

**Health of Pacific Children in New Zealand: Association between
Selected Elements, Behaviour and Body-size**

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Abbreviations for Definitions

Abbreviation	Definition
BMI	Body Mass Index
CBCL	Child Behaviour Checklist
CM	Centimeters
CNS	Central Nervous System
CRM	Certified Reference Material
CI	Confidence Intervals
FFM	Fat Free Mass
FM	Fat Mass
FFQ	Food Frequency Questionnaires
FSANZ	Food Standards Australia New Zealand
g	Grams
%	Percentage
PPM	Parts per Million
H	Height
IQ	Intelligence Quotient
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
JECFA	Joint Food and Agricultural Organisation
PTWI	Provisional Tolerable Weekly Intake
PIFs	Pacific Island Families
PUFAs	Polyunsaturated Fatty Acids

WHO	World Health Organization
NHANES	National Health and Nutrition Survey
NZ	New Zealand
NZFSA	New Zealand Food and Safety Authority
NZTDS	New Zealand Total Diet Survey
MAF	Ministry of Agriculture and Forestry
MOH	Ministry of Health
NZEO	New Zealand European and Others
EDTA	Ethylenediaminetetraacetic Acid
CBCL	Child Behaviour Check List
SES	Socio-economic Status
STATA	Statistical Software Package
IOTF	International Obesity Task Force
JECFA	Joint Food and Agricultural Organization (FAO)/WHO Expert Committee on Food Additives
IASO	International Association for Study of Obesity
OR	Odds Ratio
SD	Standard Deviation
µg/g	Micro gram per gram
µg/kg bw/week	Micro-gram per kilogram body weight per week

Abbreviations for Elements

Abbreviations	Elements
<i>Essential</i>	
Ca	Calcium
Mg	Magnesium
As	Arsenic
Co	Cobalt
Cr	Chromium
Fe	Iron
I	Iodine
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
Se	Selenium
Zn	Zinc
<i>Non-essential</i>	
Al	Aluminium
B	Boron
Sb	Antimony
<i>Toxic</i>	
Cd	Cadmium
Hg	Mercury
MeHg	Methylmercury
Pb	Lead

List of Publications and Awards Arising from the Doctoral Thesis

There were a number of publications that were drawn from this thesis and submitted for consideration to peer-reviewed academic journals for publication and to scientific committees for conference presentations. Copies of the published journal article are included in this thesis as appendix 1. The citations of the one paper, three peer-reviewed conference publications, five national and international conference presentations, three university presentations and one award for this thesis are listed below:

Peer-reviewed journal publication

1. **Karatela S.**, Paterson J., Schluter P. and Anstiss R. (2011). Methylmercury exposure through seafood diet and health in New Zealand: Are seafood eating communities at a greater risk? *Australasian Epidemiologist* 18(1), 21-25.

Peer-reviewed conference publications

1. **Karatela S.**, Paterson J., Rush E., Schluter P.J. and Ward N.I. (2011). Elemental assessment of Pacific Island children's health status: body size (Abstract). *Australasian Epidemiologist*, 18(2):106.
2. **Karatela S.**, Paterson J. & Schluter P. (2010). Health of Pacific children: environmental and nutritional determinants (Abstract). *Australasian Epidemiologist*, 17(2):109.
3. **Karatela S.**, Paterson J., Schluter P. & Anstiss R. (2009). Mercury exposure in mothers and their children within the Pacific Island Families Study (Abstract). *Australasian Epidemiologist*, 16(2):83.

Conference presentations

1. **Karatela S.,** Paterson J., Schluter P. & Ward NI. (2011). Elemental assessment of Pacific Island children's status: Obesity. (Oral presentation). *Australasian Epidemiological Association Annual Conference* 2011, Perth, Australia, 19 September-21 September.
2. **Karatela S.,** Paterson J. & Schluter P. (2010). Health of Pacific children: Environmental and nutritional determinants. (Poster presentation). *19th Australasian Epidemiological Association Annual Conference* 2010, Sydney, Australia, 30 September-1 October.
3. **Karatela S.,** Paterson J., Schluter P. & Anstiss R. (2009). Mercury exposure in mothers and their children within the Pacific Island Families Study. (Oral presentation). *18th Australasian Epidemiological Association Annual Scientific Meeting*. Dunedin, New Zealand, 30 August-1 September.
4. **Karatela S.,** Paterson J., Schluter P. & Anstiss R. (2008). A Nested Case-control study of mercury exposure and neurodevelopment in Pacific children. (Oral presentation). *Population Health Congress: A Global World – Practical Action for Health and Well-being*. Brisbane, Australia, 6-9 July.
5. **Karatela S.,** Paterson J., Schluter P. & Anstiss R. (2008). Mercury exposure and behaviour problems in Pacific children: A nested case-control study. (Oral presentation). *New Zealand Trace Elements Group Conference*. Hamilton, New Zealand, 13-15 February.

Other presentations

1. **Karatela S.,** Paterson J., Schluter P. & Anstiss R. (2010). Health of Pacific Children: Environmental and Nutritional Determinants (Healthy END) (Oral presentation). School of Public Health and Psychosocial Studies, AUT University, Auckland, New Zealand, 12 August.

2. **Karatela S.**, Paterson J., Schluter P. & Anstiss R. (2009). Health of Pacific Children: Environmental and Nutritional Determinants (Healthy END) (Oral presentation). School of Public Health and Psychosocial Studies, AUT University, Auckland, New Zealand, 29 June.
3. **Karatela S.**, Paterson J., Schluter P. & Anstiss R. (2008). Health of Pacific Children: Environmental and Nutritional Determinants (Healthy END) (Oral presentation). School of Public Health and Psychosocial Studies, AUT University, Auckland, New Zealand, 22 November.

Awards

1. The Australasian Epidemiological Association's Best Doctoral Student's Poster Award for: **Karatela S.**, Paterson J. & Schluter P. (2010). Health of Pacific children: Environmental and Nutritional Determinants. (Poster presentation). *Australasian Epidemiological Association Annual Conference 2010*, Sydney, Australia, 30 September-1 October.

Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgments), nor material which to a substantial extent, has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed.....

Date.....

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Abstract

In childhood environmental and dietary exposure to toxic and non-toxic elements may affect development and growth. The elemental concentrations of scalp hair and toenails may reflect chemical uptake via the diet, or environment. Very little is known about the relationship between elemental concentrations in scalp hair and toenails and behavioural and growth characteristics of children in New Zealand.

The Pacific Islands Families (PIF) study has provided a unique opportunity for children of age six and nine years to explore such relationships. The overall aim of this research was to collect and analyse the scalp hair and toenails of Pacific children (and in some cases from their mothers) resident in South Auckland, New Zealand (as part of the PIF population study) for calcium, magnesium, iron, manganese, zinc, copper, selenium, iodine, cobalt, chromium, nickel, molybdenum, antimony, arsenic, aluminium, boron, mercury, lead and cadmium.

Three questions were addressed: (1) what is the relationship between mercury in scalp hair, reported to originate from fish consumption to behavioural problems; (2) what is the relationship between mercury in toenails and behavioural characteristics and specific behavioural domains (particularly aggression, rule breaking, attention and social problems); and (3) in a population where three out of four children are defined as obese or overweight is there any relationship between elemental concentration in toenails and current body-size?

The first study was a nested case-control study which recruited children (with and without behavioural problems) and their mothers to study the effects of mercury through seafood diet and dental amalgams on child behaviour. Hair samples were collected from both mothers and their children as a biomarker for mercury exposure. The second and third studies were cross-

sectional studies designed to explore the association of elements on behavioural problems as identified by mothers (using the child behaviour checklist) and body-size categories (using the International Obesity Task Force's (IOTF) cut-off points). Toenail clippings were collected as a biomarker for elemental assessment for these studies. The biological measurements (scalp hair and toenail) were carried out using inductively coupled plasma mass spectrometry (ICP MS). The existing multi-disciplinary, longitudinal PIF information provided additional data on confounding factors and co-variants on these samples.

In the first study ($n=92$ mother and child pairs), almost 20% of both mothers (median hair: $0.32 \mu\text{g/g}$ mercury) and 18% of children (median hair: $0.43 \mu\text{g/g}$ mercury) exceeded the Environmental Protection Agency's (EPA) threshold for mercury of $1 \mu\text{g/g}$ Hg but were lower than the World Health Organization's (WHO) threshold of $1.6 \mu\text{g/g}$ Hg. There was no conclusive evidence on child behavioural problems and hair mercury concentrations. A direct correlation was observed between mothers and their children's hair mercury concentrations ($r = 0.79$ (95% CI 0.65, 0.88)).

In the next study ($n=278$ children; 160 boys, 118 girls), 21% of children had toenail mercury concentrations above $1 \mu\text{g/g}$ Hg with girls having higher mercury concentrations (24%) than boys (18%). Aggressive behaviour and seafood diet was associated with toenail mercury exposure after adjusting for gender, ethnicity and income levels (OR: 2.15 95% CI 1.45, 3.18 $p\text{-value} < 0.05$; OR 1.38 95% CI 0.83, 1.2 $p\text{ value} < 0.05$, respectively).

Within the final study, three out of four children were defined as obese or overweight; however, no significant correlation was found between body-size categories and toenail elemental concentrations. The elemental interaction of selenium-mercury and zinc-copper

had an association with the different body-size categories (p value 0.03; and p value 0.02, respectively). It was observed that the mean toenail selenium ($0.35 \mu\text{g/g Se}$), calcium ($868 \mu\text{g/g Ca}$) and zinc ($129 \mu\text{g/g Zn}$) concentrations were lower than the required optimal health concentrations for toenails (selenium $0.75 \mu\text{g/g Se}$; calcium $900 \mu\text{g/g Ca}$; zinc $160 \mu\text{g/g Zn}$) amongst these children. Toenail mean lead ($0.86 \mu\text{g/g Pb}$), cadmium ($0.21 \mu\text{g/g Cd}$) and mercury ($0.72 \mu\text{g/g Hg}$) concentrations were also higher than the optimal health requirements. Ethnic differences in relation to toenail elemental concentrations were observed for manganese, cobalt, iron, chromium, antimony, aluminium, mercury, lead and cadmium. Boys had higher concentrations for calcium, magnesium, manganese, copper, zinc, iron, antimony arsenic, aluminium, boron and lead than girls. Toenail clippings are a better biomarker for elemental status within Pacific people than scalp hair samples.

Overall, this research contributes to the understanding of the elemental concentrations for Pacific people using scalp hair and toenail clippings as biomarkers in terms of associations with health outcomes (particularly child behavioural problems and body-size categories). Mercury in toenails demonstrated a moderate association with a specific behavioural domain – aggressive behaviour – while elemental interactions such as zinc-copper and mercury-selenium seemed to influence the body-size categories in these children even though single elements did not show any associations on body-size categories. Furthermore, some possible explanations for both the conclusive and inconclusive results that have so far emerged, and suggestions for potential ways forward in this area of research are discussed.

Chapter 1 Introduction

1.1 Background

In New Zealand (NZ) there are large inequities in child health status especially within the Pacific population due to socio-economic inequities (Statistics New Zealand and Ministry of Pacific Island Affairs, 2011b), such as low educational attainment (Ministry of Education, 2006), poor housing (Butler, Williams, Tukuitonga, & Paterson, 2003), and limited food security (Rush, Puniani, Snowling, & Paterson, 2007). Pacific people represent one of the fastest growing population subgroups in NZ due to migration and high fertility rates (Statistics New Zealand, 2006).

Pacific peoples come from diverse ethnicities, the biggest groups being Samoan (49%), Cook Islands (22%), Tongan (19%) and Niuean (8.5%) (Statistics New Zealand, 2006). According to the 2006 census, 11.5% of Pacific children were between the ages of 0 and 14 years, and were more likely to be living in lower decile areas than other ethnic groups apart from Māori (Pacific Strategic Action Plan, 2007). Living in lower decile areas has been negatively correlated with food security (food availability, food access and food use) which in turn affects diet and hence does not meet the recommended dietary requirements (Goyer, 1990). Food insecurity has been observed in up to 50% of the Pacific households in NZ compared to 23% of New Zealand European and Other (NZEO) households (Rush & Rusk, 2009; Russell, Parnell, & Wilson, 1999).

In order to attain optimal health, the body requires a certain balance of elements (essential, non-essential or toxic elements) which is achieved through proper diet (Fiabane & Williams, 1977; Fraga, 2005). The evidence about food insecurity indicates that this is an important

public health problem within Pacific people. Adequate digestion of food and the absorption of nutrients are therefore important for maintaining the elemental concentrations required in normal health.

Elements can be classified as essential, non-essential and toxic to human health (Ward, 2000). An element is considered essential if a dietary deficiency of that element consistently results in a "sub-optimal biological function that is preventable or reversible by the addition of that element" (Nielsen, 1984). Elements are considered toxic if their presence produces an unwanted change to a biological system, often through the impairment or over stimulation of a physiologically important process (Williams, James, & Roberts, 2000). There is no conclusive evidence about non-essential elements on the effect in the human body (Ward, 2000). However, numerous studies have related these elements to deficiencies or toxicity to health (Braun et al., 2008; J. Campbell, 2001; Forte et al., 2005; MacPherson & Bacso, 2000; Rink, 2011). They are either ingested in the foods eaten or absorbed from the environment through the skin, respiratory and gastrointestinal tracts (Paustenbach, 2000).

The detrimental effects of toxic elements on children's behavioural outcomes are well documented (P. Grandjean & Herz, 2011; Oken & Bellinger, 2008); however, the effects on behaviour and distribution of toxic elements such as mercury through seafood diet have not been explored in NZ Pacific children. Only a few studies have explored the interactions of a suite of elements in relation to the body-size of children. Furthermore, the physiological effects of elements on childhood body-size are not yet fully understood.

Evaluating elemental status of children's health can be obtained through biological markers such as blood, urine, body fluids, hair and nail samples. Through elemental speciation the

extent and magnitude of concentrations of these elements can be determined in biological markers. Chemical analysis of various elements in selected human biomonitors (hair and nail samples) will provide a valuable database to explore the relationship between elemental concentrations and the health and developmental status of NZ Pacific children.

1.2 Health Outcomes Considered: Behavioural Characteristics and Body-size

Developmental disorders such as learning disabilities, attention deficit hyperactivity disorder, developmental delays, emotional and behavioural problems are found in 5-10% of the pediatric population (Kerstjens et al., 2009). However, there are a number of terms that have been used by different researchers to describe developmental disability, neurodevelopmental disorders and intellectual developmental disorders (IDD). Up until recently, behavioural problems were part of the core classification of IDD. After much debate and consideration, the World Health Organization Advisory Group has come to the conclusion that behavioural problems are an associated feature of IDD rather than subcategories or specifiers of IDD (Salvador-Carulla et al., 2011). However, the term used throughout this thesis is behavioural problems, which is a category of developmental disorder. Developmental disorders such as behavioural problems are the dysfunction of the brain and may affect behaviour, learning or memory processes (Cho, 2006).

There is limited research on behavioural problems in Pacific children in NZ (Paterson, Carter, Gao, & Perese, 2007). However, the NZ Pacific birth cohort study examined the prevalence of behavioural problems, using the child behaviour checklist, as reported by the mothers. This study found that the relationship was higher than for other international populations (Paterson et al., 2007).

Pacific people in NZ carry a larger burden of mental disorders than the general population (24.4 %) (CI 95% 21.2, 27.6) (Pollack et al., 2012). There is compelling evidence that the early adverse environment of a child can have lifelong effects on the emergence of conduct disorder, substance abuse and physical and mental health problems (P. Grandjean et al., 2008). It is likely that unbalanced nutrition or ingestion of toxic elements from food may contribute to such health problems, especially in children.

Body-size is one of the most important public health outcomes that confront the Pacific population in NZ. Latest statistics indicate that Pacific people are highly likely to have a larger body-size than the overall NZ population (Ministry of Health, 2008b). Similar observations were obtained from a recent study of 200 Pacific children which found 62% of boys and 58% of girls had larger body-sizes (Oliver, Schluter, Paterson, Kolt, & Schofield, 2009). Children who have bigger body-sizes are more prone to developing health problems including hyperlipidemia, Type 2 diabetes, and heart problems. Such problems could adversely impact on personal and public health for years to come (Guh et al., 2009). Recent evidence suggests that low concentrations of specific elements (such as iron, calcium, magnesium, zinc) or increased concentrations of toxic elements (lead, mercury, cadmium, arsenic) may be associated with fat disposition in the body (Padilla, Elobeid, Ruden, & Allison, 2010).

By studying the relationship between elements and such health-related problems for NZ Pacific children it is anticipated that additional tools would be provided with which to treat health-related problems and disorders. Within NZ, there is the large-scale, robust, PIF birth cohort that enables the investigation of growth and development of Pacific children. The next section provides a brief description of the PIF study.

1.3 Pacific Island Families (PIF) Study

This research study was undertaken within the longitudinal PIF study, which is following over 1398 Pacific children and their families. The PIF study commenced in 2000 with the overall aim of understanding family health and development on which to base appropriate Pacific-driven interventions throughout the life-course of Pacific people. It is recognised for robustness and, cultural appropriateness, with respectable participation rates (Paterson, Percival, et al., 2008). The PIF study was designed to provide information on Pacific people's health, and the cultural, economic, environmental and psychosocial factors that are associated with child health and development outcomes and family functioning. It also determines how such factors individually and interactively influence positive and negative child, parent and family outcomes over time, and to provide information that will help set quantifiable targets for Pacific peoples' health (Paterson, Percival, et al., 2008). The specific methodology of the PIF study is further detailed in Chapter 2.

1.4 Aim and Objectives: Overall Perspective

The overall aim of this research was to collect and analyse the scalp hair and toenails of Pacific children (and in some cases their mothers) resident in Auckland, NZ (as part of the PIF population study) for a range of chemical elements and, with subsequent statistical analysis and interpretation, it was hoped to add to the limited existing knowledge on elemental status and NZ Pacific children's health (especially body-size and behavioural disorders).

Overall three studies were conducted, each with a different focus on the investigation of elemental status in NZ Pacific children. These three studies explore the:

- (i) effect of elements in scalp hair (with specific reference to mercury) on behavioural problems in NZ Pacific children;
- (ii) effect of elements in toenails (with specific reference to mercury) on the behavioural characteristics of NZ Pacific children; and
- (iii) possible effect of selected elements on NZ Pacific children's development, with specific reference to body-size.

1.5 Thesis Outline

This body of work is presented as a logical progression of the different studies (presented as a series of chapters) that work together to form the thesis (Figure 1). The first chapter is the overall 'introduction' for conducting the doctoral research. This is then followed by Chapter two which provides an 'Overview of Thesis Development'. Chapter three is the 'Literature review 1 – Overall Perspective'. This chapter provides a literature review on Pacific health which falls across all the studies in this doctoral thesis, presents literature on elements in human health, and is followed by a review on the measurements of elements in human tissues. Chapter four is 'Literature Review 2 – Mercury Exposure and Behavioural Problems in Children' for Study 1 and Study 2. This is followed by Chapter five which is titled 'Literature Review 3 – Elements and Body-size' for Study 3. Next, Chapter six (Study 1), Chapter seven (Study 2) and Chapter eight (Study 3) provide an overview of the studies, materials and methods employed, results and discussions for each of the three studies respectively. This is then followed by the final Chapter nine which is the overall discussion and conclusions of the thesis.

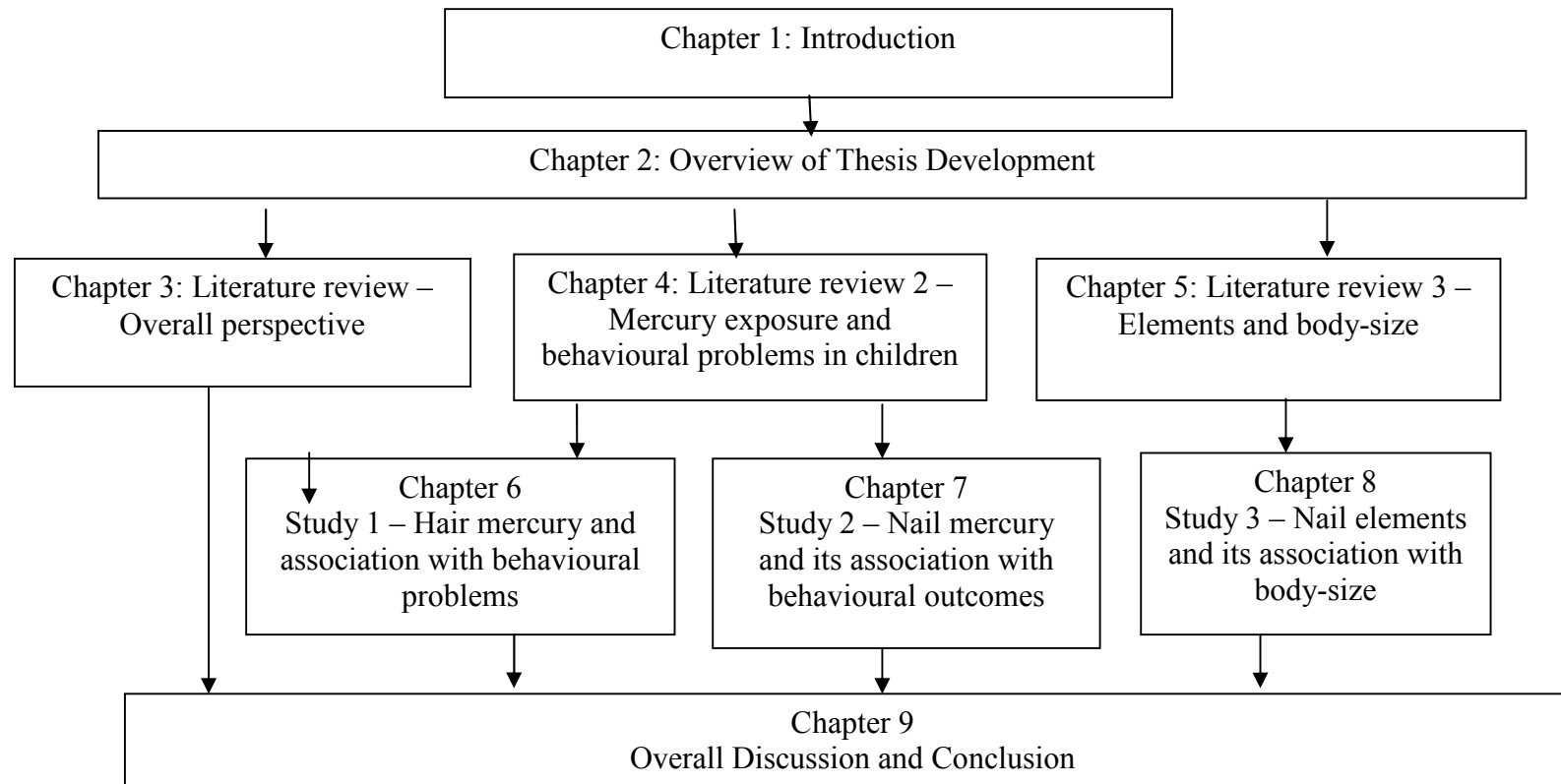


Figure 1: Flow diagram of the PhD Thesis

Chapter 2 Overview of Thesis Development

This chapter provides an overview of the research formulated around the data generated by the Pacific Islands Families (PIF) cohort. As such, the PIF methodology which is common across all three research studies undertaken in this thesis is also provided as well as a brief explanation on the choice of methods used for selecting individuals for scalp hair and toenail sample collection and subsequent elemental analysis.

2.1 The Pacific Islands Families (PIF) Cohort

The PIF study, as mentioned in section 1.3, is a birth cohort that is a culturally well recognised, robust study that is multi-disciplinary and which captures information from mothers, fathers and children. The study incorporates information about the health and development of 1398 New Zealand (NZ) Pacific children born in South Auckland, and their families (Paterson, Percival, et al., 2008). The database was developed through a process of collaboration with Pacific communities, researchers, and relevant health and social agencies (Paterson et al., 2006). The overall aims of the core PIF study are:

- (i) to provide information on Pacific peoples' health, and the cultural, economic, environmental and psychosocial factors that are associated with child health and development outcomes and family functioning;
- (ii) to determine how such factors individually and interactively influence positive and negative child, parent and family outcomes over time; and
- (iii) to provide information that will help set quantifiable targets for Pacific peoples' health.

2.1.1 PIF Methodology

The PIF study has recruited Pacific Island infants (n=1398) born at Middlemore Hospital, South Auckland, between 15 March and 17 December 2000. The eligibility criteria for an infant was if at least one parent identified themselves as being of Pacific Island ethnicity and was a permanent resident of NZ or a citizen.

Presentation of the study information to mothers and permission to visit at six weeks of birth was completed within the hospital setting. Informed consent was sought from primary care givers at each phase. Maternal home interviews were undertaken at approximately six weeks, 12 months, 24 months, four years, six years and nine years postpartum. In most cases, the interviewers were ethnically matched to the potential participant. The interviewers visited the participants in their own homes, fully described the study to the parent(s), and obtained the mother's informed consent. Once consent was obtained, the interview was carried out in their own home. For each of the phases all maternal participants were re-contacted and visited by a female Pacific interviewer. This doctoral research is based on the six and nine year measurement phases as the research took place during those phases. At the six and nine year phases, an interview protocol which took approximately one hour to complete was carried out with all maternal participants and child assessments being carried out in the school setting.

Standardised internationally developed measures and scales have been used or adapted for the PIF study. Pacific researchers have considerable input into the general measurement framework and specific measures were employed to ensure the content acceptability and validity. The use of such scales ensures constructs (universal across cultures) are measured and information considered important to child health and development is elicited. Measures employed during the pilot phase that had unsatisfactory reliability and/or validity were

removed. Some measures underwent focus group examination to check wording of items and testing procedures. However, the psychometric properties of most standardised measures used in the PIF pilot were acceptable, and few modifications were required. Various systems have been implemented to ensure data accuracy and consistency. These include: manual coding of each interview protocol to check consistency within the individual interview; accompanying interviewers to check on rapport, informed consent and on the undertaking of standard procedures; and post-interview random phone checks with participants to clarify and confirm specific details of the interview.

2.1.2 PIF Statistical Analysis

A multi-level analytical approach and cross-sectional analyses at each assessment was undertaken, allowing the prevalence of key outcomes to be estimated, as well as the association between risk factors and key outcome variables (Paterson et al., 2006).

Longitudinal analysis techniques were used in the modelling of developmental pathways including structural equation modelling, mixed linear models, and generalised estimating equations. The gathering of data from mothers, fathers, and secondary sources allowed the concordance between reports of child outcomes and parental functioning to be assessed (Paterson et al., 2006).

The Fisher's Exact Test was employed to determine whether differential attrition existed over time for a selection of basic socio-demographic variables. Differential attrition would have occurred if both 12 month and 24 month distributions of participation and non-participation were significantly different to the 6 week distributions, using a significance level $\alpha=0.05$ to define statistical significance (Paterson et al., 2006).

2.1.3 PIF Ethical Considerations

Ethical approval was granted for all aspects of the PIF longitudinal study. Ethical approval for the PIF study was obtained from the Auckland Branch of the National Ethics Committee, the Royal New Zealand Plunket Society, and the South Auckland Health Clinical Board (Paterson et al., 2006). Also, the PIF study was guided by the Pacific Advisory Board who monitored the general direction of the study. Ethical considerations for these doctoral studies are provided in the relevant chapters.

All questionnaires for the main PIF study and the doctoral study were stored in locked filing cabinets in the Pacific Research Centre. All data are stored on password-protected computer systems within AUT University.

2.2 Doctoral Study Development

This doctoral thesis is a sub-study within the PIF cohort. Some information was taken from the PIF core research but the research questions, the additional measures used, the study designs and statistical analysis were conducted separately from the PIF cohort study and were the original work of the researcher (SK). The initial doctoral research (Study 1) was a nested case-control study designed to explore the possible associations of mercury exposure on children's behavioural problems at the six year phase of the PIF project. Scalp hair samples were used as biological markers for mercury exposure; however, as these biomarkers had never been used as part of the PIF study, a thorough consultation was conducted with supervisors, Pacific researchers within the PIF, some Pacific participants and the Pacific Advisory Board, before procedures were adopted. In addition, before the first doctoral research commenced, the study was trialled in a small number of Pacific people to check the feasibility of the procedure and scalp hair sample collection. The small pilot data collection

was successful. Study 1 (presented in Chapter 6) then commenced with approval by the PIF study team, the Doctoral Committee at AUT University and Northern X Regional Ethics Committee. Despite the initial success of the pilot study, Study 1 (presented in Chapter 6) experienced difficulties due to hair sample collection and thus scalp hair sample collection was no longer feasible. The doctoral research had to be modified and Study 2 (presented in Chapter 7) and Study 3 (presented in Chapter 8) were designed. For Study 2 and Study 3, the hair biomarker was then replaced by toenail clippings to improve PIF participation rates. The revised studies had approval from supervisors, PIF researcher team, PIF Advisory Board and the AUT Doctoral Committee before commencing. The Northern X Regional Ethics Committee also provided approval to modify the new research to toenail clippings. The opportunity to investigate a selection of elements was possible through collaboration with the ICP MS (inductively coupled plasma mass spectrometry) Facility, Department of Chemistry, University of Surrey, United Kingdom (UK) who analysed the scalp hair and toenail samples for the respective studies in this thesis.

To build on the earlier work, the association between nail mercury exposure and behavioural problems was further explored in a sub-group of nine year old PIF children (Study 2). In addition, since large body-sizes within Pacific children are more prevalent, it was posited that studying the relationship between elemental status and body-size was an important aspect to examine in this doctoral research. Therefore Study 3 was designed as part of the nine year phase PIF project. The initial research (Study 1) was titled 'Health of Pacific Children: Environmental and Nutritional Determinants'. After Study 2 and Study 3 were designed the title was then changed to 'Health of Pacific Children in New Zealand: Association between

Elements, Behaviour and Body-size’ with approval from supervisors and the AUT Postgraduate Committee.

In summary, this research was based on three different studies, with two different kinds of biological markers (scalp hair and toenail samples). The next few chapters provide further details on the study designs and methodologies employed. Table 1 provides a brief overview of the different study designs employed and biomarkers used in this doctoral thesis.

Table 1: Summary of the three study methodology

Studies	Subjects	Ages (years)	Study designs	Biomarkers	Independent variables	Dependent variables
Study 1	Children & mothers	6 & 23+	Nested case-control	Scalp hair	Mercury	Child behavioural problems
Study 2	Children	9	Opportunistic cross-sectional	Toenail clippings	Mercury	Child-behavioural problems
Study 3	Children	9	Opportunistic cross-sectional	Toenail clippings	Essential, non-essential and toxic elements	Body-sizes

All the studies in this PhD research were presented through oral presentations to the Doctoral Committee and postgraduate researchers of AUT University. Any suggestions that were provided during presentations were implemented (if and when relevant) in the final plan of the thesis. The PhD was also presented in written forms which were peer reviewed by the AUT Doctoral Committee for progress approval. A review on mercury exposure in NZ was undertaken which was published in *Australasian Epidemiologist* (appendix 1). Permission

was sought and granted from the journal to use the information for this doctoral study (letter in appendix 1).

2.2.1 Data Storage across Doctoral Studies

All data that was obtained for this research thesis were stored in Microsoft Access™ and Excel™ databases (Microsoft Corporation, US) supervised by a senior biostatistician. The data was cleaned, range and consistency checks were performed, and coded for analysis. Questions with no response were not included in the analysis as a distinct coded variable. The cleaned data was then exported to STATA version 10 (StataCorp, 2007) for statistical analysis.

2.2.2 Literature Searches for this Doctoral Thesis

The doctoral literature reviews were conducted predominantly through searches on established research and library electronic databases such as Ovid, Medline and PubMed, as well as online web resources such as the New Zealand Ministry of Health and Statistics. Several searches, especially for the research on elements, were undertaken using ScienceDirect and the Web of Knowledge.

Chapter 3 Literature Review 1 – Overall Perspective

This chapter explores two health outcomes specifically around behavioural problems and large body-size in New Zealand (NZ) Pacific children. It provides an overview on elements of the periodic table in relation to human health and a review of the use of scalp hair and toenails is undertaken. These samples were employed as biomarkers for assessing elemental concentrations in the individuals under investigation in this thesis. Methods for literature searches are discussed in Chapter 2 (section 2.2.2).

3.1 Introduction

3.1.1 Pacific Children in New Zealand

Pacific people are a youthful, growing community in NZ with one in every five people being children under the age of 15 (Cook, Didham, & Khwaja, 1999). Pacific children make up 11.5 % of NZ's child population and they are culturally diverse as each Pacific group has their own language, and traditions (Statistics New Zealand, 2006). Pacific children are a priority population, as identified by the Child Health Strategy (Ministry of Health, 1998), because they are known to experience poorer health outcomes compared to the overall population mainly due to their socio-economic circumstances (Ministry of Health, 1998).

