. *Analytical and Bioanalytical Chemistry*. 2004;379(5):881-7.

33. Yuan H, Zhao J, Xie E, Yi L, Zheng Z, Geng J. Endocrine and Metabolic Diseases. In: Pan S, Tang J, editors. Clinical Molecular Diagnostics. Singapore: Springer Singapore; 2021. p. 665-716.

34. Razvi S, Bhana S, Mrabeti S. Challenges in interpreting thyroid stimulating hormone results in the diagnosis of thyroid dysfunction. *Journal of Thyroid Research*. 2019;2019:4106816.

35. Biondi B. The normal TSH reference range: What has changed in the last decade? *The Journal of Clinical Endocrinology & Metabolism*. 2013;98(9):3584-7.

36. Karbownik-Lewinska M, Marcinkowska M, Stepniak J, Lewinski A. TSH ≥2.5 mIU/l is associated with the increased oxidative damage to membrane lipids in women of childbearing age with normal thyroid tests. *Horm Metab Res*. 2017;49(5):321-6.

37. Zöphel K, Wunderlich G, Grüning T, Koch R, Döge H, Kotzerke J. [Where does subclinical hypothyroidism start? Implications for the definition of the upper reference limit for thyroid stimulating hormone]. *Nuklearmedizin*. 2005;44(2):56-61.

38. Bernhardt R, von Hippel F. Chronic perchlorate exposure impairs stickleback reproductive behaviour and swimming performance. *Behaviour*. 2008;145(4-5):527-59.

39. O'Rourke MK, Lizardi PS, Rogan SP, Freeman NC, Aguirre A, Saint CG. Pesticide exposure and creatinine variation among young children. *Journal of Exposure Science & Environmental Epidemiology*. 2000;10(6):672-81.

40. Yu LL, Jarrett JM, Davis WC, Kilpatrick EL, Oflaz R, Turk GC, et al. Characterization of perchlorate in a new frozen human urine standard reference material. *Anal Bioanal Chem*. 2012;404(6-7):1877-86.

41. Blount BC, Valentin-Blasini L, Osterloh JD, Mauldin JP, Pirkle JL. Perchlorate exposure of the US population, 2001–2002. *Journal of Exposure Science & Environmental Epidemiology*. 2007;17(4):400-7.

42. Clark LC, Jr., Thompson HL, Beck EI, Jacobson W. Ecreation of creatine and creatinine by children. *AMA American Journal of Diseases of Children*. 1951;81(6):774-83.

43. Mills PK, Zahm SH. Organophosphate pesticide residues in urine of farmworkers and their children in Fresno County, California. *American Journal of Industrial Medicine*. 2001;40(5):571-7.

44. Miller MD, Marty MA, Arcus A, Brown J, Morry D, Sandy M. Differences between children and adults: implications for risk assessment at California EPA. *Int J Toxicol*. 2002;21(5):403-18.

45. Polhamus B, Dalenius K, Mackentosh H, Smith B, Grummer-Strawn L. Pediatric nutrition surveillance; 2006 report. 2007.

46. Qin Y, Xu C, Li W, Jian B, Wu B, Chen M, et al. Metal/metalloid levels in hair of Shenzhen residents and the associated influencing factors. *Ecotoxicology and Environmental Safety*. 2021;220:112375.

47. Mehra R, Thakur AS. Relationship between lead, cadmium, zinc, manganese and iron in hair of environmentally exposed subjects. *Arabian Journal of Chemistry*. 2016;9:S1214-S7.

48. Zhu Y, Wang Y, Meng F, Li L, Wu S, Mei X, et al. Distribution of metal and metalloid elements in human scalp hair in Taiyuan, China. *Ecotoxicology and Environmental Safety*. 2018;148:538-45.

49. Aziz MY, Hussain SH, Ishak AR, Abdullah MA, Mohamed R, Ruzi II, et al. Heavy metal concentrations in Malaysian adults’ hair and associated variables in Bukit Mertajam, Penang, Malaysia. *Biological Trace Element Research*. 2022;200(8):3475-81.

50. Demers LM, Spencer CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. *Clinical endocrinology (Oxford)*. 2003;58(2):138-40.

51. Soldin OP, Soldin SJ. Thyroid hormone testing by tandem mass spectrometry. *Clinical biochemistry*. 2011;44(1):89-94.

52. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH)-releasing hormone receptor: interaction with TSH and autoantibodies. *Endocrine reviews*. 1998;19(6):673-716.

53. Rugge B, Balshem H, Sehgal R, Relevo R, Gorman P, Helfand M. Screening and treatment of subclinical hypothyroidism or hyperthyroidism. Rockville, MD: Agency for Healthcare Research and Quality; 2011. Contract No.: 1(12)-EHC033-EF.

54. English P, Blount B, Wong M, Copan L, Olmedo L, Patton S, et al. Direct measurement of perchlorate exposure biomarkers in a highly exposed population: a pilot study. *PLoS One*. 2011;6(3):e17015.

55. O’Brien KM, Upson K, Buckley JP. Lipid and creatinine adjustment to evaluate health fffects of fnvironmental exposures. *Current Environmental Health Reports*. 2017;4(1):44-50.

56. Ott WR. A physical explanation of the lognormality of pollutant concentrations. *Journal of the Air & Waste Management Association*. 1990;40(10):1378-83.

57. Bewick V, Cheek L, Ball J. Statistics review 14: Logistic regression. *Crit Care*. 2005;9(1):112-8.

58. Croghan CA, Egeghy PP. Methods of dealing with values below the limit of detection using SAS. *Southern SAS User Group*. 2003;22:24.

59. Needham LL, Özkaynak H, Whyatt RM, Barr DB, Wang RY, Naeher L, et al. Exposure assessment in the National Children’s Study: introduction. *Environmental Health Perspectives*. 2005;113(8):1076-82.

60. Canales RA, Wilson AM, Pearce-Walker JI, Verhougstraete MP, Reynolds KA. Methods for handling left-censored data in quantitative microbial risk assessment. *Appl Environ Microbiol*. 2018;84(20).