While Pacific people are culturally diverse, they have a broadly shared culture and set of beliefs. For example, as part of Pacific culture, food is regarded not just as a source of nourishment but is also a way of showing thankfulness, condolences and apologies (Tamasese, Parsons, Sullivan, & Waldegrave, 2010) and therefore food plays an important role in Pacific life (Moata'ane & Guthrie, 2005). A Pacific youth health study for the Waitamata District Health Board reported that it is generally accepted that youths will be

overweight as food is an important element in their culture (Leger, 2005). It is also suggested that migration from Pacific countries to NZ, could have brought about changes in the people's eating patterns due to changed economic, social, demographic and health structures – a phenomenon known by some authors as 'nutritional transition' (Mondini & Monteiro, 1997; Popkin, 2006, 2009). This has been reported in numerous studies which have documented major shifts in the eating patterns of specific cultural groups worldwide (Hohepa, Schofield, & Kolt, 2006; World Health Organisation, 2003) and elemental deficiencies have been observed (World Health Organisation, 2010). Pacific people are also known to consume a high-fish diet (Crump, Kjellstrom, Shipp, Silvers, & Stewart, 1998; Ministry of Health, 2012a). A recent NZ survey found that over half (53%) of Pacific people are more likely to eat fresh or frozen fish/seafood one or more times a week than those categorised as NZ European and Others (NZE0) (Ministry of Health, 2012a). Studies have shown that consuming predatory fish can expose people to toxic elements such as mercury (Hightower, O'Hare, & Hernandez, 2006). These dietary patterns can lead to nutritional deficiencies or toxicities with adverse health effects on children (Mertz, 1993).

In NZ, the burden of nutrition-related diseases is greatest among vulnerable and disadvantaged groups such as Pacific people (Eyles et al., 2008). In the last decade there has been a shift from home-prepared meals to using fast-food outlets (typically high in sugar, fat, and salt) possibly due to food advertising on television during children's viewing times (Hohepa, Schofield, & Kolt, 2004). Such food advertising has been shown to influence children's food preferences and purchase behaviour leading to high-fat diets (Story & French, 2004). According to the Ministry of Health (2008b), Pacific children have a lower mean energy intake than Māori, and a higher intake than European. As such they derive their

energy from fatty foods. In addition, the proportion of fat intake may increase with a decrease in family resources (Ministry of Health, 2008b; Utter, Scragg, Mhurchu, & Schaaf, 2007). A survey found that 45% of Pacific people ran out of food sometimes or often, compared to 9% of NZEO households (Ministry of Health, 2011, 2012a). Forty-five percent of Pacific people also reported that a lack of money has affected their choice of food consumed either sometimes or often, compared to 23% of NZEO households (Rush, 2009). Lack of a good diet and improper eating habits can lead to an imbalance of elements or ingestion of toxic elements both in adults and children. This in turn can distort an individual's physiology and damage the developing brain, which consequently could compromise their health (Amare et al., 2012; Failla, 2003). In a British study, it was reported that children from low socio-economic families, had considerably lower calcium and iron (Nelson, 2000). Health inequities do exist between Pacific children and other population groups in NZ (Ministry of Health, 2008a) thus it is vital that these inequities are understood and minimised, as all children need to have an equal opportunity to reach their highest attainable standard of health, development and wellbeing (Ministry of Health, 2008b).

In summary, the evidence outlined above shows that Pacific children are more likely than other children (apart from Māori children) to suffer from adverse health outcomes due to certain health risks such as unhealthy dietary patterns and eating habits due to their socio-economic status (Statistics New Zealand and Ministry of Pacific Island Affairs, 2011a). The two important health outcomes highlighted in the literature form the basis of this thesis, namely behavioural problems in children and childhood body-sizes.

3.1.2 Behavioural Problems in Pacific Children in NZ

Behavioural problems in childhood are increasingly recognised as constituting a serious source of health morbidity with negative consequences for adult as well as child health. There is very little research into developmental disabilities of Pacific children in NZ. Only four publications were found which were related to behavioural problems in Pacific children in NZ and these are all within the NZ Pacific birth cohort study (Gao, Paterson, Abbott, Carter, & Iustini, 2007; Paterson, Carter, Cowley-Malcolm, Gao, & Iustini, 2008; Paterson et al., 2007; Paterson, Taylor, Schluter, & Iusitini, 2012). Three more surveys conducted by the Ministry of Health were also identified on broader developmental disabilities (Ape-Esera, Nosa, & Goodyear-Smith, 2009; Ministry of Health, 2004, 2011).

In 2001, a survey estimated that 0.9% of Pacific children between the ages of 0 to 14 years had developmental disabilities, particularly intellectual disability (Ministry of Health, 2004). Another survey conducted between 2005-2009 found that the number of Pacific children and young people with developmental delays was higher than for their Māori, European and Asian counterparts (Ministry of Health, 2011). A survey conducted between 2005-2009 found that mental health issues are experienced by Pacific people at a higher rate than among the general population, especially in those Pacific people who were born in NZ, 37% of mental health disorders compared to 15% of those who migrated from Island countries (Ape-Esera et al., 2009). This places Pacific children (who are born in NZ) at a possible higher risk of mental health disorders in adulthood if developmental disorders are not treated and prevented at an early stage. For example research suggests that children who are restless, easily distracted and impulsive have a higher chance of being suicidal, having antisocial personality disorders, and engaging in criminal behaviours when they become 21 years of age

(Caspi, 1998; Trzesniewski et al., 2006). An 18 year follow-up study of 375 young adults, found that hostility/aggression and disruptive behaviours were predictors of antisocial behaviours in adulthood (Pakiz, Reinherz, & Giaconia, 1997). Although data specifically on behavioural problems in NZ Pacific children is lacking, one longitudinal study, the PIF cohort (refer to section 2.1), has identified a high prevalence of child behavioural problems (Paterson et al., 2012). However, it is not known if such behavioural problems exist in children within the wider Pacific communities in NZ. Behavioural problems are an important health outcome as there are long-term consequences of developmental disorders in children which can carry on into later life and lead to antisocial and criminal behaviour, low attainment and, high economic costs for the community with possible mental disorders (Ape-Esera et al., 2009; Griggs & Walker, 2008; LeClair & Quig, 2001).

3.1.3 Body-Sizes and Nutrition in Pacific Children in NZ

Abnormal body-size is an important public health outcome that confronts Pacific people in NZ. The Ministry of Health uses the International Obesity Task Force (IOTF) Body Mass Index (BMI) cut-off points which are sex and age-specific, and have been designed to coincide with the WHO BMI cut-off points for adults at age 18 years. Many studies have looked at BMI and mortality (Flegal, 2005; Katzmarzyk, Craig, & Bouchard, 2001; Klenk et al., 2009) though BMI has its limitations, including the inability to reflect body fat distribution. However Taylor et al., (2010) found no strong evidence to support replacing BMI in clinical or public health practice with other adiposity measures. For the purposes of this research the IOTF classification to determine large body-size is used. Many studies have been identified in relation to body-sizes using the IOTF criteria in Pacific children in NZ. Only the most relevant and recent reports and articles were included in this review.

The last census indicates that Pacific people are more likely to be in the larger body-size group than the overall NZ population and that more than 60% of Pacific children in NZ were classified as overweight or obese using the Cole's-cut-off to define overweight and obesity (Ministry of Health, 2008c). Furthermore, Pacific boys and girls were 1.50 (95% Confidence Interval (CI) 1.32–1.67) times more likely to be overweight and 2.81 (95% CI 2.32–3.31) times more likely to be obese than those in the total population (Ministry of Health, 2008c). Among children aged 5-14 years in 2006-2007, the pattern of ethnic differences in the prevalence of larger body-size was similar to that of the population aged 15 years and over, whereby Pacific children had the highest BMI of 30+ rate (26%) compared to other NZ children (Social Report, 2010).

Research has also shown that Pacific children were more likely to consume pies, hamburgers, and fizzy drinks than the rest of NZ's children, foods which are linked to body fatness (Utter, Scragg, Schaaf, & Fitzgerald, 2006). The most recent survey found that Pacific children eat less fruit and vegetables compared to other NZ children (Ministry of Health, 2008b, 2012a). Several studies have documented the fact that there is an increase in the prevalence of overweight and obesity in ethnic minorities in NZ (Dwyer et al., 2000) which could be due to possible changes in their dietary habits after migrating to their new country. Abnormal body-sizes are associated with high incidence of all-cause mortality, cardiovascular diseases, stroke and Type 2 diabetes. Consequently it is important to understand and investigate the varying body-sizes within Pacific children (Ministry of Health, 2008b).

There are two possible limitations to the body-size studies outlined above. Firstly, most of the studies have used BMI for age and gender cut-offs for Pacific children in NZ. There is constant debate on whether the BMI (which is used to identify people with excess body fat)

should be used for NZ children of different ethnic groups (Rush, Plank, Chandu ., et al., 2004; Rush, Puniani, Valencia, Davies, & Plank, 2003) as Pacific children are known to have a lower percentage of body fat and more muscle mass (Rush, Plank, Chandu ., et al., 2004; Rush et al., 2003). Secondly, most of the studies on obesity in NZ have been cross-sectional and thus it is difficult to establish temporality. The studies have used food frequency questionnaires which may not necessarily be valid or reliable measures of diet (Burrows, Martin, & Collins, 2010; Burrows, Warren, Colyvas, Garg, & Collins, 2009). However, direct markers such as biological samples are more accurate measures of dietary elements and this is reviewed in section 3.3.

3.2 Elements in Human Health

There is an increasing interest in the potential influence of toxic and essential elements on the development of the brain and the body in children. Certain elemental imbalances in the human body have been studied worldwide (Kimmons, Blanck, Tohill, Zhang, & Khan, 2006b; Park, Choi, & Nam, 2009) and relate to a number of health problems. Food is the principal pathway for sources of nutrition which helps keep nutritional balance in the body. However, changes in nutritional patterns are dependent on a number of cultural, socio-economic, climatic and ecologic factors which determine which foods are available and consumed by the community. From studies worldwide, it has been shown that the nutritional needs of people are either adequately satisfied or exceeded (Abdullah, Khan, & Reis, 1996; Iyengar & Nairn, 2000). For certain population groups (such as Pacific people), due to economic restrictions, they may not reach the necessary levels of certain essential elements required to maintain an optimum level of health, especially through the consumption of 'nutritious' food. Due to better sample preparation procedures, sophisticated analytical

techniques and quality control validation, it is now possible to determine the levels of most naturally occurring elements (especially essential) in the food chain and ultimately the human body. With a greater understanding of how the human body uses these elements, comes an appreciation of their relationship with health and disease. There are many different classifications used to express the role of elements in relation to human health and for the purposes of this research the particular classification of elements used is presented in Table 2.

Table 2: Classification of essential minor; essential, non-essential and toxic trace elements in terms of human health (Vandecasteele, 1993; Ward, 2000)

Classification	Essential minor	Essential trace	Non-essential trace	Toxic trace
Human health	Ca , Cl, Mg , P, K, Na	As , Co , Cr , F, Fe , I , Mn , Mo , Ni , Se , Sn, V, Zn	Ag, Al , B , Ba, Be, Br, Ge, Li, Rb, Sb , Sr, Ti, Zr	Cd , Hg , Pb

Note: Elements in bold were determined in this research

In terms of the classification in Table 2, a minor element relates to that found at levels of approximately > 10 mg/l or mg/kg (depending on whether a tissue or fluid) and trace as < 10 mg/l or mg/kg, in the human body.

Research has shown that certain diseases are associated with abnormal levels of particular elements, for example, imbalances in at least nineteen elements (such as lithium, boron, aluminium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, selenium, molybdenum, cadmium, iodine, mercury and lead) can cause potential health problems (Lappe, 1983). Elements are present in many different forms within the human body, namely ions, complexes, and metalloenzymes. Consequently, for a state of good health, essential elements must not only be present, but they must also be in the right location, the right

amount, the correct oxidation state and be bound to the right chemical partner (Fiabane & Williams, 1977). Human disorders of essential elements arise from inadequate intake, genetic defects, excessive exposure and impaired elimination (Patriarca et al., 1998). The human body's main source of the necessary levels of elements for good health is from nutrition (a balanced and healthy diet) although there are other, less important, exposure routes (skin surface absorption, respiratory uptake). Therefore, adequate digestion and absorption of elements or nutrients is very important in relation to optimum human health. Element absorption is related to bioavailability, i.e. "the proportion of total metal (element) in a food, meal or diet that is utilised for normal metabolic functions" (Fairweather-Tait, 1997). Certain elemental species are more bioavailable than others, as element bioavailability in the body is determined by many dietary and physiological factors, such as chemical/physical form and oxidation state. All elements and compounds can be classified as toxic; it is only the dose which distinguishes the difference between deficiency, normal and toxic status (Mertz, 1981). Figure 2 provides a schematic of the metabolism of elements in humans.

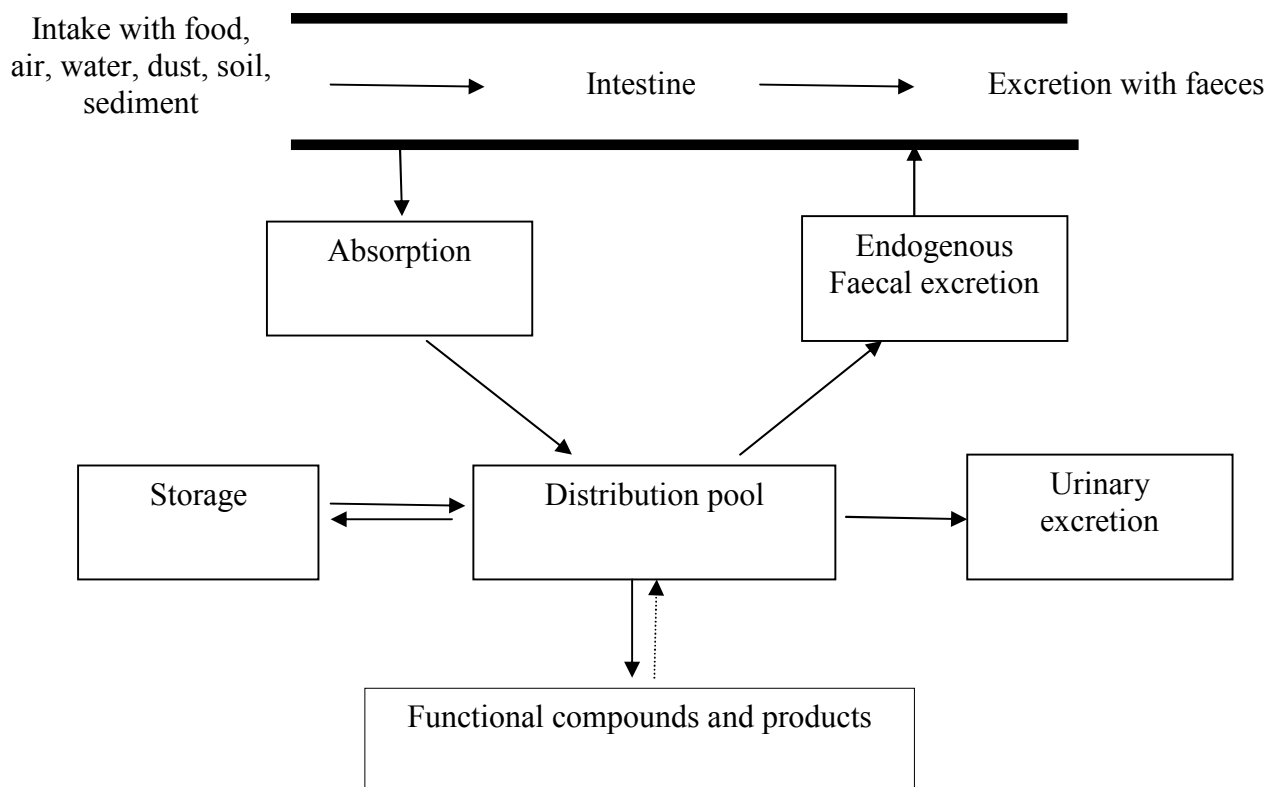


Figure 2: Model of Element Metabolism (Kirchgessner, 1993)

3.2.1 Behavioural Problems and Mercury

Behavioural problems in children are due to a number of factors that are multi-systematic in nature (Brown, Copeland, Sucharew, & Kahn, 2012; S. B. Campbell, 1995; Pachter, Auinger, Palmer, & Weitzman, 2006a; Sikora et al., 2013). However toxic elements such as mercury have been demonstrated to affect the bioavailability of various essential elements, potentially leading to subclinical developmental disabilities, including behavioural problems (P. Grandjean, Satoh, Murata, & Eto, 2010; Neuman, Winterton, Lu, & Roja, 2006). Individuals with elevated MeHg levels do not always show clinical symptoms and so these can be difficult to detect and measure (Miodovnik, 2011). Concerns are also growing about the health impact on adults due to MeHg exposure, as studies are showing that MeHg exposure might interfere with vision, motor function, and memory (Lebel et al., 1998). In NZ, an

important route of MeHg exposure is through fish and seafood consumption (Crump et al., 1998; Vannoort & Thomson, 2005). As children are more vulnerable to the effects of MeHg, there are growing concerns which are being raised by media and health authorities about fish/seafood consumption during childhood and its hazardous effects on the health and development of exposed children (Nielsen, 1984; Williams et al., 2000; World Health Organization, 2007). However, the benefits of fish consumption to children have also been well recognised (Mozaffarian, 2009; Oken & Bellinger, 2008). Behavioural problems in children are measured using many kinds of tests; however, the child behaviour checklist (CBCL) can evaluate emotional and behavioural problems of children (Visser, Huizinga, Hoekstra, VanDerGraaf, & Hoekstra-Weebers, 2006). The CBCL has been used to investigate the effects of each of Polyunsaturated B, MeHg, and lead on behavioural problems (Burns, Baghurst, Sawyer, McMichael, & Tong, 1999a, 1999b; Davidson et al., 1998; Myers et al., 2000; Myers et al., 2009; Wasserman, Staghezza-Jaramillo, Shrout, Popovac, & Graziano, 1998). A detailed literature review on mercury is included in Chapter 4.

3.2.2 Body Fatness and Elements

New evidence suggests that deficient levels of elements or increased toxic metals may be associated with increased fat deposition in the body (Goyer & Clarkson, 2008; J. S. Huang, Lee, & Lu, 2007; Padilla et al., 2010; Padmavathi, Rao, Venu, Ganshan, & et al., 2010; Satarug & Moore, 2004; J. B. Vincent & Rasco, 2010; Yanoff et al., 2007). Larger body-sizes have been recognised as a risk factor for several nutrient deficiencies (Garcia, Long, & Rosado, 2009; Xanthakos, 2009). The cause of these nutritional deficiencies in children with a BMI of over 25+ could be due to the quality of diet such as the higher intake of fatty

processed foods associated with poor nutritional quality. This is particularly problematic in highly developed countries in which there is an abundance of relatively cheap, energy-dense, but nutrient-poor food (Manios et al., 2009; Washi & Ageib, 2010; Xanthakos, 2009).

Elemental deficiencies have been observed in obese individuals across age groups worldwide (Garcia et al., 2009). There are many studies that have researched the effects of elemental deficiencies on human functions in different populations; however, there is limited information on how these elemental deficiencies affect those with BMI >30 especially children. A detailed literature review on body fatness and elements is provided in Chapter 5.

3.3 Measurements of Elements

As reviewed earlier, it has been demonstrated that the concentration of elements at certain levels can affect the human physiology with the potential to have both positive and negative effects on health. Therefore the accurate measurement of elements becomes very important in public health research. Such measurements can be used to detect the total elemental concentration of a particular material (i.e. the sum of elemental concentrations in all chemical species containing that particular element) or look more specifically at individual chemical species. Furthermore, choosing the right biological marker for elemental analysis is very important and depends on the investigation at hand, its feasibility and biological implications. For example, hair and nails are less invasive, can be stored at room temperature until chemical analysis, and provide direct long-term measurements of exposures or risk factors.

Elemental concentrations can be measured in body tissues and fluids. These can be good diagnostic tools if the results are interpreted properly. One way of interpreting trends in element concentrations can be to compare results from the same subject over a reasonable time period (six months for example), but this is not always practical. Another way is to

compare the results with a corresponding reference concentration range for a well-defined population. Reference ranges are established through the careful evaluation of literature sources (Iyengar & Woittiez, 1988). In undertaking such research, reliable and valid analytical techniques become necessary. There are several modern techniques for determining low concentrations of elements in biological samples (Prohaska, Pomazal, & Steffan, 2000; Rodushkin, Axelsson, & Burman, 2000) one of which is the inductively coupled plasma mass spectrometry (ICP MS), as this was the technique available for this doctoral research. Reliable and valid elemental analysis can be achieved through the use of quality control procedures. These procedures cover all aspects of an analysis, from record keeping, sample handling and storage through to instrument calibration and the presentation of results. Quality control for biological elemental analysis will often include the use of certified reference materials to check the accuracy of an analytical procedure. Checks can also be made through the use of different instruments and by inter-laboratory comparisons (Cornelis, 1991).

3.3.1 Tissues and Fluids Used as Elemental Biomarkers

Many biological tissues have been used to analyse elements such as blood (whole, plasma, serum, erythrocytes), hair (scalp and pubic), nails (toes and fingers), liver, kidney, lung tissue, urine, milk, saliva, sweat and cerebrospinal, seminal, tear and bronchoalveolar fluids (Caroli et al., 1994). The use of biomarkers in health research is of great importance in both the clinical and epidemiological settings especially to identify health issues related to trace elemental disorders (both toxic and beneficial elements). Biomarkers are also known to reduce bias thus improving validity in the measurement of exposures (or risk factors) for health disorders. Biomarkers provide direct measurement of the level of exposures and

therefore lessen the possibility of misclassification of exposure. Thus, the use of biomarkers improves the sensitivity and specificity of the measurement of the exposures or risk factors.

The choice of biomarkers is based on practical considerations and depends upon the focus of the investigation i.e. the most appropriate biomarker that could be used to best demonstrate the validity of a hypothesis. Other factors which may influence the choice of a biomarker to be used include: ease of sampling (both for the participants and researcher), financial feasibility, easy storage and available analytical techniques for that sample. Also, different kinds of biomarkers provide information on exposures that may be long term or short term. For example element concentrations may only give a 'snap-shot' of the body when the sample was taken, or, they may reflect longer-term body stores. Blood, for example, is subject to homeostatic regulation, and any conclusions drawn from element analysis of blood or its components should take this into consideration.

For this doctoral research taking into account all the above factors, hair samples were used as biomarkers for mercury exposure in Study 1 and toenail clippings were used in Study 2 and 3 for determining exposure to 19 selected elements. These biomarkers are further reviewed in the next section below.

3.3.2 Hair Samples as a Biomarker for Mercury

Scalp hair is a fibrous material derived from skin which has two main parts: the root, which lies beneath the surface of the skin, and the shaft, which protrudes out from it. Hair is formed from a group of matrix cells in the root called the bulb and grows at between 0.2 and 0.5 mm per day, and during its formation is exposed to circulating blood, lymph and extracellular fluids. As the hair grows, it hardens to form the shaft in a process called keratinisation. The

effect of this hardening process is such that the contents of the hair are sealed in. (Katz & Katz, 1992). Hair samples are easy to collect, and non-invasive.

Mercury exposure can be measured in scalp hair samples and is well recognised as the best biomarker for methylmercury exposure (United Nation Environmental Programme, 2010).

Mercury provides a long-term marker of exposure to methylmercury as once it is incorporated in the hair, it does not return to the blood system (United Nation Environmental Programme, 2010). Methylmercury is incorporated into hair as it is formed and has a direct relationship with blood mercury levels (Cernichiari et al., 2007; United Nation Environmental Programme, 2010). Hair allows for peaks in mercury concentration to be detected and can put exposure in a temporal context as hair grows about 1 cm each month (United Nation Environmental Programme, 2010).

Inorganic and elemental forms of mercury are not excreted to any significant amount in scalp hair, making hair an inappropriate biomarker of inorganic or elemental mercury exposure.

Among fish consumers, 80% of the mercury in hair is from methylmercury. Age, hair colouring and treatments, and ethnicity (hair type varies by ethnicity) may affect the uptake of mercury by hair. Mercury concentrations of 1 parts per million (ppm) in hair is within the normal range while those consuming contaminated fish may have 10 ppm or higher mercury in hair (United Nation Environmental Programme, 2010). A disadvantage of hair samples is that it can become difficult to distinguish mercury that has been exposed externally on the hair and mercury deposited during hair growth (Morton, A, & Gardiner, 2002). This can be overcome by washing before analytical analysis to prevent external contamination (Morton et al., 2002).

3.3.3 Nail Samples as Biomarkers for all Elements

Human toenails are a specialised keratinous skin appendages (as in hair) that grow approximately 1 mm per month and the growth cycle is completed at 6 to 9 months (Cashman & Sloan, 2010). Keratins are fibrous proteins that contain disulfide bridges (Raab & Feldmann, 2005) which are thought to chelate (prevents absorbed nutrients from precipitating) elements present during formation (Slotnick & Nriagu, 2006). Depending on age, gender, health conditions and metabolic rates, nail growth rates differ (He, 2011). A wide variety of elements are found in human toenails and many tend to exist at approximately the same order of magnitude in each type of toenail (Aguilar & Saiki, 2001; Mehra & Juneja, 2003). Elements in nails provide a time-integrated measure of body intake, especially toenails clippings which reflect a long exposure time frame given the relatively slow growth rate (Yaemsiri, Hou, Slining, & He, 2010). A 1 mm of nail sample corresponds to roughly 1 month of body nutritional status (He, 2011). For example, the mean Se intake in time, spans from one month to a year (Longnecker et al., 1993). In addition, concentrations of toxic elements in nail tissues have been reported in order of magnitude higher than those of body fluids and other accessible tissues (Hussein Were, Njue, Murungi, & Wanjau, 2008; Sukumar & Subramanian, 2007).

Nail samples, particularly toenails, are also less exposed to external contamination (Barbosa, Tanus-Santos, Gerlach, & Parsons, 2005). Just like hair, they are simple to collect, easy to analyse and store well (Brockman et al., 2009). Also, elements deposited into the nails are not subject to additional metabolic processes and many elements are present in the nail at substantially larger concentrations than in urine or blood (Hordinsky, Sawaya, & Scher, 2000). There have been studies that have examined the reliability of toenail measurements

(Garland et al., 1993; Krogh et al., 2003). Although attenuation in measures of association may occur, the toenail concentrations of most elements are suggested to be useful biomarkers of exposure in which a single sample is assumed to represent long-term exposure (Garland et al., 1993). Mercury levels in nails have been compared with blood with significant correlations observed between toenail-Hg and blood-Hg (Alfthan, 1997) and toenail-Hg and Methylmercury in blood (Bjorling-Poulsen, Andersen, & Grandjean, 2008).

Humans encounter exposures (some beneficial and some harmful) to elements in various chemical forms on a daily basis. As mentioned earlier, sources of exposure can be through ingested foods, multivitamins, and beverages, inhaled airborne particulates and absorbed compounds. The advantage of biomarkers is their potential for integration of all absorption pathways, yielding information on what is purported to be the most important actual body burden or status of the toxin or nutrient.

The concentrations of elements in nails have been studied in relation to health problems such as skin diseases, hypertension, diabetes, obesity, diet, smoking and drinking habits (Bougle, Bureau, & Laroche, 2009; Flores-Mateo, Navas-Acien, Pastor-Barriuso, & Guallar, 2006; Mehra & Juneja, 2005; Mordukhovich et al., 2012; Rajpathak, Rimm, Morris, & Hu, 2005; Tascilar, Ozgen, Abaci, Serdar, & Aykut, 2010).

Chapter 4 Literature Review 2 – Mercury Exposure and Behavioural Problems

This chapter provides a background on the toxic element mercury and its effects on human health. In addition it details what is known on the scope of mercury and behavioural problems in children. The overall intent was to gather a sufficiently detailed level of understanding of published work available on the topic under investigation (refer to section 1.4) with a view to highlighting gaps in the research. Literature searches were conducted as discussed in Chapter 2 (section 2.2.2). The review paper was published in *Australasian Epidemiologist* (appendix 1).

4.1 Mercury

Mercury (Hg) is a naturally occurring element (with atomic number of 80), found in air, water and soil. It is usually released into the environment through weathering of soil and rocks, from volcanoes and forest fires, and is found in lakes and oceans. Hg is present in the environment in three forms namely: elemental Hg which can be in liquid or gaseous state; inorganic Hg which occurs in the forms such as mercurous nitrate, mercuric chloride, mercuric sulphides, and mercuric acetate; and organic Hg in the form of methylmercury (MeHg), ethyl, phenyl and dimethylmercury (Risher, Murray, & Prince, 2002). The effects of exposure depend on the type of Hg, the amount and frequency of Hg intake. Each of the forms of Hg has a different toxicological profile and clinical symptom (Clarkson, Magos, & Myers, 2003).

There are a number of ways in which humans can be exposed to the different forms of Hg. Although Hg is present in water and air, the concentrations are extremely low and are not a significant source of Hg exposure in humans (Clarkson et al., 2003). Rather, exposure in the

general population primarily occurs from fish/seafood consumption, which is in the form of MeHg, (Anon, 2000; Bjornberg et al., 2005; Clarkson et al., 2003; National Research Council, 2000), dental amalgams absorbed through Hg vapours in the form of elemental Hg, (DeRouen et al., 2006; Levy et al., 2004; Trepka, Heinrich, Krause, & al., 1997), and from vaccines in the form of thiomersal (Clarkson & Strain, 2003). In 2000, NZ phased out all thiomersal-containing vaccines in its childhood schedule vaccines, although some influenza vaccines still contain thiomersal (Immunisation Advisory Centre, n.d.). Hg containing products, such as Hg thermometers and fluorescent lighting, which have been damaged, can also be a cause of Hg exposure to the public. Occupational settings may also expose people, including manufacturers of electric equipment, medical devices or automotive parts that contain Hg, chemical processing plants that use Hg, metal processing, medical and hazardous waste incineration plants, and medical facilities where equipment may contain elemental Hg (Santos et al., 2000; Teaf & Garber, 2012; Yard et al., 2012). Traces of Hg are present in all food but uptake by plants from soil is low and therefore concentration of Hg in fruits and vegetables is low. An international survey has shown that pastures and crops contain less than 0.1 microgram (mg) Hg/kg dry matter (European Commission, 2003). The NZ Total Dietary Survey (NZTDS) 2003/04 of a large nationally representative sample, similarly found that vegetables and fruits such as lettuce, potatoes, tomatoes, prunes, pumpkin, raisins/sultanas, mushrooms, melons to name a few, all had Hg levels well below 0.0001mg/kg (Vannoort & Thomson, 2005).

4.2 Methylmercury exposure

The consumption of fish (especially predatory fish) and seafood is the major source of exposure to methylmercury (MeHg) in humans, (Environmental Protection Agency, 1997; World Health Organisation, 2008a) but there can also be other sources of MeHg exposure.

(a) Fish consumption: In the environment, especially lakes, sea, and waterways, released elemental Hg is converted through methylation to MeHg by anaerobic bacteria in the aquatic ecosystem. Its chemical properties allow it to rapidly diffuse and tightly bind to proteins in aquatic biota, including the proteins in the muscle tissue of fish (Health Canada, 2007).

MeHg bioaccumulates in bigger fish, and consequently larger predatory fish (such as mackerel, pike, shark, swordfish, barracuda, large tuna, marlin, whales and trout) have higher Hg concentrations (Goldman & Shannon, 2001). It is known that almost all of the Hg in fish muscle is MeHg (Bloom, 1992; Maycock & Benford, 2007). The level of Hg varies in different fish species because each has different habitats, lifecycles and feeding patterns. In NZ, many fish contain levels of MeHg higher than the WHO recommended tolerable MeHg intake level (0.5 mg/kg Hg) due to volcanic and geothermal activities (Environment Waikato Regional Council, 2008; Johnston & Savage, 1991) or naturally occurring Hg deposits (Hoggins & Brooks, 1973). Freshwater fish in geothermal lakes and rivers in NZ may also accumulate high concentrations of MeHg e.g. > 36 ppm (Environment Waikato Regional Council, 2008; Sabadell & Axtmann, 1975).

(b) Dental amalgams: One very important and common source of elemental Hg exposure to humans is through dental amalgam in the form of Hg vapour. Methylation of elemental or inorganic Hg does not take place to a significant extent in either human or animal tissues, even at very high toxic levels of elemental Hg (Clarkson & Margos, 2006). Exposure to other

kinds of Hg has not shown an increase in the MeHg burden of individuals or animals (Barregard, Lindstedt, Schutz, & Sallsten, 1994). However, people with dental amalgams are at increased exposure to elemental Hg, which may account for a total Hg exposure equal to or greater than MeHg exposure (World Health Organisation, 1991). This increases their risk of various health conditions, such as cough, dyspnea, fever, tremors, malaise, gingivitis, delusions and hallucinations (National Research Council, 2000; World Health Organisation, 1991). It is known that elemental Hg can also cross the placenta and accumulate in the foetus (Yoshida, 2002). In NZ, the prevalence of dental cavities within Māori and Pacific people has been shown to be relatively high (Schluter, Durward, Cartwright, & Paterson, 2007) thus exposing them to Hg vapours through dental amalgams. However, the estimated risk from dental amalgam is low (World Health Organization, 2007).

4.3 Transport of Methylmercury

Approximately 90–100% of the MeHg is absorbed through the gastrointestinal tract where it enters into the blood stream and is distributed throughout the body (Clarkson, 2002). The pattern of this distribution is relatively uniform, except in red cells where the concentration is 10–20 times greater than the plasma concentration. The half-life of MeHg in people is dependent on the exposure length in addition to the dose of MeHg. For example, for people exposed to high doses over a short term, the half-life of MeHg is approximately 44 days (Balshaw, Edwards, Daughtry, & Ross, 2007), whereas for people exposed to high doses over a long term, the half-life is approximately 90 days (Balshaw et al., 2007). Also, MeHg readily crosses the blood-brain and placental barriers and thus is accumulated and concentrated in the foetus, especially in the brain. (P. Grandjean, Jørgensen, & Weihe, 1994). MeHg also accumulates in hair during its process of formation (Virtanen et al., 2005). The vascular

effects of MeHg include oxidative stress, inflammation, thrombosis, vascular smooth muscle dysfunction, endothelial dysfunction, dyslipidemia, immune dysfunction and mitochondria dysfunction (Houston, (2007). MeHg has great affinity for body fats and for sulfhydryl groups in proteins thereby resulting in a high retention rate within the human body (Health Canada, 2007).

4.4 Adverse Health Effects of Methylmercury

The historical Hg outbreaks in Iraq and Minamata resulted in severe impairments in speech, hearing and neurological disorders of those exposed (Harada, 1995). In addition, a pregnant mother who was exposed to such high levels of Hg gave birth to children with severe developmental disabilities (Amin-Zaki et al., 1974; Harada, 1995). Many longitudinal studies since then conducted in fish-eating populations have shown mixed effects from prenatal Hg exposures (Davidson, Myers, & Weiss, 2004; P. Grandjean et al., 1999; Oken & Bellinger, 2008). The recognised, well-designed Faroe Islands study showed a maternal hair Hg geometric mean of 4 µg/g and 23 µg/l in cord blood. At these Hg levels deficiencies were seen in 14 year old children's motor function, attention, and verbal domains but no associations were observed for postnatal exposure (geometric mean = 3 µg/g in hair and 9 µg/l in blood at 7 years of age) (Debes, Budtz-Jorgensen, Weihe, White, & Grandjean, 2006). However, factors such as whale blubber eaten in these communities could have been a possible confounding factor that had not been taken into account. The Seychelles study did not find a consistent pattern of association between prenatal (mean Hg 7 µg/g in maternal hair) or postnatal MeHg (mean Hg 6 µg/g in hair at nine years of age) and neurodevelopmental disorders (Davidson et al., 2010; Myers et al., 2009). The NZ study showed a greater percentage of children in a high MeHg group (maternal hair MeHg levels

>6 µg /g) who had lower developmental scores (a three-point decrement in intelligence quotient (IQ) than children from the low MeHg groups (maternal hair MeHg <3 µg /g); however, this study focused on a small sample and many confounding factors were not taken into account. An integrative analysis of three cohorts (Faroe Islands, Seychelles, and New Zealand) found an overall child IQ change of −0.18 points (at the 95% confidence interval - CI), that is a −0.38 to −0.01 change for each microgram per gram increase of maternal hair MeHg (Axelrad, Bellinger, Ryan, & Woodruff, 2007). Table 3 provides an overview of some important studies that show adverse effects and non-adverse effects of prenatal Hg exposures on children.

Table 3: Prenatal mercury exposure with and without adverse effects on children

Country	n	Biomarker	Age (years)	Median/Mean	Reference
<i>Adverse effects</i>					
Faroe Islands	917	Cord blood	7	22.9	Grandjean et al.,(1997)
Faroe Islands	878	Cord blood	14	22.5	Debes et al.,(2006)
Seychelles	229	Maternal hair	9 months	5.7	Strain et al.,(2008)
US	341	Maternal blood	3	3.8	Oken and Bellinger (2008)
US	212	Maternal hair	4.5	0.5	Stewart et al.,(2006)
NZ	31	Maternal hair	4	8.8	Kjelstrom et al., (1986)
NZ	73	Maternal hair	6-7	8.3	Kjelstrom et al., (1989)
<i>No adverse effects</i>					
Faroe Islands	917	Cord blood	7	24.2	Grandjean et al.,(2001)
Poland	374	Cord blood	3	NS ^a	Jedrychowski et al.(2007)
Seychelles	613	Maternal hair	10	7	Davidson, Strain, et al.,(2008)
Seychelles	87	Maternal hair	10	7.8	Davidson et al.,(2000)
Seychelles	217	Maternal hair	5.5	7.1	Myers, et al.,(1995)
Seychelles	643	Maternal hair	9	6.9	Myers et al.,(2003)

* Mercury concentrations (µg/g) ^aNot specified

Recently there has been an interest in understanding postnatal Hg exposures at low levels in children. Very few studies have been conducted on postnatal Hg effects. A recent study of the US background level of MeHg exposure ($\sim 0.5 \mu\text{g/l}$ in whole blood at two years of age) did not reveal any significant associations with neurodevelopmental outcomes in children at age 2, 5, and 7 years (Cao et al., 2010). The most studied population on postnatal exposure was the Seychelles and the Faroe Islands studies. A review highlighted that postnatal Hg exposure may influence children's development (Myers et al., 2009). This study found several associations between Hg and certain developmental tests; however, the results differed amongst different ages and developmental domains. None of the studies explored behavioural problems specifically in children.

There have been no studies in NZ that have investigated postnatal Hg effects in Pacific children. The international studies using hair samples (most had mean Hg levels of about $6 \mu\text{g}$) (Davidson et al., 2010) as biomarkers are presented in Table 4 below. As seen the results have varied ages, neurodevelopmental endpoints and biomarkers as well as populations studied.

Table 4 provides information on some studies that have used the Child Behaviour Checklist (CBCL) and other kinds of tests for identifying postnatal neurodevelopmental/developmental disorders in children. A variety of outcome measures were used in all the different studies that included neurologic examination, developmental rating scales, neuropsychological tests, and attainment tests which again makes comparison difficult.

Table 4: Studies that have used tests for identifying developmental effects on children in relation to hair mercury

Reference	n	Age (years)	Tests associated
Myers et al., (2000)	711	5.5	CBCL – No adverse effect
Myers et al.,(2004)	643	9	CBCL – Thought problems – adverse effect
Axtell et al.,(2000)	711	5.5	MSCD, GCI (improved below 10 µg/g and worsened above 10 µg/g)
Huang et al.,(2003)	711	5.5	MSCD, GCI – beneficial effect present

CBCL (Child Behaviour Check List), MSCD (McCarthy Scales of Child Development), GCI (General Cognitive Index)

Generally, many studies on developmental disorders in children exposed to MeHg from maternal consumption of fish have primarily measured cognitive abilities. These include effects on memory, language, cognition, and sensory motor functions. However, various studies have used different endpoints such as Denver development scores, Conner's developmental scores, Wechsler Intelligence Scale for Children (WISC), and scholastic achievements. From the literature review it is noted that not many studies have used the CBCL for investigating developmental effects on mercury exposure. This doctoral study has used the well-established CBCL for assessing the effects of Hg exposure.

4.5 Control Measures and Risk Communications in NZ

The NZ Food and Safety Authority (NZFSA) manages a national monitoring programme for heavy metals in fish while the Food Standards Australia New Zealand (FSANZ) prescribes a maximum level of MeHg in fish and seafood (0.5 ppm for most fish and 1 ppm for certain fish). (World Health Organisation, 2008b) The Joint Food and Agricultural Organization

(FAO)/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for MeHg as 1.6 µg/kg body weight(bw)/week, (World Health Organisation, 2003) which is the equivalent of 0.23 µg/kg bw/day. This value is considered protective of developing foetuses, the most sensitive sub-group in the population. Therefore with the evidence, NZFSA adopted the 2003, JECFA revised PTWI for MeHg (Vannoort & Thomson, 2005). The NZFSA estimated the average daily intake of MeHg by adults, children, toddlers and infants from food sources which ranged from approximately 0.60 to 0.74 µg/kg bw/week (Vannoort & Thomson, 2005).

The dietary exposures in the 2003/04 New Zealand Total Diet Survey (NZTDS) were based on average energy diets for each of the age-sex groups. According to Vannoort & Thomson (2005), some people might have significantly higher exposures, especially within the high fish-eating groups (Vannoort & Thomson, 2005). There is specifically recommended dietary information provided by the Ministry of Primary Industry (2009) on its website . However, it is unclear whether this information reaches pregnant women or at-risk sub-populations (such as Pacific people). Investigation is needed within NZ to identify the best method to inform and educate vulnerable sub-populations so these people can make well-informed choices on consuming fish for good health whilst avoiding fish which may be harmful due to high MeHg levels. Also, with emerging evidence that even low MeHg levels can potentially cause adverse health effects, further studies need to be conducted to verify the extent of MeHg exposure in people, types of fish being consumed and levels of MeHg in fish/seafood that is being consumed, within NZ populations.

4.6 Susceptibility to Mercury

Many other studies suggest that there are different influences that determine the concentrations of Hg. A number of factors such as genetic susceptibility, socio-economic, nutritional and cultural factors could exacerbate the effects of Hg exposure on fetuses, children and adolescents (Neuman et al., 2006). Researchers are only beginning to understand that a number of psychosocial factors such as poverty, poor nutrition, crowding, social disorganisation, racial discrimination, fear, economic deprivation, and cultural factors can make certain groups more susceptible to effects from neurotoxins (Bellinger et al., 2008). This is so because psychosocial stress can lead to acute and chronic changes in the functioning of body systems (e.g. immune system) and also lead directly to illness (Kreiger & Higgins, 2002). Earlier studies on lead have shown that the adverse effects appeared greater among children from lower than higher social economic backgrounds (Bellinger, Leviton, Wateraux, Needleman, & Rabinowitz, 1988; Lansdown, Yule, Urbanowicz, & Hunter, 1986). Moreover, economically privileged children have been shown to recover faster from cognitive functions due to lead exposure than the poorer children with similar levels of neurotoxin.

Scientists also believe that gender may play a crucial role in determining how toxic elements, such as Hg, are manifested due to the differences in kinetics and mode of action. However there is little research in this area (Davidson et al., 2004; Vahter, Akesson, Liden, & al, 2007). Based on a small study carried out in Iraq, an outbreak of MeHg from contaminated grains indicated that women were more affected than men following exposure at adult age (Magos et al., 1981). Two studies on children have shown greater developmental effects in males, than in females (P. Grandjean et al., 1994; Mckeown-Eyssen, Ruedy, & Neims, 1983).

Also, many animal studies have shown that Hg clearance in females was faster than males but females attained higher peak percentages of MeHg dose in the kidney and brain than males (Thomas, Fisher, Sumler, Mushak, & Hall, 1987). Another explanation by Airey, (1983) has shown that females have a shorter biological half-life of Hg than their male counterparts due to the fact that the monthly loss of blood in females creates a distinct hormonal and biochemical mechanism which in turn results in lower Hg levels than males.

It is believed that metabolic excretion rates might vary according to ethnicity (Ballatori, Wang, & Lieberman, 1998; Canuel et al., 2006; Walsh, Feulner, & Reilly, 2001; Yokoyama et al., 2002). For example Vietnamese and Chinese experience a genetic polymorphism for enzymes involved in ethanol metabolic excretion and Hg export from cells (Walsh et al., 2001; Yokoyama et al., 2002) which could affect elimination rates. Animal studies have found this evidence too (Ballatori et al., 1998). Differences in MeHg concentrations have been found among children from different ethnic groups within a population not considered at risk of MeHg exposure (Innis, Palaty, Vaghri, & Lockitch, 2006). Thus different sub-groups may have different metabolic processes of MeHg. Also, traditional food consumption, and or cooking habits may decrease the metabolic absorption or increase the excretion of MeHg; however, this theory has yet to be proven (Canuel et al., 2006; Perello, Marti-Cid, Llobet, & Domingo, 2008). There is also some evidence that selenite has a protective effect on inorganic Hg levels but there is no evidence regarding protection against MeHg (Canuel et al., 2006). All this evidence provides motivation to further study Hg exposure in specific groups and provide safe fish/seafood consumption guidelines accordingly.

Other studies have concluded that the beneficial influence of nutrients (such as selenium and omega-3 fatty acids) from fish may counter any adverse effects of MeHg on the developing

nervous system (Mahaffey, Clickner, & Jeffries, 2008). Again the results are equivocal. For example, a Faroe Islands birth cohort study investigated omega-3 fatty acids and selenium as a potential modifier of the effects of MeHg exposure through fish/seafood diet but found no such associations (P. Grandjean & Landrigan, 2006). Accordingly, Choi & Grandjean, (2008) suggested in their review that to assess the full impact on the toxicity of MeHg and the beneficial effects of nutrients, both the good and bad effects should be assessed at the same time in order to separate opposite impacts on the outcomes.

4.7 Conclusion

Evidence suggests that the effects of Hg prenatally are potentially profound and debilitating. However, very little research has been conducted on the effects of postnatal Hg in children and more research is required to understand these effects. There have been no studies in NZ since the last birth cohort on prenatal Hg exposure by Kjellstrom et al., (1986, 1989) nor any on postnatal Hg exposure (Kjelstrom et al., 1986; Kjelstrom et al., 1989).

Chapter 5 Literature Review 3 – Elements and Body-size

This chapter examines the available literature reporting possible associations between: (1) elemental abnormalities and large body-sizes for different populations; and (2) certain elements and the incidence of large body-size and fat disposition. The literature search was undertaken using a number of different databases, as detailed in section 2.2.2.

5.1 Introduction

There may have been a change in the eating patterns of Pacific people due to their migration that may have contributed to major changes in the body compositions of their children in New Zealand (NZ). Abnormal or excess fat accumulation in adipose tissue can lead to derangements of essential elements (Anon, 2000). The etiology of large body-size is complex; however, it is related to a combination of environmental and genetic factors which in turn can disrupt the metabolic system, especially due to possible elemental deficiencies or toxicity (Garcia et al., 2009).

There is now new evidence showing that elemental concentrations (deficiencies or increased levels) may be associated with increased obesity (L. S. Huang, Yu, Kirschke, Gertz, & Lloyd, 2007; W. J. Lee, Wang, Wei, & Huang, 2006; McClung & Karl, 2009; Padilla et al., 2010; Padmavathi et al., 2010; Satarug & Moore, 2004; Yanoff et al., 2007). Large body-sizes in individuals has been recognised as a possible risk factor for several nutrient deficiencies (Garcia et al., 2009; Xanthakos, 2009) and across different age groups worldwide (Garcia et al., 2009). It has also been shown that the cause of these nutritional deficiencies in larger body-size people could be due to the quality of diet in children, such as the higher intake of calorific-rich processed foods associated with poor nutritional quality (Ebbeling, Pawlak, &

Ludwig, 2002). This is particularly problematic in places where there may be an abundance of relatively cheap, energy-dense, but nutrient-poor food (Manios et al., 2009; Washi & Ageib, 2010; Xanthakos, 2009). There are many studies that have explored the effects of elemental deficiencies on human functions in different populations but most are focused on adults rather than children.

Many studies have shown that larger body-sized individuals have lower concentrations of elements than non-obese cases (Bougle et al., 2009; Kimmons, Blanck, Tohill, Zhang, & Khan, 2006a; Singh, Beegom, Rastogi, Gaoli, & Shoumin, 1998; Tascilar et al., 2010; Wojciak, Mojs, & Stanislawska, 2010). Some studies have suggested that the rates of larger body-sizes are increasing more rapidly in some regions of the world where elemental deficiencies are more prevalent (Monteiro, Conde, & Popkin, 2004, 2007; Monteiro, Moura, Conde, & Popkin, 2004; Usfar et al., 2010). Many possible pathophysiological mechanisms are involved in the development and maintenance of large body-size (Usfar et al., 2010) and therefore proper elemental balance is important. Imbalance of elements can lead to oxidative stress and this has been suggested to be a potential promoter of inflammatory processes. Such imbalances may be involved in the susceptibility to develop large body-size and related diseases (H. K. Vincent & Taylor, 2006) such as diabetes (Deboer, 2013; No Authors listed, 2013; Ohkuma et al., 2013), atherosclerosis (Dick, Lesser, Leipsic, Mancini, & Lear, 2013; Recio-Rodriguez et al., 2012) and inflammation (Ferroni, Basili, Falco, & Davi, 2004; Ferroni et al., 2008; Vazzana et al., 2012). Several studies have suggested that obesity is strongly associated with a state of chronic oxidative stress obesity (H. K. Vincent, Innes, & Vincent, 2007). Keaney et al., (2003) suggested that large body-size is associated with a state of excess oxidative stress. Body-size may independently decrease the activities of the body's

protective antioxidants and enhance the systematic oxidative stress and may be particularly relevant to obesity-related renal cancer development (Gago-Dominguez, Castela, Yuan, Ross, & Yu, 2002). This represents yet another contributing mechanism for excess cardiovascular disease with body fatness. Oxidative stress has also been observed in children where adipokine levels increase throughout the continuum of obesity (S. Sun, Ji, Kersten, & Qi, 2012).

5.2 Specific Elements in Relation to Large Body-size

Only two studies have investigated elemental levels and childhood body-sizes (Bougle et al., 2009; Tascilar et al., 2010). However, a few others were identified that have studied body fatness and the effects of elements in adults and animals. The following summarises the various studies between specific elements and body-size.

Iron (Fe): The association between iron levels and different body-sizes in adolescents was first reported in the early 1960s (Seltzer & Mayer, 1963; Wenzel, Stults, & Mayer, 1962). Since then a number of studies have confirmed an association between reduced blood iron concentrations and large body-size in both children and adults (Chambers et al., 2006; del Giudice et al., 2009; Nead, Halterman, Kaczorowski, Auinger, & Weitzman, 2004; Pinhas-Hamiel et al., 2003; Sanad, Osman, & Gharib, 2011; Thethi et al., 2011). For example, the National Health and Nutrition Examination Survey (NHANES) III study in the US showed that overweight children or those at a risk of being overweight were twice as likely to be iron deficient (Nead et al., 2004). There might be a number of reasons why iron deficiency is seen in large body-sized individuals. This could be from low iron intake due to an unhealthy diet, reduced absorption, and the sequestration of iron as a result of chronic inflammation (Yanoff et al., 2007; Zimmermann et al., 2008). It was also reported that high BMI-z-scores were

associated with decreased iron absorption in women and reduced improvement of iron status in iron-deficient children following intake of iron-fortified foods (Zimmermann et al., 2008). In contrast, Menzie et al., (2008) reported that obesity related hypoferrremia is not associated with differences in the reported intake of heme and non-heme iron or intake of factors that affect iron absorption. However, the intake of phytic acid, oxalic acid, eggs, coffee, tea, and zinc among others can inhibit iron absorption (Garcia et al., 2009). Hepcidin, which regulates iron homeostasis by inhibiting iron absorption by erythrocytes and sequestering iron by macrophages, may be a mechanism linking obesity and iron deficiency (Garcia et al., 2009).

Zinc (Zn): Zinc is a component of many enzymes and is also involved in appetite control (Payahoo et al., 2013). It is involved in the synthesis, storage and release of insulin (Hashemipour et al., 2009). Zinc deficiency has been related to insulin resistance, glucose intolerance and obesity (Garcia et al., 2012; Hashemipour et al., 2009). Since insulin resistance plays an important role in the pathophysiology of obesity and metabolic syndrome it is important to understand its effects (Mikhail, 2009). Many studies have shown that obese individuals have lower plasma zinc levels (Marreiro, Fisberg, & Cozzolino, 2004; Tungtrongchitr et al., 2003). A study in the US demonstrated that higher intake of zinc supplementation reduced the risk of type 2 diabetes (Q. Sun, van Dam, Willett, & Hu, 2009); while other studies have shown that zinc supplementation increases high density lipoprotein cholesterol and reduces triglyceride in Type 2 diabetes patients (Farvid, Siassi, Jalali, Hosseini, & Saadat, 2004; Kelishadi et al., 2010). Furthermore in children with a metabolic syndrome, a randomised cross-over trial significantly reduced insulin resistance by supplementing 20 mg of zinc in obese children (Kelishadi et al., 2010). A recent study

indicated that supplementation of 30 mg/day of zinc resulted in the reduction in weight and BMI indices with increase in serum zinc concentrations (Payahoo et al., 2013).

Calcium (Ca): Calcium is the most abundant essential nutrient found in the human body. It is required for many physiological functions, including the contraction of muscles and blood vessels, secretion of hormones and enzymes, and the transmission of impulses in the nervous system (Straub, 2007). Most of the calcium in the body is stored in the bones and teeth (Shills, Shike, Ross, Caballero, & Cousins, 2006). It has been suggested that calcium intake could cause changes in body weight via a decrease in the production of parathyroid hormone and vitamin D (Major et al., 2008; Zemel, 2004; Zhu et al., 2013). Calcium increases the breakdown of fat and decreases fat accumulation. Calcium has also been demonstrated to increase faecal fat excretion, which could lead to a reduction of body weight (Buchowski et al., 2010; Heaney, 2011). This has also been supported in a recent review (Heaney & Rafferty, 2009) and a meta-analysis (Onakpoya, Perry, Zhang, & al, 2011) favouring calcium in the reduction of body weight. There have been other studies that have not found any association between increased calcium intake or dairy products and weight loss (Bowen, Noakes, & Clifton, 2005; Thompsen & Thompson, 2006). Furthermore, a systematic review conducted by Trowman, Dumville, Hahn, & Torgerson, (2006) did not find any statistically significant effect of calcium supplementation on body weight or body fat.

The effect on calcium metabolism in adipocytes and thermogenesis may underlie the association between calcium intake and obesity (Garcia et al., 2009). Low plasma calcium concentrations associated with low calcium intake can lead to a calcitriol (1, 25-dihydroxyvitamin D) mediated increase in intracellular ($[Ca^{2+}]_i$) concentrations (Gruff & Gropper, 2000). The increased ($[Ca^{2+}]_i$) concentrations, in turn, stimulates the expression

and activity of fatty acid synthesis and inhibits lipolysis (Jones, Kim, Zemel, & et al., 1996; Zemel & Miller, 2004). An increased intake of calcium and dairy products inhibits lipogenesis and promotes lipolysis and lipid oxidation, thereby possibly inhibiting induced obesity (Zemel, 2004; Zemel & Miller, 2004). In another study, it was found that an increase in calcium intake accompanied by normal protein intake in humans resulted in increased fat content in faeces and an excretion of approximately 350 kJ of energy per day (Jacobsen, Lorenzen, Toubro, Krog-Mikkelsen, & Astrup, 2005). High-calcium diets may increase energy expenditure and regulate body weight by suppressing calcitrio-mediated inhibition of adipocyte uncoupling binding protein 2 (UCP2), which is implicated in thermogenesis (Fleury, Neverova, & Collins, 1997; Shi, Dirienzo, & Zemel, 2001).

Magnesium (Mg): Low magnesium status in larger body-sizes has been observed in several studies and this may be associated with chronic inflammation indicators, or with diseases with a chronic inflammation component (Neilson, 2010). Corcia, Allegra, Lentile, & Buemi, (1997) found that larger body-sized individuals had significantly lower plasma magnesium concentrations than lean healthy individuals, whilst normotensive obese individuals did not. These findings are consistent with magnesium supplementation lowering blood pressure in hypertensive individuals, but not in normotensive, overweight Korean adults (S. Lee, Park, Son, Lee, & Kim, 2009). Huerta et al.,(2005) found that serum magnesium was significantly lower in large body-size children than in sex and puberty-matched lean controls. These children had increased fasting insulin and homeostasis model assessment of insulin resistance and decreased quantitative insulin sensitivity check index.

In animal models, severe magnesium deprivation rapidly decreases extracellular magnesium and this results in an inflammatory response in animals (Mazur et al., 2007). The

inflammation response most likely is caused by an increase in intracellular calcium and the priming of phagocytic cells, which results in the release of inflammatory cytokines (Mazur et al., 2007). However the likelihood of severe dietary magnesium deficiency being a cause of these mechanisms is unlikely in humans. More studies are needed to clarify the role of magnesium in obesity and comorbid conditions and factors that affect that role (Neilson, 2010).

Selenium (Se): This element is involved in a number of important enzymes and selenoproteins, and has been shown to be involved in protection against viral infections and cancer, in immune function and in the aetiology of I-deficiency disorders (Combs & Lu, 2001; Rayman, 2002). A few recent studies have shown a reduction in obesity in humans with adequate selenium levels (Maranhão et al., 2011; Savoury et al., 2011). In contrast a current study on toenail selenium and inflammation as measured by fibrinogen, hs-CRP and IL-6 found no associations between the various measures (Xun et al., 2010).

Chromium (Cr): This element regulates carbohydrates and fat metabolism (Padmavathi et al., 2010), similar to manganese. Investigation in humans and animal models has suggested that chromium supplementation reduces body weight, regulates hunger, and also decreases body fat (Grant, Chandler, Castle, & Ivy, 1997; Mertz, 1969). Chromium supplementation is reported to decrease plasma total cholesterol and triglycerides, increases high density lipoprotein (HDL) cholesterol, and lowers body weight in diabetic people (Mertz, 1969). However the effect of chromium deficiency on lipid/fat metabolism has not been studied. A recent investigation conducted on animals demonstrated that chronic maternal Cr deficiencies increased visceral adiposity and modulated adipose tissue function in animal offspring (Padmavathi et al., 2010). This study suggested that the peri-postnatal period in the

development and function of adipose tissue in offspring may predispose them to body fat and insulin resistance in later life.

Other elements: There is a limited literature on the effects of copper, manganese, iodine, cobalt, molybdenum, boron, antimony and aluminium on large body-size. Copper deficiency is known to cause neurological diseases and hematologic abnormalities including anemia with neutropenia, in adults (Angotti et al., 2008; Kumar, Gross, & Ahlskog, 2004; Prodan, Holland, Wisdom, Burstein, & Bottomley, 2002). Copper deficiency and large body-size are intertwined (Relling et al., 2007). Genetically obese ob/ob mice exhibit lower hepatic copper content despite adequate dietary copper intake (Kennedy, Failla, & Smith, 1986). However, there is lack of research in determining an association between large body-size and copper in children.

Manganese is involved in controlling sugar levels and carbohydrates and is involved in the function of numerous organ systems and is needed for normal immune function, regulation of blood sugars, production of cellular energy, reproduction, digestion and bone growth (Aschner, Guilarte, Schneider, & Zheng, 2007). Low blood sugar level leads to an increased volume of fat in one's body. However, no studies have been done to suggest a role for manganese in the development of obesity. Further research is required to study the effects of manganese on body-size in children.

Antimony is naturally occurring and is similar in its chemical and toxicological properties to arsenic. It is used in industries for hardening lead products like batteries. People have been exposed to antimony through tap water and it has been shown to cause cancers (Gebel, 1997). However, not many studies were identified that investigated antimony on body-sizes. Only

one US study found an association with BMI and antimony (Padilla et al., 2010); however, more studies are required to understand the affects antimony may have on children's health.

The physiological role of iodine in the human body is in the synthesis of thyroid hormones by the thyroid gland. However, there have been no reports on the effects of iodine on fat disposition. Cobalt is part of vitamin B₁₂ and so is an important element. At high levels it can damage the lungs and heart (Agency for Toxic Substances and Disease Registry, 2004).

Cobalt is used to treat anaemia in pregnant women, because it stimulates the production of red blood cells. However its effect on fat disposition is still unknown. Molybdenum is an essential element and is a component of metalloflavoproteins, including xanthine oxidase, sulphite oxidase and aldehyde oxidase (Novotney, 2011). A deficiency of molybdenum results in headache, mental disturbance and coma (Gupta, Srivastava, & Gupta, 2011).

However, no studies have researched the effects of molybdenum and obesity. Not much is known about the effects of boron on large body-size. One study conducted by Tascilar et al., (2010) found no significant association between boron and large body-size. Animal studies have indicated some reproductive effects of boron, both from toxicity and deficiency (Devirian & Volpe, 2003; Sayli, 1998, 2001) but relatively little is known about the effects on childhood fat disposition. Also, there is some evidence that a certain amount of boron is beneficial for bone and brain health (Chapin et al., 1997; Dourson et al., 1998; Nielsen, 2008). The effects of fat disposition and aluminium have not been explored very much except for one recent study which found no difference in aluminium levels between large-body sizes and the control group (Tascilar et al., 2010).

5.3 Conclusion

Deficiencies or excessive (above optimum) levels of some elements can lead to undesirable pathological conditions that can be prevented or reversed by careful monitoring and proper nutrition. Studies have associated elemental imbalance in the development of obesity and related clinical manifestations with higher plasma concentrations of inflammatory biomarkers (Dandona, Aljada, & Bandyopadhyay, 2004). No such elemental studies have been undertaken in NZ in evaluating possible levels in human tissues or fluids with regards to large body-size. Moreover, many overseas studies have only used blood plasma elemental levels, which is problematic in that: (1) the levels for most essential elements are homeostatically regulated and reflect only a small metabolic window (of hours or days in terms of food and water consumption); and (2) it may not always be acceptable to use blood collection for studies on children. The aim of this study is to focus on using toenails as biomarkers to assess the elemental concentrations of Pacific children in NZ and explore the possible link of elemental concentrations with factors relating to the assessment of body-size.

Chapter 6 Study 1 – Hair Mercury and Association with Behavioural Problems

6.1 Introduction

Evidence from the literature (Chapter 4) indicates that low levels of mercury in the form of methylmercury, a well-known neurotoxin can cause damage to the developing brain (P. Grandjean & Landrigan, 2006). As explained in Chapter 4 section 4.1, along with social and economic factors, mercury has been known to cause damage to the developing brain and nervous system which could lead to developmental and behavioural disabilities especially prenatally or during early childhood (Ronchetti et al., 2006) Children are more vulnerable than adults to mercury because their developing brains are susceptible to chemical interference, their immunological pathways are immature and they undergo rapid growth and development which can easily be disrupted by mercury (Neuman et al., 2006). They also have a longer life span in which to express illness (Neuman et al., 2006). Many epidemiological studies have found evidence of dose-related adverse effects of exposure to Hg (particularly in the form of methylmercury) on developmental disorders in children from high-risk communities in which fish is a major staple diet (Crump et al., 1998). At cellular level, mercury damages the membrane structure of the cell, forms oxidative distress, impairs protein synthesis and neurotransmitter balance in neural tissues, furthermore it is toxic for the mitochondria (Rutchik, 2013; Sarafian, 1999).

Maternal consumption of seafood during the gestational period can pass on the methylmercury to the foetus with adverse developmental effects (Myers et al., 2003).

Evidence has shown that the developing foetal brain is 10-fold more susceptible to damage from exposure to mercury (Ball, Ball, & Pratt, 2001). Its distribution in blood and tissues is

well known and it can easily pass through the placenta (Unuvar et al., 2007) and blood-brain barrier. Methylmercury is preferentially stored in the brain and central nervous system ; overtime brain levels may exceed blood levels by three to six times (Ronchetti et al., 2006). Therefore mothers and young girls are also an important group for environmental exposure studies. In addition exposure can occur through amalgam fillings (in the form of elemental mercury) in both mother and child. There is a concern on the possible effects of amalgam on children and foetuses during the period in which the risk of cavities is greatest, and thus the placement of amalgam is most frequent (Davidson et al., 2004).

6.2 Aims and Objectives

The overall aims of this study were to explore the effects of a toxic element, mercury, on behaviour problems in Pacific children. The primary aims of the study were:

- (i) to assess the distribution of mercury in a sample of six year old Pacific children and their mothers;
- (ii) investigate the association between children's hair mercury concentrations and behavioural problems of children after accounting for known confounders such as gender and ethnicity;
- (iii) to assess the distribution of hair mercury in a sample of six year old Pacific children and their mothers.

The secondary aims were to examine the consumption of fish and the presence of amalgam fillings in children and their relationship to mercury in the children. Specifically to:

- (i) describe the frequency of different fish types consumed in mothers and children and investigate the relationship of type of fish consumed and mercury in children's and mothers' hair;
- (ii) describe the frequency of amalgam fillings in children;
- (iii) investigate the relationship of type of fish and amalgam fillings with mercury in children's and mother's hair.

6.3 Materials and Methods

6.3.1 Study Design and Setting

The study was conducted between June 2007-June 2008 within the PIF cohort when the children were six years of age and living in Auckland, New Zealand (NZ). All children were born in and residents of NZ. A nested case-control design was employed in that children with behavioural problems (as identified by the child behaviour checklist (CBCL)) were identified as cases and those exhibiting normal behaviour were controls.

6.3.2 Participants

Eligibility criteria: All children and mother participants were included who were part of the PIF cohort study, children aged six years old (both male and female), and adult females who were mothers of child participants. Children were excluded if participants (both children and their mums) had very short hair, those who may have dyed their hair (mothers in particular) as colouring effects the mercury concentrations, and participants with wigs or who had artificial hair attachments especially among the mothers.

Ascertainment of cases: The cases were children with behavioural problems as identified within the cohort (at six years) using the internationally recognised CBCL and reported by

mothers (Achenbach & Rescorla, 2001). This study utilised the parental version of the 120-item CBCL/6-18 from maternal PIF study participants regarding the behaviour of their six year old children and was administered by the PIF interviewers. The CBCL questionnaire was completed by the mothers or the child carer. The internationally recognised CBCL questionnaire has obtained ratings on behavioural/emotional problems of children by parents or caregivers. The time frame for item responses was within the past six months. The analysis of the CBCL provides one overall total problem score (T- score), two broad-band syndrome scores – internalising and externalising; and seven narrow-band syndrome scores: emotionally reactive, anxious/depressed, withdrawn, somatic complaints, sleep problems, attention problems and aggressive behaviour (Achenbach & Edelbrock, 1981; Achenbach & Rescorla, 2001).

Scores for internalising behaviour reflect mood disturbance including: anxiety, depression, and social withdrawal, as displayed by the child, whilst 123 scores were used for externalising behaviours (which reflects conflict with others and violation of social norms). Within the CBCL measure, the score for internalising behaviour was derived as the sum of scores for 32 questions within three syndromes: anxious/depressed, withdrawn and somatic complaints; and externalising behaviour scores were derived from 35 questions within two syndromes: aggression and rule breaking. The CBCL is assessed on a 3-point Likert-type scale: 0=not true, 1=somewhat or sometimes true, and 2=very true or often true. Higher scores indicate greater degrees of behavioural and emotional problems. In order to determine children in the clinical range, the cut-off values recommended by Achenbach and Rescorla (2001) were applied; the 83rd and 90th percentiles were used to define the borderline and clinical ranges for the total problem scores respectively. Here cases were defined as children

having been clinically screened as having behavioural problems, including children in the borderline at six years.

The CBCL is a widely adopted and accepted research tool that provides a formal clinically interpretable measure of children's behaviour in ten specific areas (Achenbach and Rescorla, 2001). The internationally recognised CBCL questionnaire (which is most commonly used across many countries and cultures (Rescorla, Achenbach, Ginzburg, Ivanova, & al at., 2007; Rescorla, Achenbach, Ginzburg, Ivanova, & el at., 2011) has obtained ratings on behavioural/emotional problems of children by parents or caregivers. The CBCL has been found to be appropriate across different cultures and languages (Crijnen, Achenbach, & Verhulst, 1997, 1999). Moreover, it has good test, retest (0.95 to 1), inter-tester reliability (0.93 to 0.96), and an internal consistency of 0.78 to 0.97 (Achenbach & Rescorla, 2001) and race and socio-economic status account for minimal variability (Myers et al., 2004). Furthermore, parents readily accept CBCL; it is easy to administer, is brief, reliable and efficient to score. It correlates highly with other behavioural indices such as the Connor's Parent Questionnaire and the Quay-Peterson Revised Behaviour Problem Checklist (Achenbach, Howell, Quay, & Conners, 1991). Within the PIF cohort, internal consistency was determined from repeat measures from the same caregivers. Cronbach's alpha for internalising was 0.82, for externalising was 0.86 ,and for total behaaviour problems was 0.93. Therefore the internal consistency within the PIF cohort the CBCL was considered a reliable measure to identify children with and without behaviour problems. Within the total PIF cohort 125 children were identified at age six years with problem behaviour (children above the 90th percentile). The aim was to recruit all children with problem behaviour from the cohort i.e. cases.

Ascertainment of controls: For every case a control was selected (ratio was 1:1). The controls were randomly selected from all the remaining child participants who were not identified as having behavioural problems.

6.3.3 Measures

In addition to the core PIF measurements a number of additional measurements were undertaken for this case-control study. The measures used for this study are provided in detail below:

Exposure measure: Total mercury concentrations

Hair samples were used for determining mercury exposure as it is the recommended biomarker for estimating the methylmercury dose received by the child's brain (National Research Council, 2000; World Health Organisation, 2008a). Hair closest to the scalp represents the latest mercury exposure (approximately one month's growth) (Srogi, 2007). As elements such as mercury occur in hair at higher levels, it allows for more sensitive and more analytically accurate results (Oken et al., 2005).

The vast majority of systemic exposure to organic mercury is to the methyl species. But the methylmercury is converted to inorganic mercury before it is bound to hair (Magos & Clarkson, 2008), and so total mercury in hair samples was measured for this research. Additionally, there is a strong correlation between long-term systemic exposure to organic mercury and its hair concentrations (Clarkson & Margos, 2006; Groth, 2010), so therefore hair samples provided a direct measurement of mercury burden in children and mothers in this sample. Hair mercury concentrations are measured as a continuous variable in µg/g.

The FAO/WHO Expert Committee on Food Additives (JECFA) has a provisional tolerable weekly intake (PTWI) for MeHg to 1.6 ($\mu\text{g/g}$ body weight which is much lower than their previous limit of 3.3 ppm body weight) (World Health Organisation, 2003). This was based on the data provided by the studies in the Faroe Islands and Seychelles which investigated the relationship between impaired neurodevelopment in children and Hg concentrations (Yorifuji, Debes, Weihe, & Grandjean, 2008). The criteria for the tolerable intake within this study (Study 1) is 1.6 $\mu\text{g/g}$ body weight.

Exploratory variable: Seafood diet and amalgam fillings measurements

An interviewer-based food frequency questionnaire (FFQ) examined the frequency of seafood by participants. The seafood questionnaire designed for the case-control study was adapted from the original seafood consumption questions developed by the English study cohort called the Avon Longitudinal Study Team (Hibbeln et al., 2007). The questionnaire has been validated by the Avon longitudinal study (Newson & ALSPAC, 2003). Local fish shops in South Auckland (where most of the participants resided) were visited and the species of fish and seafood sold and their local names recorded. All the varieties of fish and seafood sold were included in the mother's and children's seafood consumption questionnaires. There were two sets of questionnaires (one for mother and one for child) that were developed and administered at the six year phase and answered by mothers (questionnaires included in the appendix 4).

The questionnaires (for both mother and child) were divided into two parts: Part A consisted of 25 seafood questions which were yes/no, others were closed ended questions (never or rarely, once in the last four weeks, once in two weeks, 1-3 times a week, 4-7 times a week, more than 7 times a week). Mothers answered seafood questions as a proxy for their children.

The exposure information for both mother and children was obtained on fresh or frozen fish (fish fillet, fish steak or whole fish), canned, processed or pre-prepared fish food, shellfish/crustacean consumption. Questions were also asked about their fish oil supplement intake. Mother's questionnaires had extra questions on if they fished and gathered shellfish and the types of fish they would usually catch if they did. However, for the current study, only the types of fish consumed and the frequency of fish consumed by mother and child was included in the final analysis. Other questions such as tinned fish had a low response rate and given the small sample size (n =45 children; n=45 mothers) further analysis was not justified.

Part B was the dental questions: The questions were adapted from the main PIF questionnaires. This consisted of two questions (with sub-sections) that elicited information on dental hygiene and the amount of silver/amalgam fillings they had obtained from both mother and child. The questions were yes/no (appendix 4). The dental questions were pre-tested by Pacific researchers before administering it to the study participants to pilot the procedure and questionnaires.

Dental health records

The dental questions were complemented by collecting dental health records of child participants (from those who had consented/assented) from the Auckland Waitemata Regional Dental Health Services where all records of child participants treated in the local dental schools are kept. The Waitemata Regional Dental Health Services were provided with names and birthdates of children used for matching to obtain their dental records. Mother's dental records were not able to be obtained as for most there was a poor history of attendance at dental services and no record of where (or if) they had their teeth checked or treated.

Confounding variables: Demographic variables from the PIF study

Demographic variables were selected from the main PIF study at the six year phase and included: ethnicity, gender, household income levels at six years, maternal education levels, and marital status information.

Measurement of demographic characteristics was based on family income at the six year phase (categorised into \$0-\$20,000, \$20,001-\$40,000, >\$ 40,000) which was reported by mothers, and current maternal educational achievements (categorised into no formal qualification, secondary school qualification, post-secondary qualification). The mother's current marital status was divided into non-partnered, de-facto, and legally married.

6.3.4 Data Collection Procedures

Hair samples were chosen and collected as biomarkers since these were less invasive (especially in child populations) than blood or urine samples and therefore would be easily collected.

In the first instance all selected potential participants were telephoned by a Pacific researcher to explain the study, invite them to participate and to check for their eligibility which excluded short hair, dyed hair or those mothers with wigs or artificial attachments.

Appointments were made for all eligible participants who agreed to be visited at home to discuss the study, provide information sheets and get consent from mothers and assent from children. Reminder calls were made to these potential participants a day before the interview and if for some reason they could not be available on that day another appointment was scheduled. All visits were made by Shamshad Karatela (SK) who was accompanied by a Pacific researcher. Once consent/assent was obtained, questionnaires were administered and

hair samples collected at that same visit if possible. Other appointments were made for those participants who could not answer the questionnaires at the time or provide hair samples during the first visit.

Hair sample collection, storage and handling

Scalp hair samples were collected as a biomarker for determining mercury. The procedure for hair sample collection was adapted from the (World Health Organisation, 2008a) method.

These hair samples were cut from the back of the head close to the scalp with stainless steel scissors which was cleaned with steriliser after every cut for hygiene reasons. Approximately 50- 100mg (50 strands) of scalp hair were collected from all participants. The part of hair closest to the scalp was tied with a cotton thread to the scalp end of the hair so that it could be identified for sample analysis. The samples were then placed in a polythene bag closed with a zipper (all samples were given code numbers so that participants were not directly identifiable). These were then stored in a locked cabinet at room temperature until transportation to the UK's Inductively Coupled Plasma Mass Spectrometry (ICP MS) Facility, at the University of Surrey for chemical analysis.

6.3.5 Sample Size

The number of research participants was determined by carefully considering a number of constraints, in addition to the power calculations. Based on six years of age figures, we had identified 125 children who could have been classified as cases. Ideally, it was anticipated that, after exclusion criteria and consent gained to participate in this preliminary sub-study, there would have been approximately 100 cases recruited. The choice to use a 1:1 case-control ratio was governed by the fact that this ratio is the most statistically efficient and was achievable.

Assuming 5% of the control group exceeds the mercury threshold, (Crump et al., 1998) a nested-case control study with 100 participants in each group will have 70% power to detect at the 5% significance level a detectable difference of 0.1. It was believed that a difference of at least this size was clinically meaningful and would have had important public health implications which was an essential reason for conducting this study as part of the doctoral thesis.

6.3.6 Hair Sample Laboratory Analysis

Chemical analysis of hair material was conducted by Professor Neil Ward and Gillian Lord, University of Surrey, UK. The ICP MS Facility of the University of Surrey is internationally recognised for the invention of inductively coupled plasma mass spectrometry, and researchers of the facility have over 30 years developed new analytical techniques for detecting elements in biological samples (including hair and toenail clippings) suitable for epidemiological purposes. The ICP MS Facility has been involved in many international quality control inter-laboratory proficiency or round-robin tests and justifies all sample analyses by using internationally recognised certified reference materials (to established acceptable levels of accuracy and precision). A brief outline of the hair sample preparation method and ICP MS technique is mentioned here.

The hair samples were washed with neutral detergent (1:100) in 20 ml glass vials and rinsed well with distilled water. They were washed with acetone and dried under reduced pressure. The samples were then cut into fine pieces in the vial using a pair of dissection scissors. They were stored in a desiccator until analysis.

6.3.6.1 Chemical Hair Analysis

All instrumental data for mercury (recorded according to the selected isotope used) is reported as counts per second. The value is corrected for a reagent blank signal (to correct any contribution from the digestion procedure) and ratioed with the internal standard isotope value (to correct any instrumental drift or signal enhancement/depression caused by the matrix). Data for the calibration standards are handled in the same manner and an Excel (Microsoft Corporation, US) calibration curve is produced for each element, with ratio signal (y-axis) and concentration of five standards (x-axis), from which the calibration equation is determined for calculation of the unknown hair sample elemental concentration. The elemental values for each hair sample are corrected for the dilution factor resulting in the final values for statistical analysis.

6.3.7 Statistical Analysis

All data obtained for this study were stored in Microsoft Access and Excel databases (Microsoft Corporation, US) supervised by a senior biostatistician. The data was cleaned, range and consistency checks were performed, and coded for analysis. Questions with no response were not included in the analysis as a distinct coded variable. The cleaned data was then exported to STATA version 10 (StataCorp, 2007) for statistical analysis.

Summary statistics for mercury mothers and children was calculated to include median, upper (75%) and lower (25%) quartiles and minimum, maximum concentrations. Descriptive statistics was also provided for demographics, fish consumption and amalgam fillings which

included frequencies and percentages. Non-normally distributed data was log transformed to normalise the data.

Spearman correlation was computed to understand the concordance between mothers and children. Bivariate analysis such as chi-square tests or Fisher's exact tests was performed to test for differences in proportions of categorical variables among two or more groups. The $p < 0.05$ (95% confidence interval) was considered as the cut-off value for statistical significance.

Logistic regression analysis with binary outcome of behaviour problems (cases, controls) was performed to investigate the relationship between behaviour in children and hair mercury concentrations after adjusting for ethnicity and gender in our model.

6.3.8 Ethical Considerations

This part of the doctoral study was granted by the Northern X Ethics Committee which is a national committee (NTX/07/05/050) (letter attached in appendix 2). Consent for this study was sought from mother and assent was sought from children. Information sheets were provided for both mother and child separately. The Pacific Advisory Board was also consulted for this part of the study before commencement (letter attached in appendix 2).

Questionnaires and data were stored as discussed in Chapter 2, section 2.2.1. Any leftover hair samples were destroyed after the laboratory analysis. All questionnaires as part of this research will be destroyed after 10 years as per the ethics requirement.

There were ethnic considerations that needed to be considered before these data collection procedures were carried out. One of the criteria of data collection within the PIF study was

that a non-Pacific researcher had to be accompanied by a Pacific person. Therefore for all data collections here, SK was accompanied by a PIF researcher.

6.4 Results

6.4.1 Recruitment and Response Rate

There were 125 cases (children with behaviour problems) and 125 controls (children without behaviour problems) that were eligible to take part in this research. From those only 55 families ($n=110$ mother child pairs) were approached from whom three (mother-child pairs) refused to participate in this study. The remaining participants who were eligible were no longer contacted due to difficulties encountered in collecting hair samples.

Among the 55 families, 46 children and 46 mothers took part in this study. There were two children and their mothers who were no longer at their original address so could not be traced, three of the children had short hair and so their mothers were also not included. Another mother-child pair who were eligible initially both had very short hair when visited so they were excluded. Three mother-children pairs did not want to participate in the study .

As shown in Table 5 below most of the mother-child pairs ($n=86$) preferred to have the hair samples collected from them at the time of questionnaire administration.

Table 5: Hair sample and questionnaire collection

Participants	<i>Mother-child n(%)</i>	<i>Child n(%)</i>
Hair sample re-visits	3(7)	3 (7)
Hair sample & questionnaire	43 (93)	43 (93)
Total	46	46

6.4.2 Demographic Characteristics

More than half of all the participants (mother and children) were of Samoan ethnicity, (63%) followed by Tongan (13%) and the remaining (3%) were from the others (European, Māori and Niuean) group within this sample. The majority of the mothers had no post-secondary school qualification (69%). Their average annual household income was between \$20,001-\$40,000 at the six year phase. Out of the 46 children, there were more girls (57%) than boys (43%). Details of the demographic characteristics are presented in Table 6.

Table 6: Demographic characteristics of mother and children (cases (n=11) and controls(n=35)

	Total <i>n</i> (%)	Cases <i>n</i> (%)	Controls <i>n</i> (%)
<i>Children</i>			
Females	26 (57)	4 (36)	22 (63)
Males	20 (43)	7 (63)	13 (37)
<i>Ethnicity</i>			
Samoan	29 (63)	5 (50)	24 (69)
Tongan	14 (13)	6 (60)	8 (23)
Others*	3 (4)	0	3 (9)
<i>Maternal education</i>			
No formal qual	16 (35)	4 (36)	12 (34)
Secondary qual	13 (28)	3 (27)	10 (29)
Post-secondary	12 (26)	4 (36)	8 (23)
Unknown	5 (11)	2 (18)	3 (9)
<i>Annual household income</i>			
\$0-\$20,000	12 (26)	2 (18)	10 (29)
\$20,001-\$40,000	19 (41)	6 (60)	13 (37)
>\$40,000	8 (17)	2 (18)	6 (17)
Unknown	1 (2)	0	1 (3)

* European, Māori and Niuean

6.4.3 Description of Mercury

Mothers had slightly higher median concentrations of hair mercury (0.43 µg/g, min, max 0.08 µg/g, 2.18 µg/g) then their children (0.32 µg/g; min, max 0.03 µg/g, 1.02 µg/g) as shown in

Table 7 below. There was no significant difference between gender and hair mercury concentrations in these children (P value 0.64). The median hair mercury concentrations for children between cases and controls were the same (0.3 $\mu\text{g/g}$).

Table 7: The median (25th, 75th percentiles), minimum (min), maximum(max) mercury concentrations ($\mu\text{g/g}$) of all participants (mother and children) including cases and controls

	<i>n</i> (%)	Median (25th ,75th)	Min, Max
Mother	46	0.43 (0.21, 0.78)	0.08. 2.18
Children	46	0.32 (0.18, 0.45)	0.03, 1.02
Girls	26 (57)	0.32 (0.05, 0.45)	0.05, 1.02
Boys	20 (43)	0.25 (0.18, 0.44)	0.03, 0.98
Cases	11 (24)	0.3 (0.13, 0.6)	0.03, 1.02
Controls	35 (76)	0.3 (0.2, 0.5)	0.05, 0.98

Figure 3 and Figure 4 represent whisker plots of hair mercury concentrations between mothers and children and cases and controls.

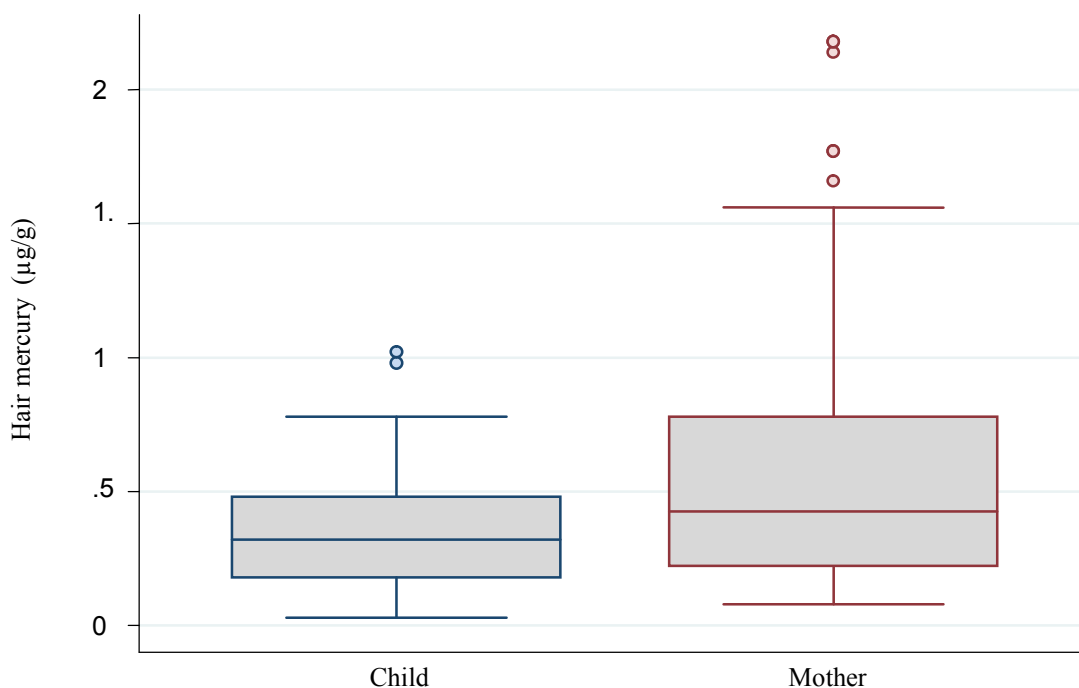


Figure 3: Box whisker plot of hair mercury concentrations ($\mu\text{g/g}$) in mother and child

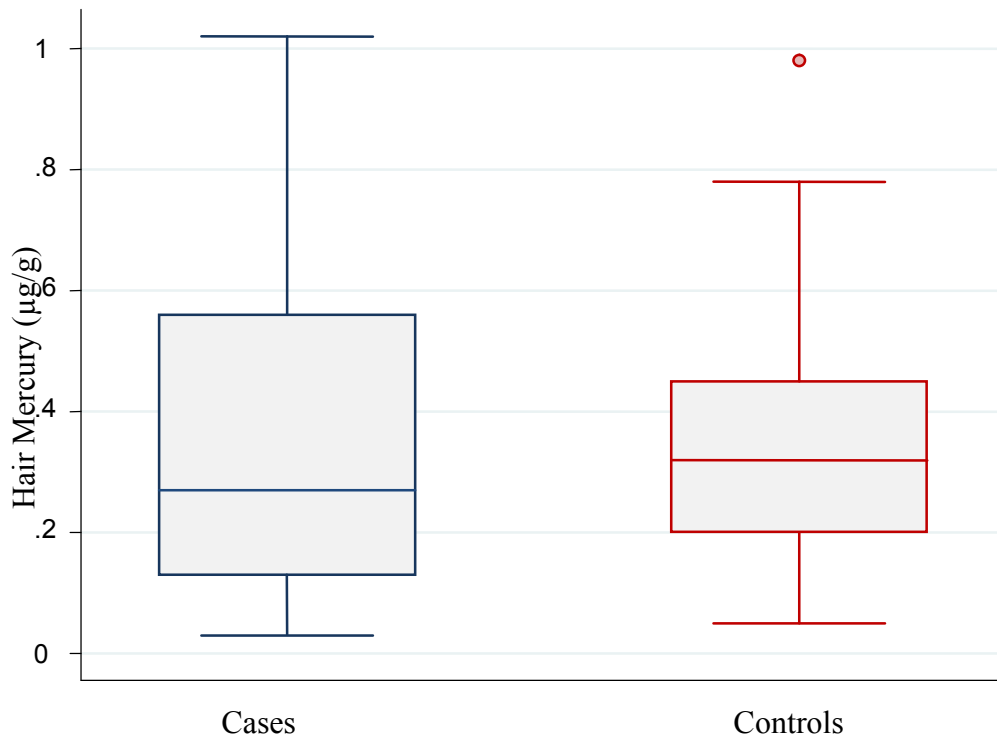


Figure 4: Box whisker plot of hair mercury concentrations ($\mu\text{g/g}$) of cases and controls

The hair mercury concentrations in participants are further shown in Table 8 below. Almost 20% of mothers and 18% of children exceeded the EPA threshold of $1 \mu\text{g/g}$ in this sample . However the majority of participants fell below the WHO's threshold of $1.6 \mu\text{g/g}$ except one mother whose concentrations reached up to $2.2 \mu\text{g/g}$.

Table 8: Hair mercury concentrations of mothers and children

	<0.5 $\mu\text{g/g}$	0.5 to <1 $\mu\text{g/g}$	>1 $\mu\text{g/g}$
Mother <i>n</i> (%)	26 (57)	11 (24)	9 (20)
Children <i>n</i> (%)	27 (59)	11 (24)	8 (18)
Cases <i>n</i> (%)	6 (13)	3 (7)	2 (4)
Controls <i>n</i> (%)	21 (46)	8 (18)	6 (13)

A significant correlation was observed between mother and child hair mercury concentrations (Rho 0.79 (95% CI 0.65, 0.88) (Figure 5).

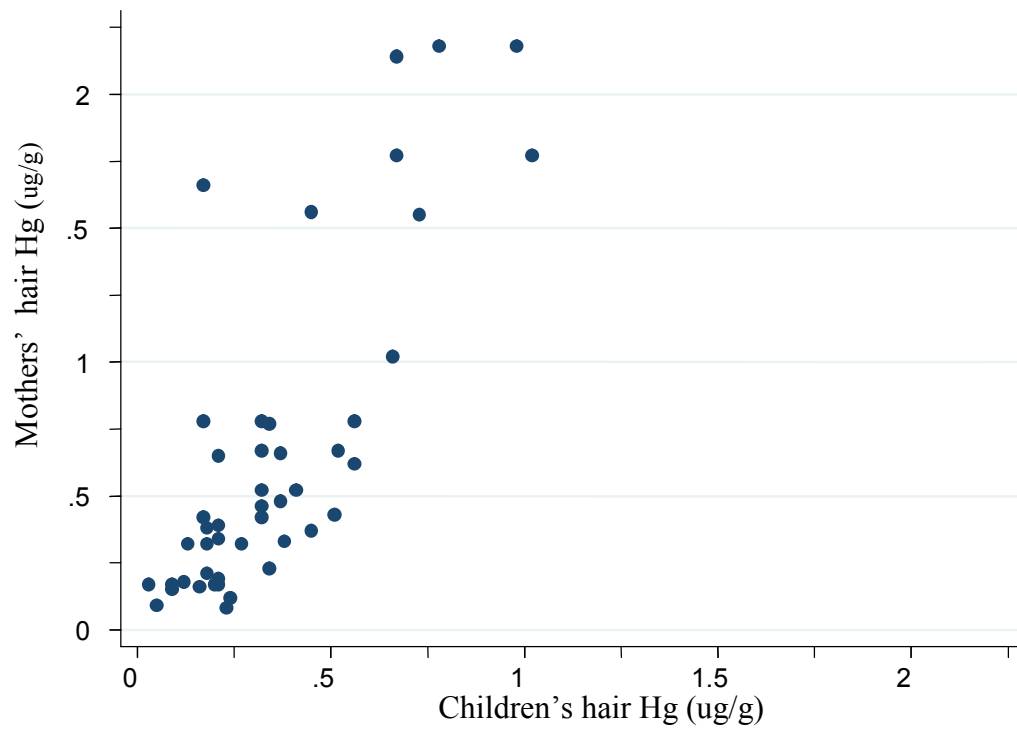


Figure 5: Scatter plot of hair mercury concentrations in mother and child

6.4.4 Fish and Mercury Concentrations

Mullet was the type of fish consumed most frequently by both children (46%) and their mothers (59%), followed by the other fish as shown in Table 9. Amongst the cases and controls the fish most eaten was the mullet (36% and 44% respectively).

Table 9: Types of fish eaten by mothers and children; cases and controls

	Mothers (n=46) n(%)	Children (n=46) n (%)	Cases (n=11) n (%)	Controls (n=35) n (%)
Mullet	27 (59)	21 (46)	4 (36)	17 (44)
Flounder	6 (13)	10 (22)	3 (27)	7 (20)
Kawahai	4 (9)	4 (9)	1(9)	3 (9)
Snapper	6 (13)	6 (13)	3 (27)	3 (9)
Other fish ^a	3 (7)	5 (11)	1 (9)	4 (11)

^aSalmon, tuna, cream fish, frost fish

Table 10 provides the mercury concentrations based on types of fish consumed in cases and in controls. However, no associations were found between mercury concentrations in the cases and controls (Kruskal Wallis P value 0.65).

Table 10: Hair mercury concentrations in cases (n=11) and controls (n=35) in relation to types of fish

	Children n (%)	Cases Hg ug/g	Controls Hg ug/g
Mullet	21 (46)	0.4	0.4
Flounder	10 (22)	0.4	0.2
Kawahai	4 (9)	0.2	0.2
Snapper	6 (13)	0.5	0.3
Other fish ^a	5 (11)	0.2	0

^aSalmon, tuna, cream fish, frost fish

6.4.5 Dental Fillings and Mercury Concentrations

All children and their mothers had provided consent and assent for access to children's dental records. All the children were successfully identified by their birth records and names. All the children had some kind of fillings with the majority having composite fillings (76%) as shown in Table 11 below. Mercury concentrations in those with temporary restoration fillings were higher than the rest. There was no significant difference observed in the mercury concentrations between cases and controls with regards to the different fillings (p values >0.05).

Table 11: Types of dental fillings in children and median hair mercury ($\mu\text{g/g}$) concentrations

Dental fillings	<i>n</i> (%)	Children (Hg $\mu\text{g/g}$)	Cases (Hg $\mu\text{g/g}$)	Controls (Hg $\mu\text{g/g}$)
Temporary	2 (4)	0.54	0	0.54
Amalgam	13 (28)	0.45	0.34	0.2
Sealed restoration	6 (13)	0.43	0.1	0.4
Glass ionomer	14 (30)	0.27	0.3	0.3
Composite	35 (76)	0.33	0.23	0.3

6.4.6 Association between Behavioural Problems and Mercury Concentrations

In the unadjusted logistic regression model, hair mercury concentrations were not associated with behavioural problems (OR) = 0.66; 95% confidence interval (CI), 0.03, 13.8; p value 0.23). The results showed that the study was highly underpowered as seen in the very wide confidence intervals. In order to determine if mercury exposure alone was independently associated with behaviour problems in this sample there needed to be an adjustment by ethnicity and gender but because of the small sample size this could not be undertaken.

6.5 Discussion

This research first attempted to study hair samples as biomarkers to explore the relationship between mercury concentrations and behavioural characteristics in children within the Pacific Island Families study. This study was not completed due to difficulties encountered in hair sample collection and therefore the study was underpowered. However, the analysis was undertaken and some interesting results are provided.

6.5.1 Summary of Results

6.5.1.1 Mercury Concentrations in Participants

Most of the participants in this study had hair mercury below the WHO recommended concentrations (1.6 µg/g Hg) but some were higher than the US EPA concentrations (1 µg/g Hg). Mothers had higher median mercury concentrations than children and this is so because adults are bigger and eat bigger portion sizes than children. The highest range of hair mercury concentration was 2.18 µg/g Hg which was observed in a mother and this could be a problem in women of child-bearing age as the foetus may be vulnerable to developmental disorders due to mercury exposure at this mercury concentration. A NZ study conducted in the late seventies where maternal hair sample were collected on 1000 mothers consuming three fish meals a week on the North Island had average hair mercury concentrations above 6 µg/g (Kjelstrom et al., 1986). This is much higher than this current study with average hair mercury concentration being 0.43 µg/g Hg in mothers and 0.32 µg/g Hg in children. However, those participants ate sharks (high in Hg concentrations) but shark is no longer eaten in NZ as noted in the review by (Karatela, Paterson, Schluter, & Anstiss, 2011) and the participants in this current study also did not eat shark.

In Table 12 below, the mercury concentrations in our sample were higher than the US but lower than other countries. The participants in the US and Spain studies ate no fish and hence the lower mercury concentrations. The Faroe Islands and Brazil studies had elevated fish consumption (more than three serves a week).

Table 12: International comparison of postnatal mean hair mercury concentrations in children and mothers

Country	Population	Fish-eating population	Total mercury ($\mu\text{g/g}$) ^a	Reference
NZ	Children (6 yrs)	Yes	0.32	<i>Karatela et al.(2011)</i>
(Current study)	Mothers (18+)	Yes	0.43	
USA	Children (1-5 yrs)	No	0.12	McDowell et al., (2004)
	Women (16-49 years)		0.20	
Spain	Children	No	0.8	Batista, Schuhmacher, Domingo, & Corbella,(1996)
Faroe Islands	Children (14years)	Yes	3	Debes et al., (2006)
Brazil	Indigenous children (7-12 years) and women (14-44 years)	Yes	14.5	Santos et al.,(2000)
			15.7	

^aMedian

6.5.1.2 Types of Fish and Mercury Concentrations

Mullet was the most eaten fish in this population followed by flounder and then snapper.

These types of fish are safer to eat as they are known to have lower mercury concentrations

(B. Thomson, Horn, & van Abel, 2009) but it seems that these mullet-eating participants had

more mercury concentrations than those that ate other kinds of fish. Other food items also contain higher mercury concentrations so fish may not be the only exposure factor. For example the 2009 NZ Total Dietary Survey found that some adults consumed certain kinds of teas that had higher mercury concentrations (New Zealand Total Diet Study, 2009). Perhaps future research should also consider other food items that may expose participants to mercury.

The benefits and risks of eating fish should be well communicated to high fish-eating communities such as Pacific Island people. The Ministry of Health, (2012b) recommends that preschoolers and children aged 2 to 12 years eat at least 1–2 servings of seafood (along with other food items) each week; however, it does not mention the type of fish to consume and the possible risk of mercury concentrations in certain kinds of fish consumed especially for pregnant women and children. This has been highlighted in the review by Karatela et al., (2011). As noted in this sample, the mercury concentrations were below the recommended WHO levels which could mean that they are eating the right type and correct amount of fish. Mercury concentrations and effects could be affected by eating other kinds of food that may have been rich in selenium and this may counteract the effects of mercury concentrations. Selenium and its effects on mercury have been demonstrated in other research (M. J. Berry & Ralston, 2008; Choi et al., 2008); however, more research is required on this aspect within Pacific people.

6.5.1.3 Dental Amalgam Fillings and Mercury Exposure

All of the children in our sample had some sort of dental treatments with fillings ($n=46$) with the majority having composite fillings ($n=35$) followed by amalgam ($n=13$). Dental amalgam, which is a mixture of mercury and silver alloy powder, has been used to restore decayed

tooth surfaces. However the use of dental amalgam in children has been reduced in recent years in NZ. However in some cases mercury is still being used especially in those children with substantial tooth decay (personal communication, Auckland Hospital dental services).

It has been established that mercury is released from amalgam fillings and that the amount of mercury that passes through the human body is sometimes very high (Vahter et al., 2007; World Health Organisation, 1991). Amalgam fillings were observed in 26% of children in this small sample and they had total hair mercury concentrations of 0.4 µg/g Hg. It is below the WHO recommended mercury level but the long-term effects of mercury due to dental amalgam on these children is not yet known. Certain studies have shown that mercury concentrations in hair are not elevated or correlated with the number of dental fillings as the mercury from amalgam fillings is not high enough to accumulate in hair (Mortada, Sobh, El-Defrawy, & Farahat, 2002), therefore the best biomarker for dental amalgam is urine (Counter, Buchanan, & Ortega, 2007). Given the widespread use of amalgam dental restorations in children with substantial decay, concern over the health risks of mercury from amalgam prompted the funding of two randomised clinical trials in the US (Bellinger, Daniel, Trachtenberg, Tavares, & McKinlay, 2007; DeRouen et al., 2006). These trials consistently found no adverse neuropsychological effects of amalgam in children, but controversy over the safety of amalgam liners (Fuks, 2002; Needleman, 2006; Osborne, Summitt, & Roberts, 2002).

6.5.1.4 Behavioural Problems and Mercury Exposure

No significant association ((OR) = 0.66; 95% confidence interval (CI), 0.03, 13.8; p value 0.23) was observed; however, due to the small sample size of the study the results are not interpretable nor are they representative of the general Pacific population in NZ.

6.5.1.5 Methodological Aspects

The strength of the present study is the nested case-control study design which was thoroughly thought out and well designed. As a non-Pacific researcher within a Pacific research a number of cultural practices and consultations were carried out. A pilot study was conducted on a few Pacific participants to check for feasibility of scalp hair sample collection. It also involved collaboration with Pacific researchers, supervisors and the AUT Doctoral Committee. A preliminary study (not presented in this thesis) was initially undertaken in 2007 on a few Pacific children to assess hair mercury concentrations in relation to behavioural problems before progressing to the main study. The mercury analysis for that preliminary study was carried out at the Harvard School of Population Health using their internationally recognised laboratory facilities. This also provided the opportunity to learn and understand the technique used for hair mercury analysis. The mean concentrations in hair were 0.9 µg/g (range = 0.13 to 3.5 µg/g), indicating both high variability and high concentrations when compared to the US EPA concentration of 1 µg/g (Environmental Protection Agency, 1997). Even within this small sample size, a significant correlation was observed between mercury concentration and child-behaviour checklist scores (Pearson correlation = 0.64, $p = 0.01$). After approval was sought, this study commenced. However, data collection became difficult and the following lessons were learnt and observations made.

An initial sample of 46 mother-child pairs was collected which took almost a year; however, resistance by some participants to the cutting of hair was observed. A decision was made to change the biomarker to something more feasible because it was difficult, slow and unpopular with the participants. There was also concern that it was burdensome on the

longitudinal cohort and could have some repercussions in future phases. It was observed in this sample that Pacific women and girls were less likely to provide hair samples and that certain ethnic groups for religious and cultural reasons were not allowed to cut their hair. Some children in the group had never had their hair cut since they were born. For some maternal participants, only their family members could cut their hair and this made it difficult for many of the women to participate. However, most of the participants that were contacted at the time all agreed and were happy to be a part of this study. Some were curious about the fact that hair can help determine health status of people. Due to the cancellation of the study, the sample size remained small and this affected the variables in the regression models which made the CIs of the ORs wide. The results are also not generalisable nor a representative of the Pacific population.

The lesson learnt from this study was the importance of cultural awareness in carrying out such research. Even though it was deemed okay and feasible to carry out such research it proved difficult as participants were not involved in its development. Cultural awareness is the foundation of any communication between researchers and participants. People generally see, interpret and evaluate things in different ways which can lead to misinterpretation between participants and researchers. It is said that it is better to see differences rather than similarities until clarified (Alder, 1991). The best way to counter this issue is by creating a dialogue between different cultural groups and to understand the differences and unique contributions of biology and environment to human development. The use of focus groups with participants to identify the feasibilities of biomarker use would be useful.

6.5.2 Strengths and Limitations

Apart from the small sample size leading to very low statistical power, the strength of this study was that it paved the way for the next research whereby appropriate measures were taken to increase participation rates. Thus, it delineated criteria for subject inclusion, suggested the modifications necessary in bio-monitor collection in the Pacific population and indicated changes necessary in the research procedure for the next research which is presented in Chapters 7 and 8.

Chapter 7 Study 2 – Nail Mercury and its Association with Behavioural Outcomes

This study followed the previous study and was modified to toenail clippings as the biomarker to determine mercury exposure in nine year old children. This chapter provides an overview of mercury exposure, materials and methods used, results, discussion and conclusion.

7.1 Introduction

There is little research in the predictors of behavioural problems in Pacific children in New Zealand (NZ) (Paterson, Taylor, Schluter, & Iusitini, 2013). Along with household socio-economic status, parental physical and mental health, parenting style, as well as family structure (Chen, Langer, Raphaelson, & Matthews, 2004; Pachter, Auinger, Palmer, & Weitzman, 2006b), prenatal mercury exposure can exacerbate the effects of behavioural problems in children (P. Grandjean & Heindel, 2008; P. Grandjean & Weihe, 2008).

Although the association between behavioural problems and postnatal low concentrations of mercury is less clear (Myers et al., 2009), it highlights the uncertainty as to the real impact mercury can make on children's behavioural development. There are many different measures used to identify behavioural problems in children one of which is the Child Behaviour Checklist (CBCL).

The child behavioural outcomes using the CBCL have been measured extensively to describe problematic behaviour in early childhood in a number of countries (Ivanova & al, 2010; Rescorla & al, 2007, 2011). The CBCL also measures internalising behaviours (such as anxious, depressed, and withdrawn and somatic complaints) which are directed towards oneself while externalising behaviour (aggressive and rule breaking behaviour) is more about

inner conflicts or unpleasant feelings towards another person or external circumstances.

Generally girls have more internalising behaviour problems and less externalising problems than boys (Bongers, Koot, van der Ende, & Verhulst, 2003; Verhulst, van der Ende, & Koot, 1997). Large cohort studies such as the Faroe Islands and Seychelles studies have also employed some/all of the CBCL to identify behavioural problems and have related it to mercury exposure. According to G J Myers et al., (2004), the CBCL appeared to be a valid behavioural measure in the Seychelles methylmercury study.

Experimental studies in animals have described subtle behavioural and developmental changes arising from toxic exposures to heavy metals at levels below those that impair organ systems or affect overall cognition, memory, or language (Feldman, Ricks, & Baker, 1980; Spyker, Sparber, & Goldberg, 1972; Stollery, 1996). Behavioural domains such as conduct or social behaviours may be a more sensitive and specific way to measure neurotoxic exposures than traditional tests of cognitive abilities and language (Spikier, 1975; Vorhees & Mollnow, 1987).

The CBCL has been reported to be sensitive and specific enough to determine the effects of exposure to low-level lead, cocaine and polychlorinated biphenyls (Burns et al., 1999a; Needleman, Riess, Tobin, Biesecker, & Greenhouse, 1996; Phelp & Cottone, 1999; Richardson, Conroy, & Day, 1996; Sciarillo, Alexander, & Farrell, 1992) and therefore CBCL would be an appropriate measure for mercury exposure.

In most exposure epidemiological studies, mercury is assessed by using such biomarkers as hair, blood, or urine. Though nail analysis has been extensively used in determining concentrations of elements in nutritional studies such as mercury concentrations and fish

consumption (Rees, Sturup, Chen, Folt, & Karagas, 2007); only 13 studies were identified with nail mercury exposure and health but most of those studies were looking at different outcomes such as cardiovascular diseases (Miodovnik, 2011; Mozaffarian, Peilin, et al., 2011), hypertension (Xun et al., 2010) and cancer (Koriyama et al., 2008; O'Rourke et al., 2012) or the populations were different such as dental workers. (Al-Saleh, Al-Sedairi, & Elkhatib, 2012).

Toenails as a biological marker for mercury exposure in children is still in its infancy. Variability between hair, nails, plasma, serum and blood is often observed as biological markers, which may suggest uncertainties in the reliability of the biomarkers. (Berglund et al., 2005). However the choice of biomarker is usually dependent on its feasibility and toxicokinetics of the chemical to be studied (Esteban & Castaño, 2009).

7.2 Aims

- i) To identify the distribution of mercury concentrations and behavioural characteristics in a sub-sample of 9 year old Pacific children.
- ii) To investigate associations of mercury concentrations among major demographic groups categorised by ethnicity, gender, income and maternal education.
- iii) To explore the association between mercury and behavioural problems of Pacific children.
- iv) To explore the association between mercury and different behavioural domains to determine if specific behaviours particularly aggression, rule breaking, attention and social problems are related to mercury exposure.

Secondary aims:

- i) To determine the distribution of seafood diet in children.
- ii) To investigate the association of seafood diet and mercury concentrations

7.3 Materials and Methods

7.3.1 Study Design and Participants

The cross-sectional study was conducted between July 2010 and July 2011. A sub-sample of nine year old children was recruited from within the PIF longitudinal cohort. The participants were not randomly selected as it was a convenience sample dependent on the recruitment for the core PIF study which was already underway. Maternal interviews were conducted in the home setting while biological markers from the children were collected in the schools. Children with very short toenails were excluded from the study. Further details on recruitment numbers and demography are in section 7.4.1.

7.3.2 Measures

Total mercury exposure: Toenail clippings were collected from all toes and used for determining mercury exposure. Like hair samples, toenails are a reliable biomarker for mercury, though it is only recently that nail clippings have been considered as such. Mercury concentrations in nails have been compared with blood with significant correlations observed between toenail-mercury and blood-mercury (Alfthan, 1997), and toenail-mercury and methylmercury in blood (Bjorkman et al., 2007). In addition toenails have a high correlation with mercury intake (Pearson correlation r : 0.54) (Ohno et al., 2007). The growth of toenails is between 3-12 months (Karagas et al., 2000). Since all the toenails were collected and analysed at the same time, it reflects the incorporation of mercury that has occurred over approximately one year (Yoshizawa et al., 2002). It is relatively easy to collect, less invasive

and easy to store. The collected toenail clippings were stored dry at room temperature in marked zip-lock bags before chemical analysis. Toenails are less contaminated externally than hair samples (He, 2011). Total mercury concentrations were determined as methylmercury is converted into inorganic mercury when bound to tissues (Magos & Clarkson, 2008). Within this part of the research, mercury is reported in $\mu\text{g/g}$ (ppm) and used as continuous measure. The WHO's threshold of $1.6 \mu\text{g/g}$ (World Health Organization, 1990) and EPA's threshold of $1 \mu\text{g/g}$ (Environmental Protection Agency, 1997) were used as comparison.

Child behaviour check list (CBCL): The CBCL questionnaire is designed to obtain parental ratings of problem behaviours in children (Achenbach & Rescorla, 2001). The administration procedures and questions for the CBCL's were the same as for Study 1 (details in section 6.3.2) but the age was 9 years not 6. Briefly, the CBCL is a measure of children's behaviour in ten specific domains (Achenbach & Edelbrock, 1981). Many researchers have used the CBCL and the appropriate use of this measure across different cultures and languages (Bird, 1996; Crijnen et al., 1999) has been established. Further details of the CBCL are explained in section 6.3.2.

Seafood questions: The seafood questions were administered at the nine year measurements wave and were maternal reported. The question of whether they had eaten seafood in the past month was a yes/no question. The food frequency questions were adapted from the questionnaire developed for NZ children in 2000 which shows good short-term repeatability (Metcalf et al., 2003). The seafood questionnaire for mothers and children that was developed as part of Study 1 was not administered for this study in order to reduce the burden the PIF participants. Therefore the types of fish and frequency of fish at 9 years was not measured.

Measurements of other variables: Ethnicity of the children was reported by the mothers and was collected at baseline. Mothers were asked during the interviews to identify themselves and their children's ethnicity and in the case of multiple ethnicities to specify the main ethnicity. The main ethnicities were Samoan, Tongan, Cook Island, Niuean, Other Pacific, and Non Pacific.

Household income was based on family income at the nine year phase (categorised into \$0-\$20,000, \$20,001-\$40,000, >\$ 40,000) and was reported by mothers. Maternal educational achievements were categorised into no formal qualification, secondary school qualification, post-secondary qualification.

7.3.3 Procedures

The children and their mothers were contacted by telephone by a trained Pacific researcher, to invite them to consider taking part in this sub-study and provided both verbal and written information about the study. With the agreement of the parent or legal guardian, an appointment for a face-to-face meeting with a PIF interviewer was made. At the meeting, information sheets were provided, explained and written assent from the children and consent from their guardian (usually the mother) was obtained so that toenail clippings could be collected by PIF child assessors from the child when the child was visited at school. The PIF child assessors reaffirmed verbal assent from the child before they clipped their toenails.

7.3.3.1 Toenail Sample Collection, Handling and Storage

The two PIF child assessors were trained on the correct method of collecting toenail samples. Approximately 50 mg of toenail clippings (quarter of a teaspoon) from feet (big toes and little toes) were placed, as they were clipped and collected onto a tissue. These were then placed in

a sealed zip-lock polythene bag and the bag identified with a unique code identification number to ensure anonymity when analysed. The samples were then stored at room temperature until transportation to the UK's ICP MS Facility at the University of Surrey for laboratory analysis.

7.3.4 Toenail Laboratory Analysis

(1) Toenail sample analysis

Chemical analysis of toenail material was conducted by the ICP MS Facility of the University of Surrey which is an internationally recognised research centre for inductively coupled plasma mass spectrometry following the pioneering work of Dr Alan Gray. This facility has for over 30 years developed new analytical techniques for detecting trace and ultra-trace elements in biological samples (including hair and toenail clippings) suitable for epidemiological purposes. The ICP MS Facility has been involved in many international quality control inter-laboratory proficiency or round-robin tests and justifies all sample analyses by using internationally recognised certified reference materials for quality control assessment (to established acceptable levels of accuracy and precision). A brief outline of the toenail sample preparation method and ICP MS technique operation parameters follows.

All toenail samples were washed prior to digestion to remove potential exogenous elemental contaminants derived from cosmetic treatments, 'dirt', etc. The washing procedure involved: (1) five steps using acetone, deionised distilled water (DDW, 18.2 MΩ) (x3) then acetone again; (2) at each washing step enough liquid was added to cover the sample and sonication (rasssonic water bath (T460/H)) for 5-10 minutes; and (3) decantation. Following the washing procedure the nail samples were dried overnight at 60°C in a drying oven (LTE Scientific). Once dried, the sample was weighed (four decimal place analytical balance) and

transferred to a pre-acid/DDW washed/dried Kjeldhal™ tube for digestion where 0.5 ml of concentrated nitric acid (Fisher Scientific, Trace Analysis Grade nitric acid) was added and the tube sealed with PVC Clingfilm. The Kjeldhal™ tube was placed in a hot block (Tecator 2012 Digestor) and heated at 160°C for 30-60 minutes. Once the digestate was visibly clear, the Kjeldhal™ tube was removed from the heat and cooled and the digest solution transferred to a clean, weighed 15ml centrifuge tube. The digested sample was weighed again (to four decimal places) and diluted 250 times (volume/weight) using DDW based on a dilution factor of 250. Due to some small toenail sample masses the 250 dilution factor was not sufficient to ensure there was enough volume of sample (4 ml) to be analysed, thus for some samples a higher dilution factor was used and noted. Before analysis the digest was filtered using a 0.22 µm syringe-driven filter unit (Millex®-GP, Millipore, Bedford, USA).

Analysis of all the washed and digested nail samples was carried out by ICP MS.

(2) Elemental toenail analysis

All instrumental data for each element (according to the isotope selected) was reported as counts per second. The value was corrected for a reagent blank signal (to correct for any contribution from the digestion procedure) and ratioed with the internal standard isotope value (to correct any instrumental drift or signal enhancement/depression caused by the matrix). Data for the calibration standards was handled in the same manner and an Excel™ calibration curve produced for each element, with ratio signal (y-axis) and concentration of five standards (x-axis), from which the calibration equation was determined for calculation of the unknown toenail sample elemental concentration. The elemental values for each toenail sample were corrected for the dilution factor and the final values used in data analysis.

7.3.5 Data Analysis

For data storage refer to Chapter 2, section 2.2.1. Bivariate analysis (Chi-square tests) was performed to test for differences in proportions of categorical variables among two or more groups. The $p < 0.05$ (95% confidence interval) was considered as the cut-off value for statistical significance in this research.

Summary statistics for mercury in children were calculated to include median, upper (75%) and lower (25%) quartiles and minimum, maximum concentrations. Descriptive statistics were also provided for demographics, gender, behavioural domains frequencies and percentages. Non-normally distributed mercury was log transformed to normalise the data. Box whisker plots were also plotted where required.

Logistic regression analysis with ordinal outcome of behavioural problems was performed to investigate the relationship between behavioural characteristics in children and toenail mercury concentrations.

7.3.6 Ethical Considerations

The Northern X Ethics Committee granted permission for this part of the research whereby the hair samples were modified to toenail clippings (NTX/07/05/050) (letter attached in appendix 3). Consent for this study was sought from the mother and assent was sought from children. Information sheets were provided for both mother and child separately.

Questionnaires and data were stored as discussed in Chapter 2, section 2.2.1. Any leftover toenail samples were destroyed after the laboratory analysis. All questionnaires as part of this research will be destroyed after 10 years as per the ethics requirement.

7.4 Results

7.4.1 Recruitment of Participants

Of the 891 children recruited to the nine year measurement phase of the longitudinal Pacific Island Families study 360 were contacted and 310 participants agreed to take part in the study. However, 278 children provided samples of their toenails collected for analysis for 19 selected elements, and also had height and weight reliably measured; 30 participants were excluded as they had short toenails and two children had missing height and weight.

7.4.2 Demographics

All the children were nine years of age at the time of measurement of which 160 were boys (58%) and 118 (42%) girls (Table 13). More than half of the children and their mothers identified themselves as Samoans ($n=148$; 53%). Around 85% of these children's household income was less than \$20,001-\$40,000 per annum. More than half of the children's mothers had high school as their highest qualifications ($n=205$; 74%).

Table 13: Socio-demographic characteristics of the study population

Variable	<i>n</i>	<i>(%)</i>
<i>Gender</i>		
Male	160	(58)
Female	118	(42)
<i>Ethnicity</i>		
Samoan	148	(53)
Tongan	29	(10)
Cook Island	53	(19)
Others ^a	48	(17)
<i>Household income (yearly)</i>		
\$0-20,000	93	(33)
\$20,00-40,000	143	(51)
> \$40,000	32	(12)
Unknown	10	(4)
<i>Maternal Education</i>		
No formal	102	(37)
Secondary school	103	(37)
Post-school	73	(26)

^aEuropean, Niuean, Māori

7.4.3 Description of Behavioural Characteristics

The prevalence of total behavioural problems (which includes both internalising and externalising behaviour problems) in the clinical range was 24% within the sample (Table 14). The majority of the children in the clinical range were higher in the externalising groups (32%) than internalising groups (19%). Within the specific behaviours, attention seeking had more clinical cases (59%) than any other specific domains. Aggressive and rule breaking were the next within the clinical range (11% and 14% respectively).

Table 14: Behavioural outcomes of children

Behavioural Outcomes	Normal <i>n</i> (%)	Clinical cases <i>n</i> (%)
Specific behaviours		
Social	262 (96)	12 (4)
Rule breaking	237 (86)	37 (14)
Aggressive	244 (89)	30 (11)
Attention seeking	113 (41)	163 (59)
Child behaviour check list combined scores		
Internalising behaviour	224 (81)	54 (19)
Externalising behaviour	188 (68)	90 (32)
Total behaviour	211 (76)	67 (24)

7.4.4 Description of Mercury

Figure 6 shows a non-normal distribution of mercury concentration in toenails. The overall median of toenail mercury concentration within the sample is 0.02 µg/g. Both boys and girls had mercury concentrations of 0.02 µg/g.

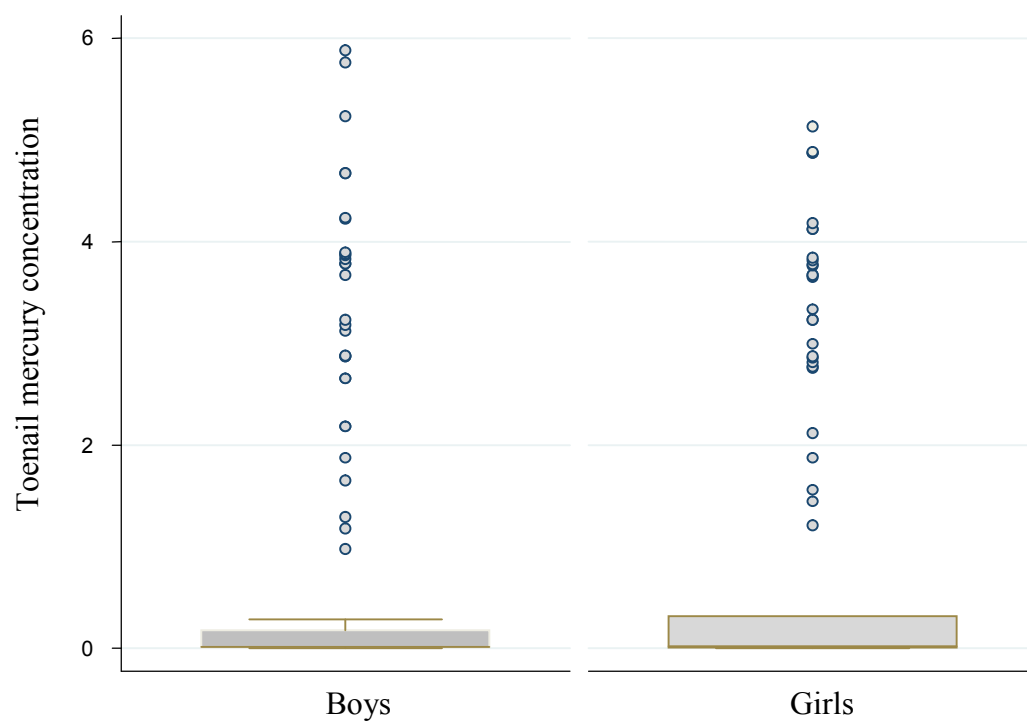


Figure 6: Box whisker plots of toenail mercury in relation to boys and girls.

There were no statistically significant associations between toenail mercury concentration and ethnicity (p value 0.95), gender (p value 0.42), income levels (p value 0.68) and maternal education (p value 0.78) (Table 15).

Table 15: The median, 25th and 75th percentiles, minimum, maximum of mercury concentrations ($\mu\text{g/g}$) in toenails

	Median	25th percentile	75th percentile	Min, Max ^c	P value ^a
<i>Ethnicity</i>					0.95
Samoan	0.2	0.008	0.02	0.002, 5.23	
Tongan	0.03	0.007	2.2	0.005, 5.76	
Cook Island	0.02	0.008	0.2	0.004, 5.9	
Others ^b	0.02	0.007	0.21	0.004, 4.1	
<i>Gender</i>					0.42
Boys	0.2	0.008	0.2	0.002, 6	
Girls	0.3	0.008	0.32	0.003, 5	
<i>Income</i>					0.68
\$0-20,000	0.34	0.08	0.22	0.004, 5	
\$20,001-40,000	0.2	0.008	0.02	0.002, 6	
>\$40,001	0.12	0.007	0.18	0.005, 5	
<i>Maternal education</i>					0.78
None/secondary	0.02	0.008	0.2	0.002, 6	
Post-secondary	0.03	0.008	0.2	0.004, 5	

^aKruskal Wallis test ^bEuropean, Niuean, Māori ^cMinimum, maximum

The toenail mercury concentrations of the children are presented in Table 16. In comparison to the WHO's threshold for mercury of 1.6 µg/g and EPA's threshold of 1 µg/g, 21% of the children exceeded those limits. The girls had higher toenail mercury concentrations of above 1 µg/g (24%) than boys (18%).

Table 16: Toenail mercury concentrations for children in categories

	<0.5 µg/g	0.5 to <1 µg/g	>1 µg/g
Children <i>n</i> (%)	161 (58)	60 (23)	57 (21)
Girls <i>n</i> (%)	67 (57)	23 (20)	28 (24)
Boys <i>n</i> (%)	94 (59)	37 (23)	29 (18)

7.4.5 Association between toenail Mercury with Behavioural Problems and Specific Behavioural Domains

The internalising group had the highest toenail mercury concentrations compared to the externalising and total behaviour groups (Figure 7).

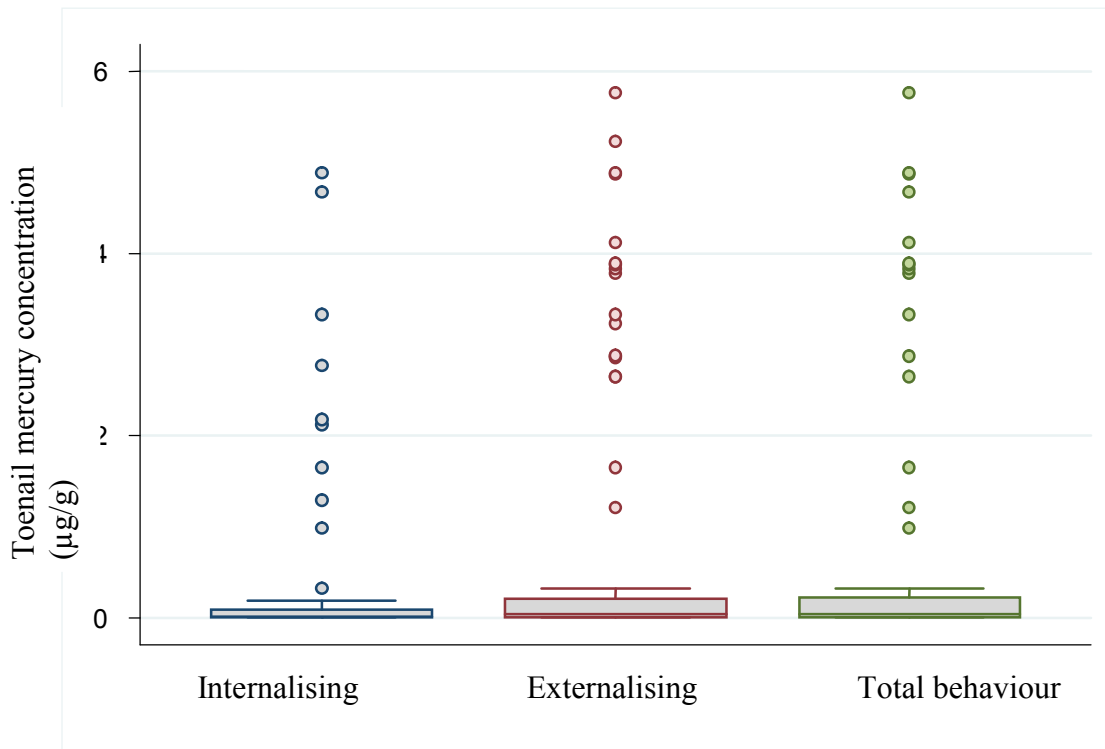


Figure 7: Box-whisker plots of toenail mercury concentration in internalising, externalising and total behaviour problems

Statistically significant associations were observed between toenail mercury and externalising (p value 0.05), aggression (p value < 0.05), rule breaking (p value < 0.001), attention problems (p value 0.05) behavioural outcomes. However, no significant association was found between toenail mercury and total behavioural scores (p value 0.5) (Table 17).

Table 17: The median, 25th, 75th percentiles, minimum(min) and maximum(max) mercury concentrations ($\mu\text{g/g}$) in toenails of participants by the different behavioural outcomes (clinical range)

Clinical range CBCL outcomes	<i>n</i> (%)	Median	25th percentile	75th percentile	Min, Max	P value^a
Internalising	90	0.2	0.008	0.9	0.003, 3	0.3
Externalising	211	0.04	0.008	0.2	0.003, 6	0.05
Total behaviour	67	0.04	0.008	0.2	0.003, 6	0.5
<i>Specific behaviours</i>						
Aggression	12	0.03	0.008	3	0.005, 6	<0.05
Rule breaking	37	0.01	0.01	0.2	0.004, 6	<0.001
Attention problems	30	0.01	0.01	0.2	0.004, 6	0.05
Social problems	12	0.01	0.007	0.03	0.004, 5	0.1

^aKruskal Wallis test

The unadjusted regression analysis in Table 18 showed a significant association between aggressive behaviour and toenail mercury. However, no significant associations was observed between toenail mercury and total behaviour, internalising, externalising, social, rule breaking and attention seeking behaviour domains.

Table 18: Ordinal logistic regression of the relationship between the different behavioural outcomes and log transformed toenail mercury

Behavioural outcomes	Log transformed toenail mercury		
	OR	(95% CI)	Unadjusted P value
Total behaviour	1.02	(0.82, 1.29)	0.8
Internalising	0.85	(0.66, 1.08)	0.2
Externalising	0.96	(0.86, 1.07)	0.5
<i>Specific behaviours</i>			
Social	1.07	(0.82, 1.3)	0.7
Rule breaking	0.9	(0.79, 1.1)	0.33
Aggressive	1.2	(0.99, 1.64)	0.05
Attention seeking	0.8	(0.83, 1.15)	0.78

A significant association of mercury with aggressive behavioural scores remained after adjusting for gender, ethnicity and income levels (OR: 2.15 95% CI 1.45, 3.18 p-value <0.05).

7.4.6 Seafood Consumption in Children and toenail Mercury Concentrations

Table 19 provides details of the children's seafood consumption. Seafood was eaten by the majority of the children (78%). There was significant difference between seafood consumption in children and mercury concentrations 0.035 µg/g.

Table 19: Description of seafood consumption in children

	<i>n</i>	(%)	Mercury µg/g	25th percentile	75th percentile	Min, Max	P value*
<i>Seafood</i>							0.035
Yes	218	(78)	0.3	0.008	0.21	0.008, 5.88	
No	41	(15)	0.2	0.008	0.18	0.002, 4.87	
Unknown	19	(7)	0.12	0.075	0.4	0.006, 5.13	

*Kruskal Wallis test

The unadjusted relationship between log transformed mercury and seafood consumption showed a significant association (OR 1.35 95% CI 0.89, 1.45; p value <0.05). When further adjusted for ethnicity, gender and income levels, the association still remained (OR 1.38 95% CI 0.83, 1.2 p value <0.05).

7.5 Discussion

The present study investigated the association of toenail mercury on behavioural outcomes in nine year old Pacific children. The most important findings in this study were that mercury had an association with aggressive behaviour both before (Kruskal Wallis p value <0.05) and after considering gender, ethnicity and income levels (OR: 2.15 95% CI 1.45, 3.18 p-value 0.05). Significant differences were also found in seafood consumption and mercury concentrations after adjusting for gender, ethnicity and income levels (OR 1.38 95% CI 0.83, 1.2 p value <0.05). Although the use of cross-sectional design precludes the advancement of causal statements, the findings here are suggestive of a process which merits further considerations. A detailed discussion is provided below.

7.5.1 Mercury Concentrations in Children

One out of five children had toenail mercury concentrations higher than 1 µg/g which was higher than the US Environmental Protection Agency's recommended concentrations of 1 µg/g (Environmental Protection Agency, 1997). However, the concentrations were lower than the WHO concentration of 1.6 µg/g (World Health Organization, 1990). The majority of the children (92%) consumed seafood; however, the toenail mercury concentrations were much lower than international seafood eating communities which had mercury concentrations above 3.5 µg/g in children (Freire et al., 2010; Nadal, Bocio, Schuhmacher, & Domingo, 2005; Pesch et al., 2002). The median toenail mercury in this doctoral research study was 0.02 µg/g which was much lower than other reported studies which ranged from 0.22 to 0.27 µg/g (Bjorkman et al., 2007; Mozaffarian, 2009; Rees et al., 2007).

Studies have shown that eating more tropical fruits reduces the absorption of mercury from fish possibly due to interaction of fruits and mercury through toxicokinetics (Passos et al., 2008; Passos et al., 2007; Passos et al., 2003). Pacific people in NZ according to the Ministry of Health Nutrition Survey (2012) ate the recommended guideline of two or more serving of fruits per day even though half of Pacific people (53% males and 58% females) eat fish compared to their NZEO counterparts (Ministry of Health, 2012a). As shown in the research the consumption of fruits may reduce the risk of possible Hg exposure through fish. It should be noted that it was difficult to compare this research to the main international studies such as the Seychelles mercury study (Davidson et al., 2010), the US National Health and Nutrition Examination Survey (NHANES) (Mahaffey, Clickner, & Bodurow, 2004; McDowell et al., 2004), the Baltimore study (Weil et al., 2005), and the Canadian Environment and Childhood

study (Freire et al., 2010) as they detected mercury concentrations in different biomarkers such as blood and hair.

7.5.2 Gender Differences in Mercury Concentrations

Studies have reported gender differences in mercury concentrations due to differences in kinetics, mode of action, or susceptibility (Vahter et al., 2007). The effect of gender on mercury concentrations was explored; however, there were no significant associations. This is in line with another mercury study on gender (Auger, Kofman, Kosatsky, & Armstrong, 2005). In contrast to this study, two studies have found that male children are affected more by neurotoxins than females (P. Grandjean, Weihe, White, & Debes, 1998; Mckeown-Eyssen, Ruedy, & Neims, 1998). However, there are only a few gender-related susceptibility studies on mercury exposure and the results that are available are inconclusive (Davidson et al., 2004). Further research on this aspect is required to establish the effect of gender on susceptibility to mercury exposure.

7.5.3 Behavioural Problems in Children and Mercury Concentrations

In agreement with international studies (Axtell et al., 2000; Davidson et al., 2000; Plusquellec et al., 2010; Strain et al., 2008) the effect of mercury concentrations on behavioural problems in children was non-significant in this current study (Kruskal Wallis test p value: 0.5). This also corresponds with another Seychelles child developmental study on postnatal mercury exposure presenting no association between behavioural problems assessed by the CBCL (Myers et al., 2000). When the Connor's teacher reading scale was implemented on children aged 107 months an adverse association was found on mercury and developmental disorders; however, the authors were skeptical of their results and stated that there was no clear

evidence linking behavioural disorders and mercury (Myers et al., 2009). Another recent study did not find any significant association on postnatal mercury and behavioural problems in toddlers (Cao et al., 2010). In contrast a few other studies have shown adverse affects of mercury exposure on behavioural problems (P. Grandjean et al., 2008; Murata, Weihe, Araki, Budtz-Jorgensen, & Grandjean, 1999; Myers et al., 2004; Strain et al., 2008). These contrasting findings are due to differences in population, the choice of biomarkers, the study methodologies and types of mercury explored. No studies that specifically investigated aggressive behaviour and mercury exposure in children were identified. This current study showed an association between aggression and mercury exposure (OR: 2.15 95% CI 1.45, 3.18 p-value < 0.05) which corresponds with other studies on lead exposure that identified an association with aggressive behaviour (Bellinger, 2008; Bellinger et al., 2008; Braun et al., 2008; Naicker, Chelopo, Govender, Kruger, & Maguire, 2012; Wright et al., 2008). Mercury has similar properties to environmental lead and affects the same regions in the brain so the effects of mercury are likely to be similar in children to those of lead (Bellinger, 2008).

7.5.4 Toenail Biomarker

There is enough evidence that mercury measured in toenails is a good indicator of long term mercury exposure (Brockman et al., 2009; Garland et al., 1993; Mozaffarian, Shi, et al., 2011). Similar to this doctoral research, a few other studies have used nail as a biomarker (Guallar et al., 2002; Mozaffarian, Peilin, et al., 2011; Rees et al., 2007). However the majority of the studies investigating the relationship between neurodevelopmental disorders and mercury exposures have used hair and blood samples (Davidson, Jean Sloane, et al., 2008; P. Grandjean & White, 1999; Oken et al., 2005) while those investigating cardiovascular disease and mercury exposures have used toenails (Mozaffarian, Shi, et al.,

2011; Ohno et al., 2007; Virtanen, Rissanen, Voutilainen, & Tuomainen, 2007). Unlike this doctoral research many other studies have used multiple biomarkers to compare toenail mercury with other biomarkers (Alfthan, 1997; Garland et al., 1993; Rees et al., 2007). In this research toenail mercury seemed to be a good indicator of fish consumption (OR 1.38 95% CI 0.83, 1.2 p value <0.05).

Limitations and strengths

The CBCL uses a parent report questionnaire to measure behaviour which may have resulted in measurement bias. However the CBCL has been used internationally and has been described as a valid measure of behaviour (Myers et al., 2000). The CBCL is also culturally appropriate for Pacific populations, therefore; the current study data may not be heavily influenced by confounders, sampling or measurement bias.

Behavioural disorders in children are known to be affected by maternal age at parturition, and home environment which were not addressed in this research study. Parental child rearing based on birth order has also been reported to have an effect on behaviour (Wasserman et al., 1998). Studies on other toxic elements have shown associations in which the first-born child is more vulnerable to polychlorinated biphenyl than the second born or following children (Kostyniak et al., 1999). Furthermore higher scores of CBCL have been associated with younger mothers and thus maternal age and birth order are positively correlated (Tatsuta et al., 2012). Also, Davidson et al.,(1998) observed that the quality of home environment had an impact on children's behaviour which can significantly affect the results including the CBCL scores. For these reasons future studies should consider such confounders.

7.5.5 Summary and Conclusion

In summary this research provided valuable information on mercury distribution in the nine year old Pacific children which could possibly be used as a baseline or reference value for future studies. In addition, toenail mercury at low concentrations appears to affect a specific behaviour domain which is aggression. A seafood diet was also associated with mercury concentrations in this research.

Chapter 8 Study 3 – Nail Elements and its Association with Body-size

The work reported in this chapter arose from the study of mercury in toenails where the analysis of the nails was extended to include nineteen other elements. At the same time information was collected about the relative body-size of the participants. A brief overview of selected elements present in toenails and their possible association with larger body-size is provided and aims, analysis and findings of the elemental analysis of the toenails was related to measures of body-size of the children.

8.1 Introduction

As seen in the literature review presented in Chapter 5, rapid growth associated with increased body fatness in childhood is a major health concern for Pacific people. According to the (Ministry of Health, 2008a), one in four Pacific children are obese compared to one in sixteen non-Pacific children. This has also been recognised as a health issue within the Pacific Island Families (PIF) cohort (Oliver et al., 2009) and highlights the need for understanding and targeting the drivers of the obesity epidemic in Pacific children. The consequences of increased body fatness at the level of the individual (psychological and physiological effects) and community (economic costs involved) are huge. For example, in the United States the economic costs of an obese person compared to a healthy weight individual is 37% higher (Runge, 2007). The estimated direct annual cost of adult BMI >30 kg/m² was NZ\$686m (4.5%) of NZ's total health care expenditure in a 2006 study (Lal, Moodie, Ashton, Siahpash, & Swinburn, 2012).

The etiology of the high prevalence of obesity in Pacific people living in NZ is unknown but it could be related to a combination of environmental, dietary and genetic factors which in

turn can perturb metabolic pathways and the phenotype. Some elements are cofactors in metabolic pathways but they and others may also be toxic in excess. Essential and toxic elements play an important role in maintaining optimum health in people (Astrup & Bügel, 2010; Garcia et al., 2009; Garcia et al., 2012). Deficiencies or excessive (above optimum) concentrations of some elements can lead to undesirable pathological conditions that can be prevented or reversed by careful monitoring and proper nutrition (Garcia et al., 2009). No such elemental studies have been undertaken in NZ in evaluating possible concentrations of elements in human tissues or fluids with child body-size. Moreover, many overseas studies have only used blood plasma elemental concentrations, which is problematic in that: (1) the levels for most essential elements are homeostatically regulated and reflect only a small metabolic window (of hours or days in terms of food and water consumption): (2) some elements and toxins are stored in fat and are not water soluble; and (3) it is not always ethically or culturally acceptable to collect blood from children for the purposes of research.

8.2 Aims

This opportunistic cross-sectional study was nested within the PIF cohort study and aimed to measure the presence and concentrations of nineteen selected elements (magnesium, iron, manganese, calcium, zinc, copper, selenium, iodine, cobalt, chromium, nickel, molybdenum, antimony, arsenic, aluminum, boron, mercury, lead, cadmium) using as a biomarker toenails of Pacific children. Further the aim was to explore the relationships of the concentrations of these elements with measures of relative body-size taking into account ethnicity and gender.

The specific objectives of this research were to:

- (i) describe the distribution and frequency of elements and body-size in a sample of Pacific children;
- (ii) compare toenail elemental concentrations with reported concentrations from peer-reviewed published studies;
- (iii) investigate the relationship between body-size and elements in the sample of children; and to
- (iv) investigate the association of elements with gender, and ethnicity, actual body burden or status of toxins or nutrients within Pacific people.

8.3 Materials and Methods

8.3.1 Study Design and Settings

The study was undertaken between July 2010 and July 2011 when nine year old Pacific children who were part of the PIF cohort that were having their body-size measurements taken. Toenail sample collections were conducted in the school setting whilst the mothers were interviewed in the home. This was a convenience sample and thus the participants were not randomly selected but approached at the same time they were recruited into the PIF core study. The recruitment for this part is the same as study 2 (section 7.4.1)

8.3.2 Participants

All participant children were aged nine years old at the time of data collection. Not all nine year old children could be measured as this study commenced a few months after the nine year data collection phase had already started. Children who had consented but had short nails were also excluded from the study.

8.3.3 Procedure

The procedures were the same for contacting all the child participants and collecting toenail clippings as in Study 2 (details in section 7.3.3). The potential maternal participants from the PIF cohort were telephoned by a Pacific researcher in the first instance to invite them to participate, and appointments for home visits were made to discuss the study, provide information sheets and get consent. A trained Pacific researcher then administered the maternal protocol which consisted of questions for this study.

The method for toenail clippings from children was the same as 7.3.3.1 as well as the toenail chemical analysis (refer to 7.3.4)

8.3.3.1 Questionnaires

PIF Questionnaires used for this research: Validated and reliable questionnaires were administered at the nine year phase to both mother and child. These questionnaires were interviewer administered regarding socio-demographic, cultural, environmental, child development, family and household dynamics, lifestyle and health issues. Participant characteristics and demographic variables which were of interest for this cross-sectional study were included in the analysis:

- child's gender (girls, boys);
- child Pacific ethnicity (Samoans, Tongan, Cook Island, and Others (which included Tokelau and Niuean), as determined by the mother at age two years;
- socio-economic status which was categorised into annual family income bands (\$0-20,000, \$20,001-40,000, > \$40,000);

- the mother's highest educational achievement (no formal qualification, secondary school qualification, post-secondary qualification), and
- mother's current marital status (non-partnered, de-facto, and legally married).

8.3.3.2 Anthropometric Measurements

Body-size at age nine years was measured in the school setting by the PIF assessors. Prior to data collection equipment was standardised, procedures documented in an operations manual and assessors trained. The height of children without shoes was measured to the nearest 0.1 cm. The children's weight was measured, with light clothing and shoes removed, to the nearest 0.1 kg. The height and weight measurements were undertaken in duplicate and repeated a third time if not within ± 0.5 cm for height and ± 0.1 kg for weight. Equipment was standardised and all procedures were documented in an operations manual and assessors trained. The average weight and height was then calculated. Average weight and height were used to calculate children's BMI as weight (kg) divided by height squared (m^2). Since children keep growing, the BMI for children was categorised according to age and gender specific curves developed by the International Obesity Task Force (IOTF) and extrapolated to age 18 years (Table 20). The BMI category for each child was determined from age at the time of measurement in years, gender, height and weight using an Excel™ AddIn supplied by Tim Cole.

Table 20: The IOTF^a BMI category (Coles criteria)

IOTF category	kg/m²at age 18y
Thin	<18.5
Healthy Weight	<25
Overweight	25 to 30
Obese	30+

^a International Obesity Task Force

It should be noted that BMI is not a direct measure of adiposity and therefore has limited application for an individual. It is used as a tool to determine population prevalence of obesity and overweight which may be used for international comparisons.

Centres for Disease Control BMI z scores (Kuczmarski et al., 2000) were derived using the Cole AddIn continuous measure in the analysis as it is a better measure of body fatness than body-size categories and calculated using the Cole AddIn downloaded from (<http://www.healthforallchildren.co.uk/pro.epl?DO=USERPAGE&PAGE=lmsterms>) in an Excel™ spreadsheet.

8.3.4 Statistical Analysis

Similar to the previous study all data from the main PIF study and those which were obtained for this sub-study were stored in Microsoft Access™ and Excel™ (Microsoft Corporation, US) supervised by a senior biostatistician (Professor Schluter). The data was cleaned, range and consistency checks were performed, and the data coded for analysis. Questions with no response received a distinct code and were not included in the analysis. The cleaned data was

then exported to STATA version 10 (StataCorp, 2007). Chi-square test was performed to test for differences in proportions of categorical variables among two or more groups. Dot plots were plotted to identify any possible association between BMI z scores and selected elements.

Multinomial logistic regression modelling was applied to examine relationships between elements and the three ordinal categorical variables used to describe body fatness; 'healthy weight, overweight and obese'. The categories used BMI<25 (denotes healthy weight), overweight is BMI 25-30 (denotes overweight), and BMI 30+ (denotes obese). Factors such as ethnicity and gender that were significantly associated with elements using bivariate (Chi-squared) analysis and Kruskal Wallis test were included in the multinomial logistic regression. The dependent variable (body-size categories) consisted of BMI<25 for healthy weight, BMI 25-30 for overweight and BMI 30+ for obese. The BMI <25 (healthy weight) group served as the reference category.

Multinomial logistic regression modelling was applied to examine relationships between elements and the three ordinal categorical variables used to describe relative body size; 'healthy weight, overweight and obese'. The BMI <25 (healthy weight) group served as the reference category.

Specific action for the statistical analysis was:

- 1) Summary statistics for each element were calculated to include median, upper (75%) and lower (25%) quartiles and dispersion (range). This also included tabular form summaries. Descriptives were also provided for demographics and body-size which included frequencies and percentages. The main purpose of obtaining this information was to provide interpretable

and comparable information about the variables at a glance by describing the location and dispersion of the data. Additionally the descriptive statistics helped to determine the normality of the variables, which was essential to valid parametric analysis. Non-normally distributed data was log transformed to normalise the data. Spearman rank correlation tests were performed for pair-wise correlations between overall elements and each of the body-size groups.

2) The relative risk ratio (RRR) for body-size was calculated as an estimate of relative risk, according to the toenail element concentrations, using a multinomial logistic regression model. A study was also undertaken of the interaction between elements and the possible effects on body-size. It was decided that this was the analysis of choice as there are more than two categorical body-size variables (BMI <25 for healthy weight, BMI 25-30 for overweight and BMI 30+ for obese). Multinomial logistic regression analysis is a robust technique that enables classification of participants on the basis of a set of predictor variables. The procedure breaks the regression into a series of binary regressions, in a group which is then compared to the reference group. In all of the multinomial regression analyses on body-size and elements the children with BMI <25 for healthy weight served as the reference category.

3) For this part chi-squared test was used to identify any differences between variable of interest (different body-sizes) and ethnic groups and gender. Non-parametric Kruskal Wallis test was performed to identify any associations between elements and different ethnic groups, as well as gender. Multinomial logistic regression was used to investigate the relationship between body-sizes and elements after adjusting for ethnicity and gender in our model.

A probability, p value of < 0.05 and/or the degree of overlap of 95% confidence intervals determined statistical significance.

8.3.5 Ethical Considerations

The addition of this investigation of the elemental analysis of toenails within the PIF study was approved by the Northern X Ethics Committee which is a national committee based in Auckland (NTX/07/05/050) (appendix 3). Ethics was also granted for the main PIF study by Northern Y Ethics Committee (NTY/08/12/119) in Hamilton and Northern Y Ethics Committee for the body-size research (NTY/08/12/118). Information sheets were provided to both mother and child (appendix 6). Consent and assent was sought from both mother and child respectively.

All questionnaires for the main PIF study and the doctoral study were stored in locked filing cabinets in the Pacific Research Centre. All data are stored on password-protected computer systems within AUT University. Any leftover toenail samples were destroyed after the laboratory analysis. All questionnaires as part of this research will be destroyed after 10 years as required by ethics.

8.4 Results

8.4.1 Demographics

All the children were nine years of age at the time of measurement. The study population included 160 boys (58%) and 118 (42%) girls (Chapter 7, Table 13). All children had body size measurements recorded and toenail clippings analysed. The majority of the children and their mothers identified themselves as Samoans (number, $n=148$; 53%). 85% of the family household income was less than \$40,000 per annum. More than half of the children's mothers had secondary school as their highest qualifications ($n=205$; 74%).

8.4.2 Description of Body-size

Using the IOTF cut-off points for BMI, 67% ($n=176$) of the children met the criteria to be classified as being in the larger BMI of 25-30 and BMI 30+ while only 32% ($n=89$) were within the 'healthy' BMI of <25 . There was no difference in the prevalence of larger body-sizes (BMI 25-30 and BMI 30+) between boys and girls (p-value 0.33) (Table 21)

Table 21: Descriptive characteristics of children by IOTF BMI classification (Cole et al., 2000)

IOTF category	Total n (%)	Boys n (%)	Girls n (%)	P value ^a
				0.33
Healthy weight (BMI <25)	89 (32)	51 (32)	39 (33)	
Overweight (BMI 25-30)	71 (26)	36 (23)	35 (30)	
Obese (BMI 30+)	115 (41)	71 (44)	44 (38)	

^aChi squared test

Half (50%) of the Samoan children were categorised as being in the BMI of 30+ and one quarter were within the 'healthy' BMI of <25 . In the 'Others' (Cook Island and Niuean) and the Tongan groups almost half were in the 'healthy' BMI of <25 category (48% and 45%, respectively). There was a significant association between all the ethnic groups and body-sizes (p value: 0.03) (Table 22).

Table 22: Descriptive characteristics of body-sizes of children by ethnicity

		Healthy weight	Overweight	Obese	P value ^a
Ethnicity	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	0.03
Samoans	148 (53)	36 (24)	40 (27)	72 (49)	
Tongans	29 (10)	13 (45)	5 (17)	11 (38)	
Cook Island	53 (19)	18 (34)	15 (28)	19 (36)	
Others	48 (17)	23 (48)	11 (23)	13 (27)	

^aChi squared test

8.4.3 Description of Elements

There were 278 children who provided sufficient mass of toenail clipping for ICP MS analysis of essential elements: Ca, Mg, As, Co, Cr, Fe, I, Mn, Mo, Ni, Se, Sn, Zn (have a fundamental role in relation to a body function at physiological levels), non-essential: Al, B, Sb (no known fundamental role in relation to a body function at physiological levels) and toxic elements: Cd, Hg, Pb (harmful effect to the human physiology).

Frequency distribution graph for all the nineteen toenail elements reported as µg/g or mg/kg dry weight were examined (Figures 8, 9 and 10). All elements showed left skewed non-normal distributions with a few individuals exhibiting particularly high concentrations. In the case of copper (Cu), nickel (Ni) and magnesium (Mg) most of the individuals have concentrations over a narrow range.

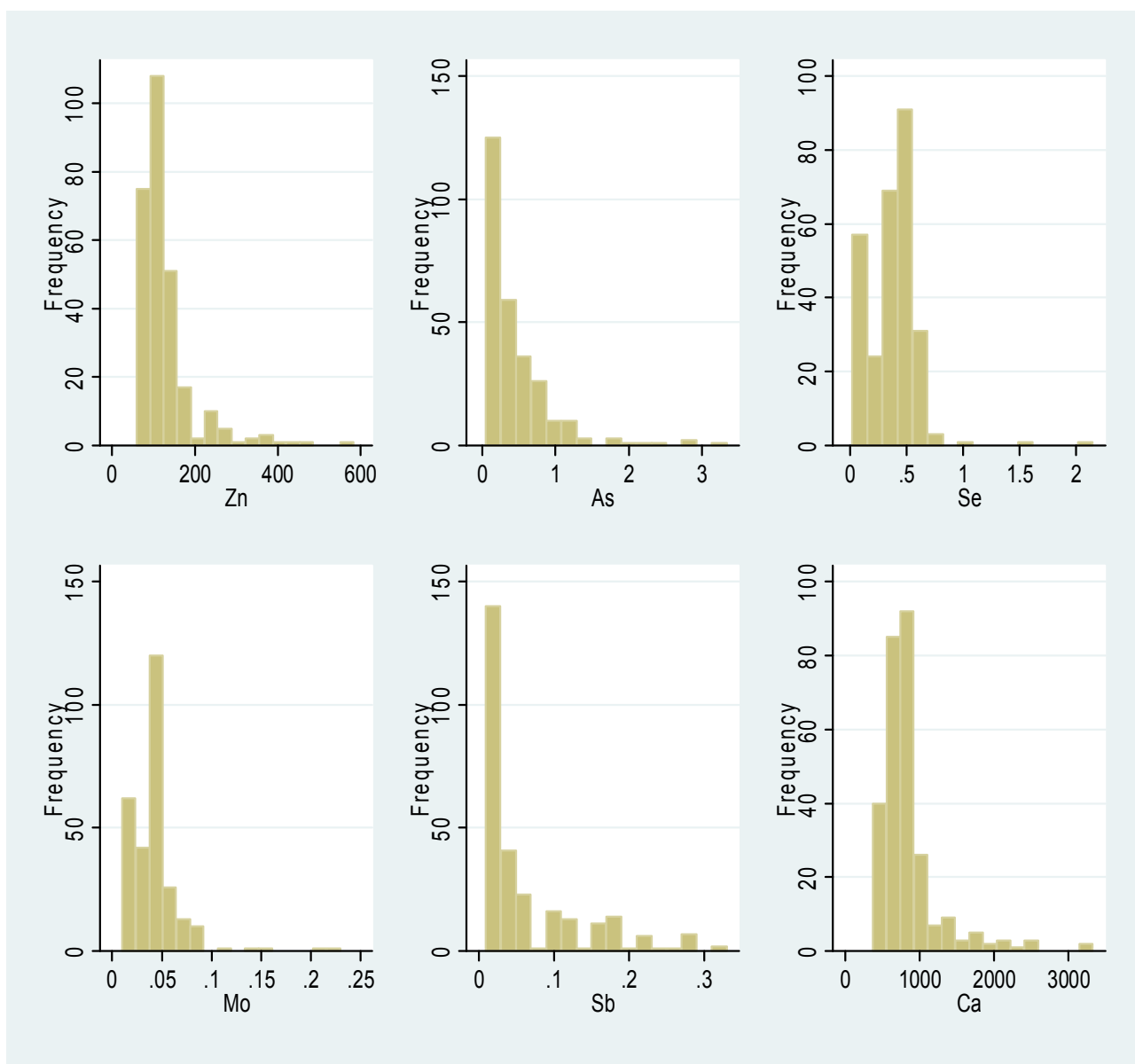


Figure 8: Frequency distribution of essential elements (Zn, As, Se, Mo, Ca) and non-essential (Sb) in nails

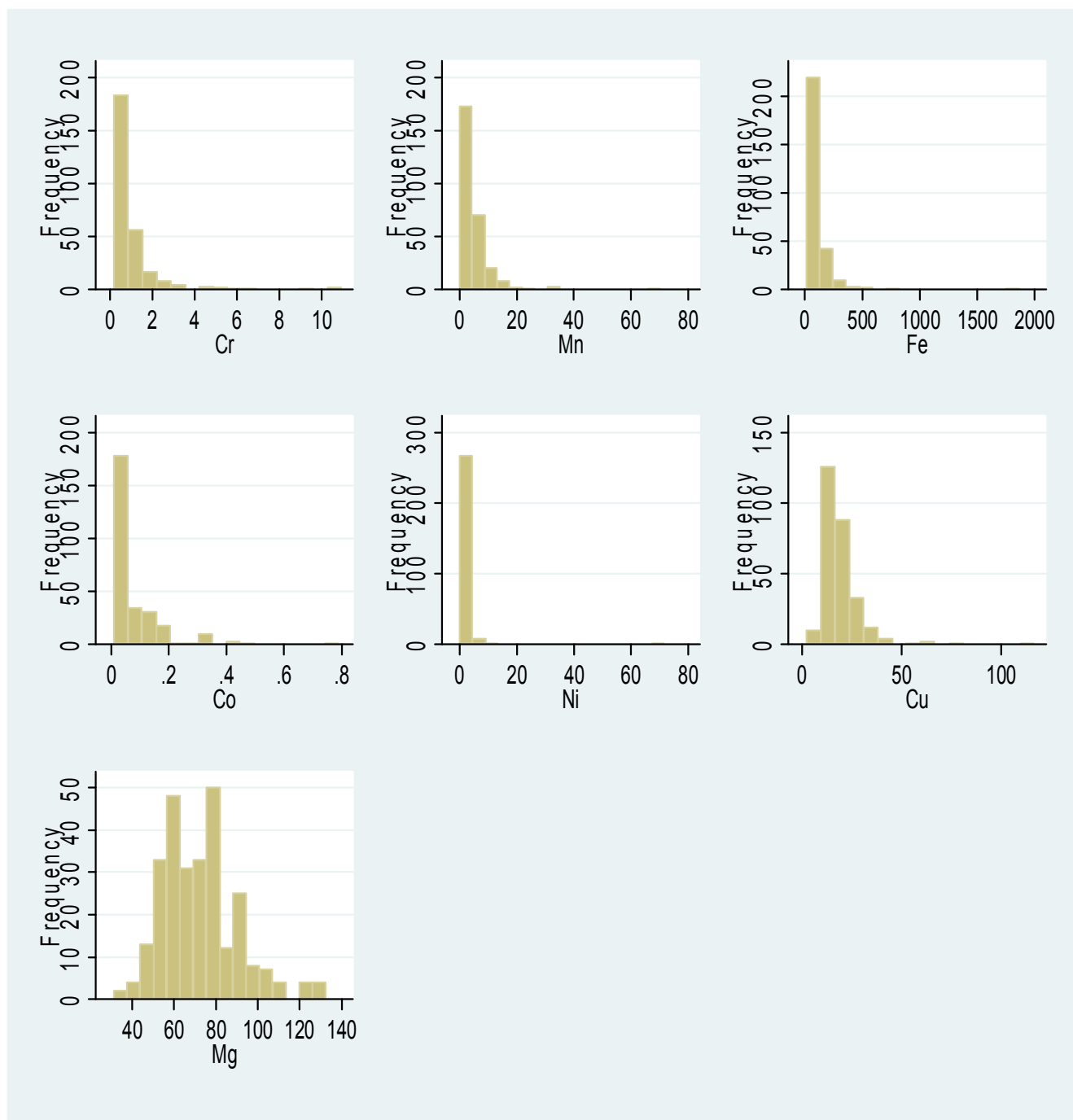


Figure 9: Frequency distribution of essential elements (Cr, Mn, Fe, Co, Ni, Cu, Mg) in nails (µg/g)

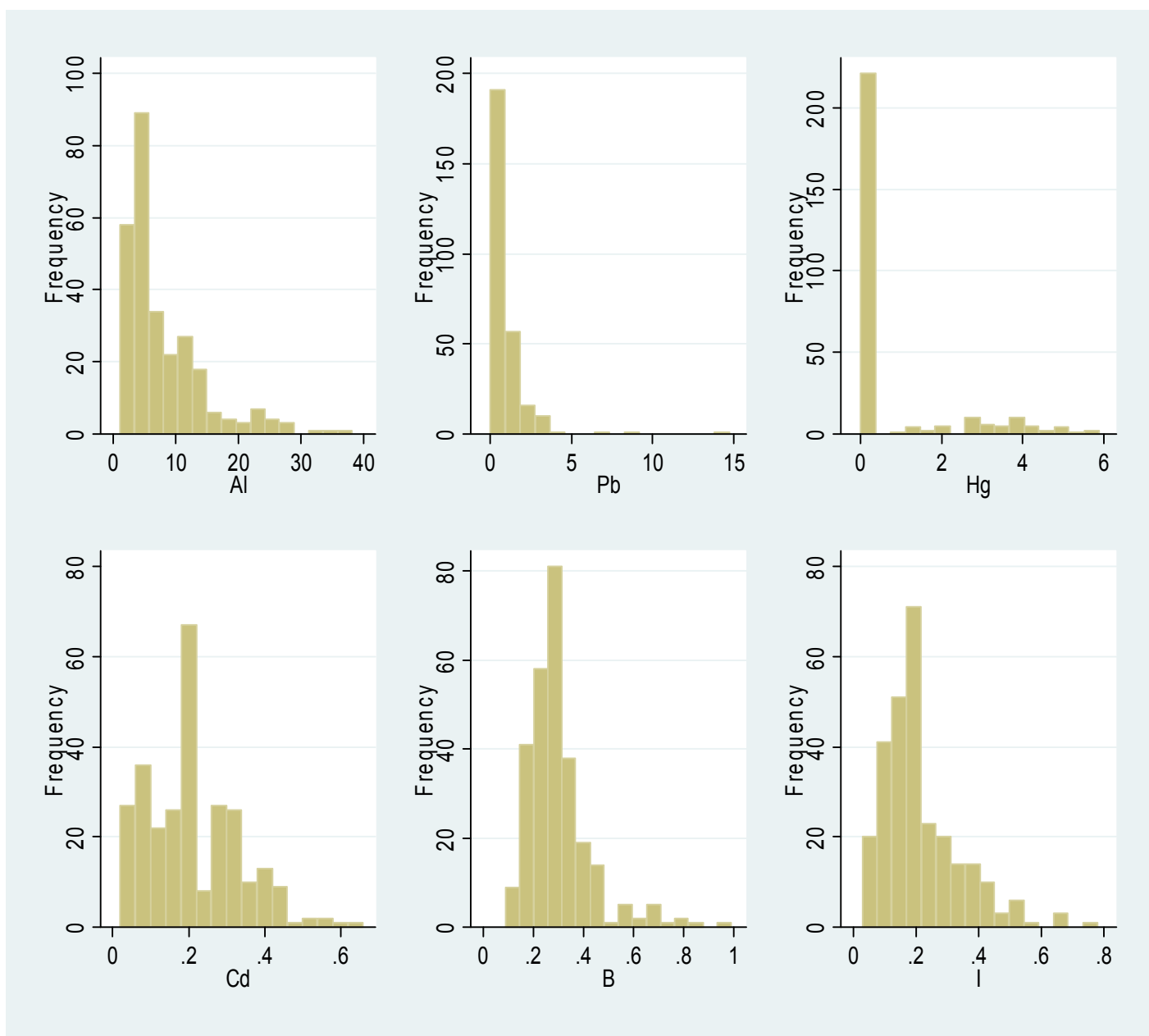


Figure 10: Frequency distribution of non-essential (Al, B), toxic (Hg, Cd, Pb), essential (I) elements in nail ($\mu\text{g/g}$)

8.4.4 Comparison of Toenail Elemental Concentrations with Reports from other Studies and Countries

Table 23 reports the elemental concentrations of this study compared with values published by (Rodushkin et al., 2000) and the references for concentrations recognised for optimal health (Ward, 2008). The 25th and 75th percentiles as well as the minimum and maximum of the distribution of the element are also provided. An important element in NZ's population is selenium which was found to be much lower than the optimal value required and also lower than the published values. Other important elements such as calcium, zinc and iron were also lower than the optimum reference values. Other elements such as arsenic, nickel and boron had lower concentrations while mercury, cadmium, lead, antimony, molybdenum, chromium, magnesium, manganese, and copper were higher than the mean for optimal health (Ward, 2008) and published values by Rodushkin et al.,(2000).

Table 23: Median, mean, minimum (min) and maximum (max) toenail concentration comparison (all mg/kg dry weight) with reported cases in other countries

Elements (µg/g)	Current study				Difference factor ^a	Published references	
	Median	(25 th , 75 th)	Mean	Min, Max		Mean reference (optimum health) ^b	Published Min, Max ^c
Calcium	776	(628, 915)	868.3	376, 3329	8.8	900	370 , 5900
Magnesium	69	(59, 80)	72.2	31 , 132	10.3	60	23, 110
Zinc	112.3	(90, 1366)	129.3	58.8 , 585.69	9.9	160	73,3080
Iron	72	(46, 121)	105.1	13.68, 1869	137	20	14,7300
Copper	17	(13, 22)	19.3	3, 117	39	8.5	9, 81
Manganese	3	(1, 6)	4.8	0.03, 70.26	2342	0.6	0.63, 3.3
Chromium	0.69	(0.47, 1.04)	1.05	0.21, 10.94	52	0.2	0.90, 6.7
Selenium	0.4	(0.26, 0.5)	0.37	0.02, 2.15	108	0.75	0.65 , 6.3
Nickel	0.33	(0.18, 0.77)	1.08	0.01, 71.84	7184	1.5	6
Arsenic	0.3	(0.12, 0.6)	0.44	0.05, 3.34	67	0.5	0.009, 2.57
Iodine	0.18	(0.13, 0.3)	0.22	0.03, 0.78	26	0.25	-
Cobalt	0.04	(0.02, 0.09)	0.07	0.01, 0.79	79	0.02	0.03, 3
Molybdenum	0.04	(0.03, 0.05)	0.04	0.01, 0.23	23	0.008	-
Antimony	0.03	(0.01, 0.09)	0.07	0.01, 0.33	33	< 0.01	0.001, 0.037
Aluminum	5.44	(3.9, 11)	8.02	1.12, 38.2	34	-	37.5
Boron	0.28	(0.2, 0.3)	0.29	0.09, 0.99	11	0.5	7, 60
Lead	0.52	(0.15, 1.1)	0.86	0.02,14.73	737	< 1.0	0.04, 240
Cadmium	0.18	(0.12, 0.29)	0.21	0.02, 0.66	33	< 0.05	0.03, 1.9
Mercury	0.02	(0.008, 0.19)	0.72	0.002, 5.88	29400	< 0.05	0.124, 2.80

^aMultiple between minimum and maximum, a measure to show how tightly it is controlled. ^b Ward (2008) ^cRodhushkin and Axelsson (2000)

8.4.5 Association of Elemental Concentrations with Body-size, Gender and Ethnicity

There were no apparent associations of body-size category and any of the elements in this sample (Table 24). To increase robustness of the tests BMI z scores were further used to determine if there might be some association with elements. The dot plots are shown in appendix 7. However, no associations were observed between BMI z scores and the selected elements.

Boys had higher toenail concentrations of Ca, Mg, Mn, Cu, Zn, Fe, Sb, As, Al, B and Pb than girls (Table 25). Other elements did not exhibit any associations between gender and elemental concentrations. Amongst the different ethnic groups, significant associations were found between the different ethnic groups and toenail elemental concentrations for Mn, Co, Fe, Cr, Sb, Al, Hg, Pb and Cd (Table 26).

Table 24: Toenail elemental concentrations by body-size

Elements µg/g	Healthy weight Median (25 th , 75 th)	Overweight Median (25 th , 75 th)	Obese Median (25 th , 75 th)	P value ^a
<i>Essential</i>				
Calcium	779 (614, 780)	729 (583, 873)	784 (673, 912)	0.23
Magnesium	69 (58, 82)	69 (58, 80)	70 (59, 80)	0.73
Manganese	4 (1.6, 6.4)	3 (1.5, 4.8)	4 (1.3, 6)	0.42
Copper	18 (13, 22)	17 (14, 21)	16 (13, 22)	0.47
Zinc	115 (88, 137)	106 (85, 133)	113 (94, 136)	0.41
Arsenic	0.33 (0.15, 0.6)	0.3 (0.1, 0.6)	0.3 (0.1, 0.5)	0.28
Selenium	0.41 (0.3, 0.5)	0.4 (0.2, 0.5)	0.3 (0.3, 0.5)	0.66
Molybdenum	0.04 (0.03, 0.05)	0.04 (0.02, 0.05)	0.04 (0.03, 0.05)	0.99
Iodine	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.2 (0.2, 0.3)	0.56
Antimony	0.04 (0.02, 0.1)	0.03 (0.02, 0.06)	0.03 (0.01, 0.09)	0.26
Nickel	0.33 (0.2, 1.1)	0.32 (0.2, 0.6)	0.33 (0.2, 0.6)	0.63
Cobalt	0.04 (0.02, 0.11)	0.04 (0.02, 0.09)	0.03 (0.02, 0.08)	0.53
Chromium	0.65 (0.44, 1)	0.8 (0.5, 1.2)	0.7 (0.5, 1)	0.51
Iron	76 (46, 121)	72 (46, 121)	70 (45, 119)	0.99
<i>Non-essential</i>				
Arsenic	0.33 (0.2, 0.6)	0.3 (0.1, 0.6)	0.26 (0.1, 0.5)	0.28
Boron	0.3 (0.23, 0.33)	0.3 (0.2, 0.3)	0.30 (0.2, 0.3)	0.33
Aluminium	6 (4, 10)	5 (4, 11)	6 (4, 11)	0.91
<i>Toxic</i>				
Mercury	0.02 (0.007, 0.2)	0.02 (0.008, 0.22)	0.03 (0.008, 0.2)	0.86
Lead	0.6 (0.3, 1.1)	0.4 (0.1, 1.06)	0.5 (0.07, 1.14)	0.86
Cadmium	0.2 (0.1, 0.3)	0.2 (0.12, 0.32)	0.2 (0.11, 0.3)	0.53

^a Kruskal Wallis test

Table 25: Toenail elemental concentrations by gender

	Boys		Girls		P-value ^a
	Median	25 th , 75 th	Median	25 th , 75 th	
<i>Essential</i>					
Calcium	804	(660, 929)	729	(601, 879)	0.03
Magnesium	71	(60, 82)	67	(57, 79)	0.03
Manganese	4.3	(2.4, 7.2)	2.12	(1.1, 4)	<0.001
Copper	18	(14, 23)	15	(13, 19)	0.001
Zinc	115	(94, 137)	107	(87,131)	0.08
Molybdenum	0.04	(0.03, 0.05)	0.04	(0.02, 0.05)	0.19
Selenium	0.4	(0.27, 0.5)	0.4	(0.2, 0.5)	0.46
Iodine	0.2	(0.1, 0.3)	0.18	(0.2, 0.3)	0.27
Cobalt	0.03	(0.02, 0.1)	0.04	(0.02, 0.1)	0.11
Iron	77	(50, 132)	59	(40, 116)	0.04
Chromium	0.71	(0.5, 1)	0.66	(0.4, 1)	0.59
Nickel	0.32	(0.2, 0.7)	0.34	(0.2, 1)	0.55
Antimony	0.04	(0.02, 0.1)	0.03	(0.01, 0.1)	0.02
<i>Non-essential</i>					
Aluminium	5.99	(4, 11)	4.71	(3, 10)	0.02
Boron	0.28	(0.2, 0.3)	0.23	(0.1, 0.3)	0.005
Arsenic	0.36	(0.2, 0.7)	0.23	(0.1, 0.5)	0.004
<i>Toxic</i>					
Mercury	0.02	(0.1, 0.2)	0.02	(0.01, 0.3)	0.41
Lead	0.62	(0.2, 1.3)	0.35	(0.05, 0.9)	0.002
Cadmium	0.18	(0.11, 0.3)	0.18	(0.12, 0.3)	0.44

^aKruskal Wallis test

Table 26: Toenail elemental concentrations ($\mu\text{g/g}$) by ethnicity (Samoan: $n=148$; Tongan: $n=29$; Cook Island: $n=52$; Others, $n=47$)

Trace elements $\mu\text{g/g}$	Samoan Median (25 th , 75)	Tongan Median (25 th , 75 th)	Cook Island Median (25 th , 75 th)	Others ^a Median (25 th , 75 th)	P-value ^b
<i>Essential</i>					
Calcium	774 (620,917)	809 (701,912)	776 (628, 886)	772 (621, 991)	0.90
Magnesium	69 (59, 80)	74 (65,88)	70 (59, 79)	72 (60, 83)	0.59
Manganese	3 (1.3, 6)	4 (2.6, 8.3)	3 (2.3,6.4)	2 (1.2, 5)	0.03
Copper	17 (13, 23)	17 (12, 22)	17 (14, 22)	16 (13, 21)	0.81
Zinc	112 (88, 136)	118 (99, 136)	109 (94, 126)	110 (88, 142)	0.85
Selenium	0.4 (0.3, 0.5)	0.3 (0.1, 0.5)	0.4 (0.3, 0.5)	0.4 (0.2, 0.5)	0.95
Iodine	0.2 (0.2, 0.3)	0.2 (0.1, 0.3)	0.2 (0.1, 0.2)	0.2 (0.13, 0.3)	0.75
Cobalt	0.04 (0.02,0.09)	0.05 (0.03, 0.12)	0.04 (0.03, 0.08)	0.03 (0.02, 0.1)	0.04
Iron	66 (42, 107)	95 (59, 178)	78 (51, 127)	62 (41, 110)	0.01
Chromium	0.8 (0.5, 1.2)	0.7 (6, 1)	0.6 (0.5, 0.7)	0.7 (0.5, 1.4)	0.05
Nickel	0.3 (0.2, 0.8)	0.5 (0.3, 1)	0.3 (0.2, 0.8)	0.3 (0.2, 0.5)	0.35
Molybdenum	0.04 (0.03, 0.05)	0.04 (0.02, 0.06)	0.04 (0.03, 0.05)	0.04 (0.03, 0.05)	0.63
Antimony	0.04 (0.02, 0.1)	0.04 (0.02, 0.1)	0.03 (0.01, 0.04)	0.04 (0.01, 0.07)	0.02
Arsenic	0.3 (0.12, 0.6)	0.2 (0.1, 0.4)	0.3 (0.1, 0.6)	0.4 (0.2, 0.8)	0.39
<i>Non-essential</i>					
Aluminium	5.3 (3.5, 9)	9 (5, 14)	6 (4, 12)	4 (4, 10)	0.01
Boron	0.3 (0.2, 0.3)	0.3 (0.2, 0.3)	0.3 (0.2, 0.3)	0.3 (0.2, 0.3)	0.97
<i>Toxic</i>					
Mercury	0.02 (0.008, 0.17)	0.04 (0.008, 2.2)	0.02 (0.008, 0.2)	0.02 (0.008, 0.2)	0.02
Lead	0.6 (0.2, 1.3)	0.7 (0.3, 1.2)	0.4 (0.1, 0.7)	0.5 (0.05, 0.95)	0.01
Cadmium	0.2 (0.1, 0.3)	0.2(0.09, 0.22)	0.2 (0.2, 0.3)	0.2 (0.09, 0.3)	0.03

^aEuropean and Niuean; ^bKruskal Wallis test

8.4.6 Spearman rho correlation between Elements

Strong positive associations (Spearman's rho) were observed between Ni-Co (rho 0.68), Se-Mo (rho 0.86), Mn-Al (rho 0.64), Al-Fe (rho 0.93), Ca-Zn (rho 0.96), Mg-Zn (rho 0.88), Ca-Mg (rho 0.92), Hg-I (rho 0.78), Cu-B ho (0.90) (Table 28). Moderate correlations were obtained for Sb-Cu (rho 0.56) and Pb-Cu (rho 0.60) (Table 28).

Pair-wise correlations between each body-size group (BMI <25 for 'healthy' weight, BMI 25-30 for 'overweight' and BMI 30+ for 'obese') were also studied and are presented in Table 27. Ca-Mg were correlated highly in both the healthy weight and overweight groups (rho value of 0.92 and 0.95, respectively) but not for the obese group. Al-Fe was highly correlated for both the 'healthy' weight and 'overweight' groups (rho value 0.95 and 0.96 respectively) and moderately for the 'obese' groups (rho value 0.89). Hg-I was highly correlated in the 'healthy' weight group (0.81) and had a low correlation coefficients for the 'overweight' and 'obese' children groups (rho value 0.72 and 0.79, respectively). Se-Mo was moderately correlated in all the body-size groups. Zn-Ca was highly correlated in all the groups. Ca-Mg in 'healthy' weight and 'overweight' was highly correlated but not in the 'obese' group.

*Table 27: Correlations (rho values) of elements in each body-size groups (**healthy weight (n=89)**, **overweight (n=71)** and **obese (n=115)**)*

Elements	Healthy Weight	Overweight	Obese
Ca-Mg	0.915	0.949	0.781
Zn-Mg	0.908	0.921	0.831
Ca-Zn	0.972	0.964	0.936
Al-Fe	0.959	0.946	0.893
Mo-Se	0.894	0.855	0.566
Pb-Sb	0.893	0.351	0.343
B-Cu	0.864	0.250	0.569
I-Hg	0.809	0.655	0.554
Mo-Se	0.894	0.403	0.828
Pb-Sb	0.893	0.649	0.937
B-Cu	0.864	0.869	0.231
I-Hg	0.809	0.451	0.450

Table 28: Pairwise correlations (rho values) between elements within the whole sample ($n=278$)

	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Sb	Pb	Hg	Al	Ca	Mg	I	B
Cr	1.00																		
Mn	0.26	1.00																	
Fe	0.47	0.68	1.00																
Co	0.02	0.05	0.22	1.00															
Ni	0.10	0.12	0.27	0.68	1.00														
Cu	0.18	0.47	0.37	0.17	0.24	1.00													
Zn	0.16	0.12	0.03	-0.06	-0.01	0.35	1.00												
As	0.25	0.38	0.28	0.05	0.14	0.35	-0.01	1.00											
Se	-0.06	0.04	-0.08	-0.19	-0.13	-0.06	-0.11	0.29	1.00										
Mo	0.00	0.07	-0.04	-0.14	-0.07	-0.02	-0.08	0.28	0.86	1.00									
Cd	-0.14	-0.15	-0.12	-0.06	-0.08	-0.31	-0.80	-0.01	0.19	0.12	1.00								
Sb	0.27	0.41	0.42	0.20	0.25	0.56	0.18	0.36	-0.10	-0.01	-0.18	1.00							
Pb	0.26	0.42	0.41	0.17	0.22	0.60	0.20	0.32	-0.13	-0.02	-0.18	0.92	1.00						
Hg	0.02	-0.03	0.07	0.18	0.13	0.06	0.09	-0.24	-0.90	-0.81	-0.19	0.06	0.08	1.00					
Al	0.42	0.64	0.93	0.20	0.29	0.38	0.05	0.28	-0.10	-0.02	-0.15	0.40	0.39	0.09	1.00				
Ca	0.13	0.14	0.04	-0.06	0.00	0.36	0.96	-0.02	-0.11	-0.08	-0.76	0.18	0.20	0.09	0.06	1.00			
Mg	0.16	0.13	0.06	-0.08	0.00	0.31	0.88	0.03	-0.07	-0.03	-0.72	0.20	0.18	0.06	0.08	0.92	1.00		
I	0.07	-0.06	0.05	0.09	0.04	0.02	0.07	-0.22	-0.84	-0.75	-0.14	0.10	0.13	0.78	0.05	0.07	0.04	1.00	
B	0.17	0.43	0.33	0.05	0.17	0.90	0.33	0.35	-0.02	0.01	-0.31	0.49	0.52	0.02	0.36	0.33	0.29	-0.01	1.00

8.4.7 Relationship between Elements and Body-size

Using an unadjusted multinomial logistic regression model there was no substantial association between risk of large body-size and toenail elemental concentrations for all the elements listed in Table 29.

Table 29: Risk of body-size category according to toenail concentrations of elements

Log transformed	Healthy weight vs Overweight		Healthy weight vs Obese		
<i>Essential</i>	RRR ^a (95% CI)		RRR ^a (95% CI)		P-value ^b
Calcium	0.75	(0.31, 1.80)	1.03	(0.49, 2.19)	0.73
Selenium	0.71	(0.66,1.32)	0.86	(0.75,1.40)	0.86
Magnesium	0.75	(0.21, 2.76)	1.31	(0.42, 4.12)	0.67
Manganese	0.84	(0.63, 1.11)	0.88	(0.69, 1.14)	0.43
Copper	0.88	(0.42, 1.86)	0.73	(0.37, 1.43)	0.65
Zinc	0.78	(0.35,1.77)	0.99	(0.49, 1.98)	0.80
Iodine	1.22	(0.71, 2.09)	1.33	(0.82, 2.16)	0.46
Cobalt	0.99	(0.72,1.35)	0.85	(0.64,1.13)	0.46
Iron	0.95	(0.62, 1.44)	1.002	(0.69, 1.46)	0.96
Chromium	1.29	(0.83, 2.10)	1.23	(0.76, 1.69)	0.52
Nickel	0.86	(0.67, 1.10)	0.87	(0.69, 1.08)	0.37
Molybdenum	0.96	(0.60, 1.54)	1.05	(0.69, 1.60)	0.93
Antimony	0.85	(0.64, 1.14)	0.83	(0.64, 1.07)	0.33
Arsenic	0.96	(0.69,1.33)	0.79	(0.58, 1.05)	0.24
<i>Non-essential</i>					
Aluminium	0.98	(0.64, 1.53)	1.11	(0.75, 1.63)	0.81
Boron	0.81	(0.34,1.91)	0.66	(0.31, 1.42)	0.57
<i>Toxic</i>					
Mercury	1.03	(0.91, 1.19)	1.01	(0.89, 1.14)	0.87
Lead	0.98	(0.79, 1.22)	0.91	(0.77, 1.11)	0.66
Cadmium	1.14	(0.74, 1.77)	0.95	(0.65, 1.37)	0.66

^aRelative risk ratio ^bmultinomial logistic regression test

Multinomial logistic regression analysis, performed to account for any potential confounding by gender and by ethnicity (Table 30 and Table 31 respectively) shows very wide confidence intervals and all had one in the confidence interval showing no apparent risk (Table 30 and 31). So therefore, for all children measured, it was observed that the odds of being in the

larger BMI after adjusting by ethnicity and gender was not related to elemental concentration of toenails.

Table 30: Risk of body-sizes according to toe-nail concentrations ($\mu\text{g/g}$) of elements after adjusting for gender

	Healthy weight vs Overweight		Healthy weight vs Obese		
Log transformed	RRR ^a	(95% CI)	RRR ^a	(95% CI)	P-value ^b
<i>Essential</i>					
Calcium	0.77	(0.32, 1.9)	1	(0.47, 2.1)	0.62
Magnesium	0.81	(0.21, 2.9)	1.2	(0.4, 4)	0.61
Manganese	0.85	(0.63, 1.14)	0.84	(0.6, 1.1)	0.41
Copper	0.92	(0.43, 1.9)	0.69	(0.44, 1.4)	0.49
Zinc	0.83	(0.35, 1.8)	0.85	(0.5, 1.9)	0.64
Selenium	0.94	(0.67, 1.3)	1	(0.7, 1.4)	0.66
Iodine	1.2	(0.7, 2.1)	1.34	(0.83, 2.1)	0.45
Cobalt	0.98	(0.71, 1.3)	0.86	(0.65, 1.14)	0.47
Iron	0.97	(0.63, 1.5)	0.98	(0.7, 1.4)	0.69
Chromium	1.3	(0.83, 2.02)	1.2	(0.75, 1.7)	0.46
Nickel	0.85	(0.66, 1.09)	0.87	(0.68, 1.1)	0.37
Molybdenum	0.98	(0.6, 1.6)	1	(0.67, 1.6)	0.67
Antimony	0.86	(0.64, 1.2)	0.81	(0.62, 1.1)	0.32
Arsenic	0.97	(0.69, 1.4)	0.76	(0.6, 1.03)	0.20
<i>Non-essential</i>					
Aluminium	1	(0.64, 1.6)	1	(0.74, 1.6)	0.65
Boron	0.84	(0.34, 1.9)	0.63	(0.3, 1.4)	0.47
<i>Toxic</i>					
Mercury	0.82	(0.3, 2.9)	1.2	(0.4, 3.9)	0.61
Lead	0.99	(0.8, 1.2)	0.91	(0.75, 1.1)	0.48
Cadmium	1.1	(0.73, 1.7)	0.95	(0.66, 1.4)	0.58

^aRelative risk ratio; ^bmultinomial logistic regression test

Table 31: Risk of large body-size according to toenail concentrations ($\mu\text{g/g}$) of elements after adjusting for ethnicity

	Healthy weight vs Overweight		Healthy weight vs Obese		
Log transformed	RRR ^a	(95% CI)	RRR ^a	(95% CI)	P-value ^b
<i>Essential</i>					
Calcium	0.75	(0.31, 1.8)	1	(0.48, 2.3)	0.23
Magnesium	0.79	(0.6, 1)	1.4	(0.44, 4.6)	0.20
Manganese	0.83	(0.62, 1.09)	0.87	(0.67, 1.12)	0.11
Copper	0.84	(0.39, 1.8)	0.68	(0.34, 1.4)	0.64
Zinc	0.77	(0.34, 1.8)	0.97	(0.48, 1.9)	0.28
Selenium	0.93	(0.66, 1.33)	1.02	(0.74, 1.4)	0.30
Iodine	1.19	(0.69, 2.1)	1.3	(0.78, 2.1)	0.21
Cobalt	0.97	(0.71, 1.3)	0.83	(0.63, 1.1)	0.11
Iron	0.95	(0.62, 1.5)	1.02	(0.69, 1.5)	0.76
Chromium	1.3	(0.81, 1.9)	1.07	(0.71, 1.6)	0.13
Nickel	0.85	(0.66, 1.09)	0.85	(0.68, 1.07)	0.54
Molybdenum	0.94	(0.58, 1.5)	1.01	(0.66, 1.6)	0.34
Antimony	0.81	(0.5, 1.1)	0.76	(0.58, 0.99)	0.08
Arsenic	0.97	(0.69, 1.3)	0.78	(0.6, 1)	0.77
<i>Non-essential</i>					
Aluminium	0.99	(0.64, 1.5)	1.13	(0.76, 1.68)	0.43
Boron	0.77	(0.33, 1.8)	0.62	(0.28, 1.4)	0.57
<i>Toxic</i>					
Mercury	1.03	(0.9, 1.2)	1.01	(0.89, 1.14)	0.30
Lead	0.94	(0.75, 1.2)	0.86	(0.71, 1.05)	0.28
Cadmium	1.14	(0.74, 1.8)	0.95	(0.65, 1.4)	0.22

^aRelative risk ratio; ^bMultinomial logistic regression test

8.4.8 Unadjusted Elemental Interactions and their Association to Different Body-sizes

Some of the elemental pairs that are known to interact with each other as a variable (Blaurock-Busch, Amin, & Rabah, 2011; Peraza, Ayala-Fierro, Barber, Casarez, & Rael, 1998; Tascilar et al., 2010) were analysed to investigate the relationship between known elemental pairs and the different body-size categories. There was some evidence that the interaction between Se-Hg as well as Zn-Cu pairs is related to the risk of being in the BMI

25-30 or BMI 30+ category compared to BMI <25 (healthy weight) (Table 32). Other elemental interactions did not show such associations to different body-sizes.

Table 32: Toenail elemental interactions and their relationship with different body-sizes

Log transformed Elements	Healthy weight vs Overweight		Healthy weight vs Obese		P-value ^b
	RRR ^a	(95% CI)	RRR ^a	(95% CI)	
Se -Hg	1.00	(0.79, 1.26)	1.00	(0.88, 1.35)	0.03
Cd-Cu	0.48	(0.17, 1.41)	0.92	(0.35, 2.40)	0.65
Se-I	0.91	(0.38, 2.18)	1.30	(0.59, 3.02)	0.49
Zn-Cd	0.62	(0.26, 1.46)	1.27	(0.55, 2.95)	0.71
Zn-Pb	1.00	(0.55, 1.86)	0.63	(0.38, 1.06)	0.47
Zn-Cu	6.06	(0.87, 2.02)	0.79	(0.12, 5.08)	0.02
I - Se	0.90	(0.38, 2.18)	1.33	(0.59, 3.02)	0.49

^aRelative risk ratio; ^bmultinomial logistic regression test

8.5 Discussion

This study, the first cross-sectional study of Pacific children in NZ which measured the concentration of nineteen elements in toenails, has shown, in a group of nine year old Pacific children, where three out of four children were either overweight or obese, that there was no apparent association between the concentration of any element and body-size category. The lack of association persisted after controlling for other known factors including ethnicity, income and gender. However, statistically significant associations were found on some elemental interactions and body-size categories.

8.5.1 Distribution of Elements in this Study

The distributions of elements within the sample were all left skewed. This is not surprising as nail material is not homeostatically regulated (as is blood) and the elemental concentrations reflected in the portion of the nail sampled would equate to the formation of the nail of 6 to 12 months. Therefore environmental and dietary exposure to the element may not reflect the

usual exposure. Moreover, each individual's toenail material may grow at a slightly different rate. However, the distribution shown for these elements confirms that the majority of the study population has similar concentrations with a few cases having higher (left skewed) elemental concentrations. This may relate to: (1) excessive dietary uptake of that element; (2) a change in the metabolism resulting in the additional release or 'excretion' of the element; or (3) possibly an environmental source of exposure. The effect of recent surface exposure was minimised because all toenail material was washed prior to analysis to remove exogenous material. In the case of arsenic (As) and antimony (Sb) it is proposed that environmental exposure is the best explanation, and possible exogenous sources are exposure to cigarette smoking or residence in a house with old paint or wallpaper coverings, soil contamination or dietary ingestion (Chung et al., 2013; Cooper & Harrison, 2009; Miklavcic et al., 2013; Wong, Chung, Chan, Ho, & Xiao, 2013). For zinc (Zn), calcium (Ca), selenium (Se) and molybdenum (Mo) diet and metabolism may be possible factors associated with increased deposition in the toenail material.

The observation of relatively low selenium concentrations in the samples is supported by other NZ studies who found serum selenium concentrations to be low (Duffield, Thomson, Hill, & Williams, 1999; C. Thomson, 2004; C. Thomson, Chisholm, McLachlan, & Campbell, 2008). This is possibly related to relatively low levels of selenium in the food supply due to low selenium in soil (Combs & Lu, 2001; Vanoort & Thomson 2011). For example whole wheat bread from the US contains selenium levels of 2 mg of selenium per kg whereby NZ breads contain only 0.1mg of selenium per kg (Combs, 2001). Furthermore, there is a difference in selenium levels between NZ North Island bread (0.111 mg/kg) and South Island bread (0.026 mg/kg) (Vanoort & Thomson 2011).

For lead (Pb), mercury (Hg), and cadmium (Cd) concentrations were higher than previously published values (Rodushkin et al., 2000) and the optimal value required for health (Ward, 2008). Possible exposures to cadmium may have been from second-hand tobacco smoke: mercury from eating fish with high mercury concentrations and lead from old paint in their home. These exposures were not measured.

The concentrations of toenail calcium in the children in this study were lower than the published values (Rodushkin et al., 2000) and recommended optimal health values (Ward, 2008). Calcium is important for strong and healthy bone development (Heaney, Abrams, Dawson-Hughes, & al., 2000). Many studies have shown that low calcium is observed in children who do not consume enough milk (David, Waddington, & Stanton, 1984; Devlin, Stanton, & David, 1989; Henriksen, Eggesbro, Halvorsen, & Botten, 2000). In NZ a few studies have shown a higher prevalence of low calcium in children (R. E. Black, Williams, Jones, & Goulding, 2002) particularly Pacific children which reflects low consumption of dairy products (Ministry of Health, 2003; Parnell, Scragg, Wilson, Schaaf, & Fitzgerald, 2003). Perhaps increasing traditional foods such as fish (which is high in calcium) could be more appropriate if dairy products are not consumed within these study children. Calcium has been also shown to reduce body-fatness hence it is an important component (National Health and Medical Research Council, 2003).

The findings of low toenail zinc concentrations (compared with the optimum reference value and published value) is concordant with previous findings in NZ and international studies which have demonstrated the existence of ongoing zinc deficiencies in school-age children (Gibson, Bailey, Parnell, Wilson, & Ferguson, 2011). If low concentrations of zinc in toenails are associated with low concentrations in the body this would have a far-reaching

implication. Zinc is a co-factor for a number of enzymes, needed for normal growth and development of sensory and immune functions (Hambidge, 1997, 2000). A recent study found that Pacific children are more likely to be zinc deficient than NZEO children when serum zinc levels were measured (Gibson et al., 2011). It is documented that Pacific children reach puberty earlier and are taller for their age than European children (Gibson et al., 2011; Gordon, Ferguson, Toafa, & al., 2003). Therefore it is possible that their zinc requirements may be higher than their European counterparts. Several studies have documented the effects of low zinc concentrations and morbidity from infectious diseases and poor cognitive development (M. M. Black, 1998).

The concentration of copper in toenails in this study for 197 out of 278 children exceeded the optimal reference range yet zinc concentrations were not high. Other research with autistic children have also reported that low zinc concentrations was associated with higher concentrations of copper (Faber, Zinn, & Kern, 2009). It is recognised that copper and zinc have both nutritive as well as antagonistic effects (Blaurock-Busch et al., 2011). Low concentrations of zinc can exasperate copper toxicity (Blaurock-Busch et al., 2011). However, more research is required to confirm this association.

Differences between boys and girls in elemental concentrations of lead, arsenic, aluminium, antimony, copper, manganese, magnesium and calcium were observed (Table 25). This is supported by other studies which found that low serum iron concentrations are higher in girls than boys due to pubertal onset or early menarche (Moayeri, Bidad, Zadhoush, Gholami, & Anari, 2006). While zinc concentrations have been found to be lower in boys possibly due to their larger body-sizes than the girls (Gibson, Skeaff, & Williams, 2000). Boys were slightly

heavier than the girls in this research which could mean that body fat and adiposity may be leading to a greater risk of lower elemental concentrations.

When the children's specific ethnicity was considered within this study of elemental concentrations, toxic elements differed significantly (Scheffe's test) between the Tongan and Samoan ethnic groups. This may be because of the differences in socio-economic status between the groups. Tongan people have lower family income than Samoans (Blakely et al., 2009). Living in poor residential areas may increase the chances of toxic element absorptions due to living in poorer homes close to motorways (Tong, von Schirnding, & Prapamontol, 2000).

The determination of child ethnicity in the present research was by parent prioritisation and Pacific people have a range of genealogical backgrounds/admixtures of ethnicity including German and European and varying acculturation in NZ (Schluter, Tautolo, & Paterson, 2011). In contrast, an international study did not observe larger variance differences between different types of biomarkers (such as elements in blood serum, plasma and hair) within ethnic groups (non-Hispanic white, non-Hispanic black, or Mexican American) (Kant & Graubard, 2008). But other studies have shown elemental absorption as being complex and highly influenced by the individual metabolism and their dietary intakes (Goldhaber, 2003; Nelson, 2000). Therefore, it is important to understand each of those elements in relation to individuals and make inferences based on that.

International studies have also shown that differences in toxic elements among groups of people could be related to residence in highly polluted areas, houses that have poor ventilation and lifestyle patterns such as high seafood diet or tobacco smoking (Bernstein et

al., 2008; Oken & Bellinger, 2008). These and other factors influence the accumulation of elements in toenails. There is some evidence that early life exposure to toxic elements such as mercury and lead can cause inflammation (Bougle et al., 2009) which might predispose for weight gain later in life. However, this is a limitation of the study as there is no information on mothers' eating habits during gestation; the mother's elemental concentrations have not been measured before or after the children were born and cannot be related to current growth patterns in these children. However, within the PIF study, it has been observed that babies who are born larger become even larger in later years and Pacific babies are generally larger at birth (Rush et al., 2010).

There are various interventions for reducing or preventing low concentrations of elements and it is usually by fortification or supplementation (World Health Organisation, 2010).

Fortifications programmes internationally and nationally have been successful in reducing the deficiency of certain specific elements (such as folate, iron, vitamin D, iodine) (R. J. Berry, Bailey, Mulinare, & Bower, 2010; Bower, 2003, 2007; Bower & Wald, 1995; Pfeiffer et al., 2012). However, the risk of toxicity for some elements is possible and thus health officials, manufacturers and policy-makers try to balance the deficiencies and toxicity risk in populations (L' Abbe, Cockell, & Lee, 2003). Studies have shown that children who took supplementation had better baseline intake of elements from their food sources than those who did not take supplements (Bailey, Fulgoni, Keast, & Dwyer, 2011). Perhaps Pacific children will benefit from supplementation but this effect is not known in NZ Pacific children.

8.5.2 Elemental Concentrations in Relation to Body-size – Comparison to Literature

Limited literature has investigated the association of elements and childhood body-size except for two recent international studies (Table 33) (Bougle et al., 2009; Tascilar et al., 2010), although, these two studies had some methodological flaws. The study by Tascilar et al., (2010) had a very small size of 33 healthy weight and 34 obese children with the children's mean age of 10.6 years. They noticed vanadium and cobalt were lower in obese children. In contrast no such association was observed in the current study. The other study by Bougle et al., (2009) investigated children between the ages of 2 to 17 years on body-size and trace elements. Their sample size was larger with 209 obese but only 33 children as the control which reduces the likelihood of finding a real difference. They did not find a relationship with any elements but did find differences in two measures of inflammation: fibrinogen and sedimentation rates. This is consistent with the current study in that no association was found with elements in nails and body-size.

Compared with this study, the two other studies (Table 33) on elemental concentrations were measured using different samples for analysis of elemental biomarkers. Plus the reliability of biomarkers or the analytical methods that were employed for the analysis in those studies have not been reported which makes the interpretation and comparison of their data even more difficult. There are many difficulties in the comparison of the results of the present study with international studies due to differences in biomarker material sampled, age groups, health conditions and geographical/environmental areas of residence. For example people living in areas close to traffic where toxic pollution may be high, tend to have higher toxic

elemental concentrations in their body compared to those living in non-traffic congested areas (Hrubá et al., 2012; Järup, 2003; Patriarca, Menditto, Rossi, Lyon, & Fell, 2000; Ward, 1989).

Table 33: Number of children recruited and type of biomarker used for elements and body-sizes

Study	Biomarker	Study type	Age	Measurements of obesity	Sample size (<i>n</i>)
Karatela et al., (2012)	Toenails	Cross-sectional	9	BMI z scores-IOTF classification	278
Tascilar et al.,(2010)	Serum	Case-control	10	BMI z scores-IOTF classification	67
Bougle et al.,(2009)	Plasma	Case-control	2-17	BMI, BMI z-scores, fat free mass	242

Furthermore, Tascilar et al., (2010) did not find an association between toxic elemental concentrations and body-sizes which is similar to the present study in that the unadjusted and adjusted (for ethnicity and gender) analyses did not show that toxic elements had an association with body-size categories of the children.

Associations were found when Hg-Se and Zn-Cu interactions were considered as a factor in the multinomial logistic regression analysis of relationships with body-size categories. These interactions (and not just single elements) appeared to have an association with body-sizes. As mentioned earlier selenium has a protective effect on mercury; however, selenium concentrations were lower in these children and possibly increased the effects of mercury, perhaps leading to body fatness but further research is required to understand these effects. Zinc was lower while copper was slightly higher in concentrations again possibly leading to body fatness. Another aspect noted was that the ‘healthy weight’ group had higher correlations between elements than the ‘overweight’ or ‘obese’ group. These are new

findings not seen in the literature and further research is required to understand these relationships within children.

8.5.3 Limitations and Strengths

A clear limitation of the study is our inability to validate or compare the toenail biological marker from this sample. There is no recognised reference range for toenail elements or association with health within Pacific people. The study was cross-sectional which precluded the evaluation of temporality or causality of any associations. Toenail biomarker measurements can be subject to biologic variability, sampling error and possible measurement errors, although care was taken to prevent such errors.

This study had strengths, such as the representation of Pacific ethnic groups in NZ and a reasonable sample size, valid and reliable measurement of body-sizes and valid/reliable methods for the elemental analysis of toenail samples. In terms of the direct and sensitive measurement of biological material, toenails were used which is more valid than other indirect measures such as exposure to pollutants using questionnaires. This prevents Type II error from occurring. Although questionnaires are relatively easy to implement and less controversial, there are numerous sources of random and systematic errors that can occur (Burrows et al., 2009; Molag et al., 2007).

8.5.4 Summary and Conclusion

In conclusion, this study has provided evidence that for these Pacific children at age nine years there is no apparent association between concentrations of elements in toenails and body-size category accounting for small differences by ethnicity and gender. However, calcium and selenium concentrations were lower in the children with slightly higher

concentrations of toxic elements. When elemental interactions such as Hg-Se and Zn-Cu were considered, an association was observed with body-size categories. This research has implications for the general NZ population as well as Pacific people.

This study has shown that the collection of samples of toenails from Pacific children aged nine years is feasible. However, for the purposes of future studies, it is recommended to validate toenail clippings within the Pacific population by using other kinds of biomarkers such as saliva/sweat. This part of the doctoral study may help to develop culturally sound and appropriate approaches to further investigations using biomarkers with Pacific people.

Chapter 9 Overall Thesis Discussion and Conclusion

This thesis sought to explore for the first time the possible association of elemental concentrations (calcium, magnesium, manganese, copper, zinc, selenium, iodine, cobalt, iron, chromium, nickel, molybdenum, antimony, arsenic, aluminum, boron, mercury, lead, cadmium), as determined in biological samples (hair and toenail clippings) on behavioural problems and body-size within Pacific children, resident in Auckland, New Zealand. A relatively new and sensitive method of analysis, inductively coupled plasma mass spectrometry (ICP MS) was used. The first study explored the association of hair mercury on behavioural problems and the presence of dental amalgam fillings. There was no association. However, the next investigation of mercury in toenails demonstrated a moderate association with a specific behavioural domain; aggressive behaviour. The final exploration on nineteen elements in toenails with current body-size categories suggested that elemental interactions such as zinc-copper and mercury-selenium seemed to influence the body-size categories in these children even though single elements did not show any associations on body-size categories.

The overall discussion of this thesis is structured to provide an overview of the research and thesis findings and place them within the wider public health and epidemiological research. Strengths, limitations, significance and implications of the findings are further discussed.

9.1 Summary of Overall Research

Within this thesis, toenail clippings and scalp hair samples were used to assess elemental concentrations of the children. Both hair and toenail biomarkers provide a good measure of long-term exposure to elements as opposed to blood or urine which reflect the exposure over a daily time period (Goyer & Clarkson, 2001; He, 2011). However, the choice of biomarkers depends on feasibility within a population along with reliability and validity of that biomarker. The initial choice for this doctoral research was scalp hair samples as it was deemed more appropriate and many researchers have used hair samples as biomarkers for elemental assessments of children. This in part is due to the ease of sample collection and it is a non-invasive method when compared with blood sample collection. However, hair samples were not ideal for this population thus toenail clippings were used for the remainder of the studies (as discussed earlier in section 6.5.2). Whilst, there is an increasing interest in the choice of a biomarker to assess the elemental profile of an individual, the choice of a proper analytical technique also becomes important in terms of producing accurate and precise elemental values that are fit-for-purpose in terms of the research aims and objectives.

Modern instruments enable the assessment of elements in biological marker tissues such that concentrations as low as parts per billion (ng/g or 10^{-9} g/g) can be detected. Within this doctoral thesis the concentrations of the important essential elements (calcium, magnesium, arsenic, cobalt, chromium, iron, iodine, manganese, molybdenum, nickel, selenium, and zinc), non-essential (aluminium, boron, antimony) and toxic elements (cadmium, mercury, lead) under investigation were measured in micrograms per gram ($\mu\text{g/g}$). Usually this can be achieved by using only a small amount (or mass) of the sample to be analysed and in a short period of time. ICP MS is the technique that is now used for trace or ultra-trace elemental

total and speciation analysis (Flanagan, Taylor, Watsin, & Whelpton, 2007; Goulle et al., 2009; Slotnick & Nriagu, 2006) and was used for the analysis of scalp hair and toenails collected from the individuals under investigation.

This research has shown that multi-elemental analysis using toenail clippings is useful for epidemiological studies, especially Pacific populations with the main emphasis being children. Once multi-elemental analysis is completed, it is recommended that the statistical analysis used should be Principle Component Analysis which is useful for searching for relationships between elements; however, this approach was not applied within the thesis as it was beyond the scope and limited expertise available. But for the purposes of this part of the study, already known elemental interactions were studied. For example, the relationship between selenium-mercury together as one variable was studied as a function of body-size categories; it was found that a clear association exists between this elemental pair. Selenium has been reported to protect the body from mercury (Ralston & Raymond, 2010). Perhaps the combination of slightly higher mercury and low selenium concentrations may have had an effect on body-sizes. The toxicokinetics of these interactions on body-size needs to be investigated further.

Elemental concentrations may be affected by genetics and environmental factors (Ha et al., 2006; Lebel et al., 1998); however, all the children recruited within this sample were of similar age, of Pacific origin and lived in South Auckland which reduced the influence of those factors. However, Pacific people are a diverse community and consist of many different ethnic groups (Ministry of Health, 2008a). The children in this study came from four different ethnicities (Samoan, Tongan, Cook Island and ‘Other groups’). In Study 3 (titled ‘Association of Elements on Body-size’), even within the similar population, statistically

significant differences (using a Kruskal Wallis test and at a probability level $p < 0.05$) were found for certain elements, particularly manganese, iron, cobalt, antimony, aluminum, mercury, and lead. It is likely that this is due to socio-economic differences. Socio-economic status can affect children's dietary intake due to lack of money which in turn can lead to imbalance in elements (Amare et al., 2012; Nelson, 2000).

Gender differences were also seen for calcium, magnesium, manganese, iron, antimony, aluminum, boron, arsenic and lead. These differences have been observed in other studies as well (Barany et al., 2002; Kristiansen, Christensen, Iversen, & Sabbioni, 1997). Genetic differences within the different ethnic groups have also been observed previously (Gellein et al., 2008; Pink et al., 2009; Sukumar & Subramanian, 2007); however, this effect is not investigated within this research. Further understanding on the influence of elements within different Pacific ethnic groups (Samoan, Tongan, Cook Island and Neuien) and gender is required.

As observed in the literature a number of terms are used to describe child behaviour e.g. developmental disability, neurodevelopmental disorders, intellectual developmental disorders (IDD) and mental retardation. It was difficult to identify research related to just behavioural problems in toxic elemental studies. This is because many terms are used in such publications. These different terms and classifications in various research papers have created a barrier to research on the basic mechanisms underlying mental disorders and symptom expression (World Health Organisation., 2011). Up until recently, behavioural problems were part of the core classification of IDD. After much debate and consideration, the World Health Organization Advisory Group have come to the conclusion that behavioural problems are an associated feature of IDD rather than subcategories or specifiers of IDD (Salvador-Carulla et

al., 2011). However, the term used throughout this thesis was behavioural problems which are a category of developmental disorder. Developmental disorders such as behavioural problems are the dysfunction of the brain and may affect behaviour, learning or memory processes (Cho, 2006). Studies that used developmental disorders and behavioural problems that were identified using the Child Behaviour Checklist (CBCL) were included for comparison with this research. For future purposes; however, researchers should now use the new term from the international classification disease and related health problems manual, which is intellectual developmental disorder with behaviour problems as a subcategory and should properly define the terms to be used in their research.

Behavioural problems are multi-factorial and identifying the causal mechanism becomes a complex task. Along with genetics and socio-economic status, toxic elements are also known to influence behavioural problems in children (Bernard, 2008; Goodlad Iii, Marcus, & Fulton, 2013; P. Grandjean & Herz, 2011). It is also known that socio-economic variables can exacerbate the effects of toxic elements (Goyer, 1997; Goyer & Clarkson, 2008). Mercury in hair and toenails did not have an association on the overall behavioural problems in children (OR: 0.66, 95% confidence Interval (CI) 0.03-13.8; p value 0.23 and OR: 1.02; 95% CI 0.82, 1.29; p value 0.8 respectively). But an association was observed within the aggressive behavioural domain (OR: 2.15 95% CI 1.45- 3.18; p value <0.05) in Study 2 (titled 'Association of Mercury and Behavioural Characteristics'). It is important to identify and understand such behavioural problems as research has shown that it can carry on to adulthood and lead to antisocial and criminal behaviour, low attainment and, high economic costs for the community with possible mental disorders (Ape-Esera et al., 2009; Griggs & Walker, 2008; LeClair & Quig, 2001). It seems that mercury may be particularly specific and

sensitive to the aggressive behavioural domain. It has been suggested that evaluation of complex aspects of behaviour such as conduct or social behaviours may be a more sensitive and specific way to measure neurotoxic exposures than traditional tests of cognitive abilities and language (Spyker et al., 1972; Vorhees & Mollnow, 1987). Therefore this part of the thesis focused on behavioural problems (with aggressive behaviour as one of the domains) in children measured using the CBCL. Furthermore, associations observed within this research were based on much lower toenail mercury (range: 0.002 µg/g to 6 µg/g) concentrations than in the literature which were above >10 µg/g. The relationship between postnatal mercury exposure at low concentrations on child behaviour is a complex question and requires further research and understanding. Additionally, mother and child's mercury concentrations in hair were highly correlated (r : 0.79 (95% CI 0.65, 0.88) in the first investigation. Both the child and mother came from the same household, and ate similar food which could explain the correlated mercury concentrations. There were some mothers (20%) with hair mercury concentrations above 1.5 µg/g. Maternal mercury concentrations at this level have been shown to cause developmental disorders (particularly attention deficient/hyperactivity disorder related behaviour) in children born to them (Sagiv, Thurston, Bellinger, Amarasiriwardena, & Korrick, 2012). Therefore monitoring and providing proper advice on mercury for mothers-to-be becomes necessary.

The final part of the research sought to determine if selected elements had an effect on children's rapid growth and development. Although this study did not show any appreciable associations between elements and body-size, elemental interactions (mercury-selenium and zinc-copper) on body-size category were evident. High selenium concentrations can counteract the effects of mercury (Ralston & Raymond, 2010). But perhaps the combination

of low concentrations of selenium and slightly higher concentrations of mercury may be associated with factors leading to body fatness within this sample. Moreover, elemental data should not be evaluated solely in terms of specific element levels or correlations between pairs of elements, as the human body does not function in such simplistic ways. It may be that a 'cluster or cocktail' effect (as would be evaluated statistically by principle component analysis) would identify the complex associations between not just trace elements but also specific chemical forms (as is already known with ferrous and ferric ion) and biochemical compounds (proteins, vitamins, amino acids, etc.).

As mentioned earlier, low zinc concentrations have been shown to lead to higher copper concentrations and *vice versa* (Hedayati & Safahieh, 2012) and this may have an association on larger body-sizes. This is a novel observation that hasn't been fully evaluated before, especially in child populations and could shed some light on these complex associations. Furthermore, elemental concentrations of a specific human tissue were evaluated against BMI z scores (as a continuous measure) but no statistically significant relationships were identified.

Other body fatness measures such as fat mass, regional fat mass and direct measurements (dual X-ray absorptiometry) could have been used to explore elemental concentrations (determined in scalp hair or toenails) in this study which may be more productive. Whilst increased body-fatness is multi-factorial, previous research has shown that it can influence the elemental concentrations of both children and adults, such that they would be linked to deficiency levels of certain elements (Dambal, Indumati, & Kumari, 2011). This is in line with most research on obesity and elemental concentrations of body tissues or fluids that have

used cross-sectional studies where temporality cannot be established. Further work in this area would be well served by longitudinal study designs to determine causality.

Knowledge about the quantitative relationships between elemental concentrations in the body is fundamental to increasing our understanding of elemental metabolism, and possible mutual interactions between elements and elements with other biomolecules (especially amino acids, vitamins, free fatty acids). In future studies evaluating body-fatness and obesity it is essential to evaluate the possible interactions between trace elements and essential free fatty acids. A major reason is that trace elements can have a direct effect on free fatty acid metabolism (Sfar et al., 2012). One study evaluated the effect of zinc deficiency on some trace elements and free fatty acids in the blood and brain of rats and showed that zinc status has an important impact on essential fatty acid levels (Liu & Gu, 2000). Furthermore, some research has already investigated a possible link between body fat distribution and specific abnormalities of free fatty acid metabolism (Jensen, Haymond, Rizza, Cryer, & Miles, 1989). Therefore, based on the ‘cluster or cocktail effect’ mentioned earlier, it is necessary to broaden the number of chemicals being investigated as such studies undertaken in this thesis, so that possible links between body fatness, element and essential free fatty acid levels may provide a clearer link to understanding the factors associated with obesity.

9.2 Research Strengths and Limitations

The major strength of this research was that it was carried out within the internationally recognised Pacific Island Families (PIF) cohort study children resident in South Auckland, New Zealand. The PIF cohort has offered an opportunity to test the possible drivers of behavioural problems and body-sizes within Pacific children. The children have been followed since birth and the researchers have gathered socio-economic, demographic and

cultural information on the children and their families. This information has provided the background information for this research. A further strength of this doctoral research was the proposal and application to use a direct biomarker (scalp hair and toenails) to determine the elemental concentrations of the children rather than just a reliance solely on questionnaires.

The findings of the doctoral thesis should be considered in the light of its limitations. There was a selection bias during the sample collection process as not all participants who were eligible agreed to have samples collected. The first study faced resistance from participants in hair sample collection; therefore further participants were not contacted as it was not appropriate. For the next two investigations using toenails, not all eligible participants could be included due to time limitations.

Secondly, statistical inference was limited by small sample sizes due to restraints in data collection in the first study ($n=92$ mother-child pair). Thus it is likely that there was insufficient power to detect any health effects. Specifically, due to the small sample size, there is a risk of Type II errors, which means that it fails to reject a null hypothesis (Hackshaw, 2008). Large sample sizes usually produce narrow confidence intervals (CI) and therefore more precise results (Hackshaw, 2008). However, the next two investigations were of reasonable sample size ($n=278$; behavioural cases $n=67$; large body-size $n=186$) considering the logistics and economic constraints. For this research more than just numerical values for sample size were considered such as cultural recognition (cultural beliefs and customs) within Pacific people. This approach is supported by Bacchetti, (2010).

Thirdly, the questionnaires used for this thesis and the biomarker evaluations were carried out through the well-established PIF interview process. The data management employed a robust

checking process that reduced variations. Within the PIF, the interviewers are of Pacific ethnicity as having interviewers of the same ethnic groups as participants is seen as a culturally appropriate way to obtain data from this population. However, there could have been minor interview errors (such as biased probing). Overall, all interviewers were well trained and monitored throughout the interview process as suggested by Davis et al., (2012) and biased probing could have been reduced.

The questionnaires used in this doctoral research were reliable and validated but they have some limitations. Inaccuracies of measurements (misinterpretation of questions) across the various samples (cases and controls) could have contributed to a non-differential measurement error in exposure and outcome which can sometimes lead to a bias towards finding no effect (Williams et al., 2000). A limitation of the Food Frequency Questionnaire is that mothers were required to recall their usual weekly consumption of foods which is subject to bias rather than measuring the actual intakes (Burrows et al., 2010; Trabulsi & Schoeller, 2001). Mothers were proxy for children's eating habits which may lead to over- or under-estimating their frequency of food intakes. Other research has shown under-reporting of energy intake by young Pacific adults and in parental reports of intake of their children (Rush, Plank, Laulu, Mitchelson, & Coward, 2004; Watson, 2001). The CBCL is a widely used tool for clinical and research purposes and is considered to be a valid and reliable indicator of children's behavioural and emotional functioning (Achenbach & Rescorla, 2001). However it is important to note that this is a screening tool and does not substitute a full diagnosis by a trained clinician.

Categorising continuous variables is a usual practice in medical science (Pocock et al., 2004; Turner, Dobson, & Pocock, 2010) especially body mass index (BMI) studies. The last part of

this thesis investigation used the International Obesity Task Force (IOTF) to classify the BMI categories in children. Categorising generally leads to loss of power due to reduced variability in the data, and other methodological challenges such as false positive results (Altman & Royston, 2006; van Walraven & Hart, 2008) and chances of misclassifications (Flegal, Keyl, & Nieto, 1991). There is a possibility that the cut-offs may have impacted the odds-ratio creating misleading associations (Altman & Royston, 2006; van Walraven & Hart, 2008). Another very important feature in categorising BMI values is having the right reference group for comparison (Frøslie et al., 2010). The choice of a reference group is equally important and thus after careful consideration and consultation with statisticians, a 'healthy weight' group was the reference category in this research. To reduce some of the BMI limitations, BMI data as a continuous measure (BMI z scores) using the Coles cut-off was carried out. However, BMI z scores are best for assessing adiposity at a single point in time but are not good for measuring change in adiposity in children (Cole, Faith, Pietrobelli, & Heo, 2005). These limitations should be considered for future studies in this field.

In addition, the last two studies (Study 2 and Study 3) in this thesis employed a cross-sectional design which was efficient and easy to conduct without burdening the PIF participants who have been followed for 11 years. As mentioned earlier cross-sectional studies do not differentiate between cause and effect nor the direction of the events (J. B. Lee, Winstead, & Cook, 2006). Other study designs to be considered include case-control studies which are quicker, less expensive and less burdensome on participants as well. Although the best study designs are experimental studies, this may not be always feasible due to financial, time and ethical restrictions. This thesis relied on nested case-control and opportunistic cross-sectional study designs.

Finally, as previously outlined the focus of this research was to explore whether elemental concentrations of tissues could provide any additional information in terms of understanding the etiology of health and behavioural problems associated with this long-studied Pacific population in New Zealand. In many ways, whilst this was a novel approach as no data was available prior to the start of this study because the possible chemical associations with such conditions are complex, it may be advisable to expand the scientific approach to include trace elements, essential fatty acids and even other biochemicals (vitamins and amino acids). This would mean that body fluids would need to be collected, and with ethical limitations on using blood collection for children, it would mean that sweat or saliva would need to be evaluated as possible biomarkers for all the chemicals mentioned. Furthermore, a major limitation is that one of the key chemicals involved in human metabolism was not investigated, namely water. Dehydration is well known to affect human behaviour (Adan, 2012; Ganio et al., 2011; A. C. Grandjean & Grandjean, 2007; Smith, Newell, & Baker, 2012; Wilson & Morley, 2003). A knowledge of the consumption of water and alternative diuretic solutions (such as tea, coffee, soft drink) beverages is essential in terms of the link between chemical levels in the diet, the body and any possible effects on health, especially behaviour.

9.3 Significance and Implications of the Findings and Future Directions

This body of work confirms evidence from the existing literature and contributes to the understanding of the role of elements of the periodic table in Pacific Island children's health in the NZ context. The results of this research suggest the need to further investigate the levels and role of elements and other essential trace chemicals using biological markers (including both tissues and fluids). While it is reasonable to assume that there is no single cause for childhood behavioural problems or body-sizes, the relative ease with which

elemental concentrations can be assessed suggests that attempting to identify such causal pathways relating to dietary deficiencies and exposure to toxins should be given routine consideration, alongside family and social factors.

Scalp hair samples were the first choice for this doctoral research but as mentioned earlier it was not a feasible study on Pacific people. In many cultures including the Pacific, hair and the head have symbolic and evocative meanings. Also, the head of a person in Pacific and Māori culture is a sacred area of the body. For example, in Māori culture the head is the most *tapu* (spiritual and social code) part of a *tapu* person (Best, 1924) and has a spiritual significance. Hair in some Pacific cultures such as Niuean is sacred and cannot be cut until the child is of a certain age. A ceremony (called *hifi ulu*) is performed before the long hair is cut which determines the status of the family. In the investigation of mercury in hair two families out of 47 participants approached had sons whose hair-cutting ceremony had not been performed yet so hair sample collection was not possible.

From the wider literature, there are biological reasons to believe that toxic and essential elements might influence children's development. Even though only some associations were observed in this body of work, the suggestion is that perhaps the exposures to toxic and essential elements should be studied prospectively and in much larger sample sizes. In order to determine if there are any associations (whether causal or not), randomised control trials should be conducted on elemental supplementations and toxic element exposure prevention programmes. Prevention of exposure to toxic elements such as mercury in certain seafood diets has been debatable. However in this study mercury exposure in children and their mothers was lower overall with some above the recommended concentrations for optimal health. Whilst some studies have shown that scalp hair may be a good biomarker for mercury

exposure, in such children it may be questioned that urine would be a better index of dietary mercury intake, especially from fish. There is a limited amount of information about up-to-date Hg levels in NZ fish, and it may be questioned whether Pacific Island people would be exposed to such fish in South Auckland in contrast to the Island communities of their forefathers. Therefore, more data is required on Hg levels in NZ foodstuffs including those prepared and sold at fast food outlets.

One of the key experiences learnt in this doctoral research was that a cross-culturally competent research with this population requires an understanding and application of Pacific people's paradigms of health knowledge, science, and research. Engaging participants in research and sharing the outcomes with the community were seen as important parts of the research process. Also, having Pacific interviewers was an important aspect in this research, as they brought the cultural competencies needed to encourage accurate responses to questions. Plus ensuring that the participants and interviewers do not know each other to provide the participants a level of confidentiality should always be considered as suggested by Panapasa et al., (2012). Thus it is important to recognise or engage in attitudes and behaviour of Pacific people and their cultures before undertaking research otherwise marginalisation occurs (Schluter et al., 2011).

9.4 Conclusion

This thesis provides considerable insights into the association of elements of the periodic table (such as calcium, magnesium, manganese, copper, zinc, selenium, iodine, cobalt, iron, chromium, nickel, molybdenum, antimony, arsenic, aluminium, boron, mercury, lead, cadmium) and Pacific children's health (particularly behavioural problems and body fatness) who are residents of South Auckland, New Zealand. Findings showed that aggressive

behaviour may be associated with toenail mercury concentrations and that ethnicity and gender largely influenced the concentrations of certain elements (particularly calcium, magnesium, manganese, copper, iron, antimony, aluminium, boron, arsenic, lead). Selenium, iron, and calcium, were found to be lower in toenail samples and thereby according to established reference data from other published studies, to be considered below that required for optimal health. 21% of children had toenail mercury concentrations (1.5 µg/g to 6 µg/g) and 18% had hair mercury levels (1 µg/g to 1.03 µg/g) higher than the recommended levels for optimal health in children. The other finding of note was that selenium-mercury and zinc-copper interactions (statistically evaluated using regression analysis) showed a moderate association with body-size categories.

Importantly this research addresses the gap that existed in the elemental influences on body-size literature through the examination of elements and Pacific children's body-size categories. With the increase in food security and lack of proper nutrition due to low socio-economic status within Pacific people in NZ, understanding elemental analysis on children's health outcomes will remain an important aspect to consider in future epidemiological and nutrition research. Furthermore, this research has shown that biological markers such as toenail clippings are less invasive and appropriate to use in Pacific research. It is hoped that this research stimulates other scientists to look further into the impact of elemental concentrations and the use of biomarkers in determining chemical (trace elements) and biochemical (essential free fatty acids, vitamins, amino-acids) concentrations in the body fluids and tissues of such children.

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Appendix 1: Peer Review



21 February 2013

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Methylmercury exposure through seafood diet and health in New Zealand: Are seafood eating communities at a greater risk?

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Abstract

Methylmercury (MeHg) is a developmental neurotoxin that presents a potential public health hazard to humans. MeHg exposure has been linked to developmental, cognitive and neurological disorders both pre and postnatally. Even very low environmental MeHg concentrations are thought to cause subclinical disorders especially in children. MeHg is highly absorbable with an important route of exposure through contaminated fish and seafood consumption. Many studies have shown dose-response MeHg effects in children whose staple diet is fish/seafood. It is also known that certain factors such as genetic susceptibility, socioeconomic, nutritional and cultural factors exacerbate the effects of MeHg exposure. This review examines the current knowledge on MeHg exposure through fish diet, our understanding of its effects on children and adults, the efforts at both national and international levels to tackle this pollutant. In particular, it raises concern that New Zealand (NZ) fish and seafood eating communities, such as Māori and Pacific population, may be at high-risk for MeHg exposure in addition to other Hg related factors

Introduction

There is substantial national and international interest in environmental contaminants, including the deleterious effects of mercury (Hg), a well known developmental neurotoxin.¹ Hg occurs in many different forms, including Hg vapour, inorganic Hg, ethylmercury, and methylmercury (MeHg). For the general population, primary exposure comes from a combination of fish/seafood consumption, dental amalgams and vaccines. There are also some occupational and local environmental (natural and man-made) exposures. Each of these forms of Hg have different toxicological profile and clinical symptoms.² This review focuses on MeHg, the most hazardous form of Hg. Most developed countries such as the United States (US), Canada and New Zealand (NZ) have strict guidelines and policies designed to protect the public from significant MeHg exposure.

In recent times there have been concerns about the health of children exposed to very low environmental MeHg concentrations, levels previously thought to be safe.³ Neurotoxins have been demonstrated to affect bioavailability

of various essential minerals, potentially leading to subclinical developmental disabilities, including behavioural problems.⁴ Individuals with elevated MeHg levels do not always show clinical symptoms and so symptoms can be difficult to detect and measure.⁵ Concerns are also growing about health impact on adults due to MeHg exposure, as studies are showing that MeHg exposure might interfere with vision, motor function, and memory.⁶

In NZ, an important route of MeHg exposure is through fish and seafood consumption.^{7,8} As children are more vulnerable to the effects of MeHg, there are growing concerns about fish/seafood consumption during childhood and its hazardous effects on the health and development of exposed children which is being raised by health authorities.⁹ However the benefits of fish consumption to children has also been well recognised.¹⁰ In the absence of widely publicised guidelines, this leaves parents and the general public to question what are appropriate and safe levels of fish consumption, particularly those in high seafood consuming sectors of the population. The purpose of this review is to provide an overview of MeHg exposure through seafood diet within the NZ context.

Hg exposure

There are a number of ways in which humans (both prenatally and postnatally) can be exposed to the different forms of Hg. Although Hg is present in water and air, the natural concentrations are extremely low and usually negligible to humans.² Traces of Hg are present in all food but uptake by plants from soil is low and therefore concentration of Hg in fruits and vegetables is also extremely low. The NZ Total Dietary (NZTD) survey 2003/04 of a large nationally representative sample found that vegetables and fruits all had Hg levels well below 0.0001mg/kg.¹¹ There are some Hg containing products, such as Hg thermometers and fluorescent lighting, which when damaged can be a cause of exposure to the individuals, however these instances are relatively rare. Also some occupational settings may expose people to Hg such as dentists.¹² However, apart from the workers directly involved, these exposures pose little risk to the general public. Instead, Hg exposure in the general population primarily occurs from fish/seafood consumption, which is in the form of MeHg.¹³ dental

amalgams absorbed through Hg vapours in the form of elemental Hg¹⁴, and from vaccines in the form of thiomersal.^{15,16} In 2000, NZ phased out all thiomersal-containing vaccines in its childhood schedule vaccines, although some influenza vaccines still contain thiomersal.¹⁷ However, the estimated risk from thiomersal-containing vaccinations and dental amalgam is low.¹

Methylmercury exposure in humans

MeHg is the most toxic form of Hg and is highly absorbable relative to other Hg compounds.¹³ The consumption of contaminated fish (especially predatory fish) and seafood is the major source of exposure to MeHg in humans,¹³ and suckling infants can be exposed through breast milk.¹⁸ In NZ, dietary exposure to Hg may account for up to 54% of a person's total exposure to Hg and nearly all their exposure to MeHg compounds.¹³

In the environment, especially lakes, sea, and waterways, released elemental Hg is converted through methylation to MeHg by anaerobic bacteria in the aquatic ecosystem which then bioaccumulates in bigger fish and consequently larger predatory fish (e.g. mackerel, pike, shark, swordfish, barracuda, large tuna, marlin, whales and trout) have higher Hg concentrations.²⁰ Almost all Hg in fish muscles is MeHg.²¹ The level of MeHg varies in different fish species because each have different habitats, life-cycles and feeding patterns. In NZ, many fish contain levels of MeHg higher than the World Health Organization (WHO) recommended maximum MeHg levels (0.5 mg/kg) due to volcanic and geothermal activities,²² or naturally occurring Hg deposits.²³ Freshwater fish in geothermal lakes and rivers in NZ may also accumulate high levels of MeHg.²⁴ For example, trout in NZ are known to contain MeHg levels that can reach 4.13 mg/kg.²² Since contaminants are high in some fish/seafood, people who consume large amounts of these kinds of fish/seafood, are vulnerable to adverse health effects.²⁵ However, currently there is no current information on the type of fish that is most eaten within Pacific, Māori or Asian people in the NZ population.

Transport of MeHg in humans

Approximately 90–100% MeHg is absorbed through the gastrointestinal tract where it enters into the blood stream and is distributed throughout the body.¹³ The pattern of this distribution is relatively uniform, except in red cells where the concentration is 10–20 times greater than the plasma concentration. The half-life of MeHg in people is dependent on the exposure length in addition to the dose of MeHg. For example, for people exposed to high doses over a short-term, the half-life of MeHg is approximately 44 days,^{26–28} whereas for people exposed to high doses over a long-term, the half-life is approximately 90 days.²⁹

MeHg readily crosses the blood–brain and placental barriers and thus is accumulated and concentrated in the fetus, especially in the brain.³¹ MeHg also accumulates in hair during its process of formation.³² The vascular effects of MeHg include oxidative stress, inflammation, thrombosis, vascular smooth muscle dysfunction, endothelial dysfunction, dyslipidemia, immune dysfunction, and mitochondria dysfunction.³⁰

Seafood diet in NZ

Seafood is an important part of a healthy diet and is a major source of protein for many communities both nationally and internationally. A survey conducted in NZ by the Seafood Industry Council (2007) found that about 88% of NZ people eat fresh or frozen fish at least once a month and approximately 45% eat fish at least once a week.³¹ Many studies have shown that fish is an important source of the omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid not found naturally in other food and could potentially contribute to a healthy heart, optimal brain function, cognition, improved eye and skin health and some protection against certain cancers,³³ though not all studies have found such benefits.^{32–35} Since seafood also contains environmental contaminants such as MeHg, balancing the risks and benefits of eating fish is an important public health issue.

Like MeHg, omega-3 fatty acid levels vary in different seafood.³⁶ Fatty fish tend to have higher omega-3 fatty acid levels than lean fish; while ocean fish are known to have higher levels of omega-3 fatty acid than fresh water fish.³³ No associations have been shown between MeHg levels and omega-3 fatty acids.³³ Large fish such as sharks and swordfish accumulate higher levels of MeHg but do not necessarily have high omega-3 fatty acids while fish such as anchovies, salmon, and herring have high omega-3 fatty acids and are known to have low MeHg concentrations.³³ The Ministry of Health (2009) in NZ has assessed various fish-types for omega-3.³⁴ In eel fillet, fish such as eel contain an average 0.6g of omega-3, flounder contain 0.2g, and Hoki contain 0.1g.³⁴ However, some fish species, such as kahawai (also known as Australian salmon), were found to contain no measurable levels of omega-3,³⁴ and thus may be a source of MeHg without any omega-3 benefits. It is thus recommended that consumption of these fish-types should be limited, especially by pregnant women.³⁴ Historically, the consumption of fish in NZ was predominantly gained through eating fish and chips, especially in lower social economic status (SES) families.³⁵ Shark was routinely used as the meat source in fish and chips, yet shark is known to have high MeHg levels of up to 1.97 mg/kg.³⁵ A survey of fish in the early 1980s on Hg levels in 33 takeaway shops in South Auckland found that about 40% of the fish levels were above the WHO recommended maximum level of MeHg (0.5 mg/kg) and if eaten in excess by vulnerable people, could cause adverse health effects from MeHg.³⁶ The more recent NZTD survey 2003/04 found that battered fish total Hg concentrations reached up to 0.85 mg/kg,⁷ however the MeHg levels were not provided. While the routine consumption of shark may have changed in the intervening years, the risk of adverse health effects still remains and also led the NZTD survey to suggest that consumption of this fish-type should be limited, especially amongst vulnerable people.⁷

Many studies have shown that some ethnic groups are at a greater risk of MeHg exposure as they generally consume relatively more fish.²⁵ Māori and Pacific populations traditionally have high fish consumption, and thus may be particularly vulnerable to adverse effects from MeHg. In a NZ study of 7331 mothers that was conducted in late 1970s, 1486 (20%) consumed fish more than three times per week while

pregnant.³⁵ Rates of fish consumption were greater for Māori (31%) and Pacific (53%) mothers. Amongst these mothers, 73 had MeHg levels (determined from hair samples) which were above 6 µg/kg; 8 (11%) of whom were Europeans, 20 (27%) were Māori, and 45 (62%) were of Pacific descent.³⁶ This study was conducted over three decades ago and the pattern of maternal fish consumption may have changed in the intervening years. No subsequent studies in NZ have explicitly examined these rates. However, a recent NZ National Children's Nutrition survey (2002/03) demonstrated that a higher proportion of Pacific children consumed fish (~58%) than their Māori (~37%) and European counterparts (~35%). Furthermore, significantly more Pacific (~27%) and Māori children (~18%) children consumed fish cakes and fish fingers, which have high MeHg concentrations, compared to children of European origin (9%).³⁷ Unfortunately, little current information is available about MeHg exposure, levels and sequelae in these populations.

Health effects of MeHg

The deleterious health effects of MeHg have been known since the 1860s.³⁸ There have been several outbreaks since then due to dietary or occupational exposure with devastating health effects both pre/postnatally and in adults too. Two such major outbreaks occurred in Japan, Minamata Bay, through contaminated fish consumption.³⁹ Another outbreak occurred in Iraq from bread made from contaminated grains whereby thousands of victims suffered neurological symptoms.⁴⁰ These symptoms were found to be more pronounced in children born to mothers who were poisoned from MeHg.⁴⁰ More recently, there has been increased concern about the health of children exposed to environmental MeHg because it has been found to cause subclinical effects at even these low concentrations.² It is demonstrated that toxic minerals can have antagonistic effect on various essential minerals leading to disturbances in metabolic utilisation which could lead to developmental disabilities including behavioural problems.

Many studies have been conducted to determine the effects of prenatal and postnatal low level MeHg exposure at subclinical and population levels and have reported poorer neurological status and slower development^{3,41} while some studies did not observe any adverse effects.⁴ Most attention has focused on three major longitudinal studies, namely: the Faroe Island (baseline n=1,022),⁴²⁻⁴⁵ Seychelles (baseline n=804),⁴⁶⁻⁴⁸ and NZ (baseline n=11,000) studies.^{35,49,46} While each of these populations consumed seafood in their diets, their findings differed. The NZ study showed a greater percentage of children in a high MeHg group (maternal hair MeHg levels >6 µg/g) who had lower developmental scores (a three-point decrement in intelligence quotient (IQ)) than children from the low MeHg groups (maternal hair MeHg <3 µg/g, 3-6 µg/g). The Faroe Island study also reported higher MeHg levels (average 22.9 µg/L) in cord blood and in maternal hair (geometric mean 4.3 µg/g) with lower developmental scores in children. The Seychelles study reported no association between maternal MeHg levels in hair (arithmetic mean maternal MeHg 6.8 µg/g) and developmental scores on neurological tests in children. There has been considerable debate over these mixed findings, however, it is likely due to the differences in study designs and sources of

exposure.⁴⁷ Nonetheless, using the information from these three major prospective studies, an attempt was made to develop a quantitative dose-response function for prenatal MeHg exposure and IQ.^{48,49} Axelrad and colleagues determined that an increment of 1ppm of MeHg in maternal hair during gestation can cause a decrease in IQ to range from 0.18 points⁴⁸ to 0.7 points.⁴⁹

Using the child behaviour check-list, a NZ cohort of 1,376 Pacific children aged 2 years found approximately 15.5% with behavioural problems within the clinical range.⁵⁰ Given these children's relatively high exposure to a sea-food/fish diet, MeHg may be one of the contributing factors. There have been many studies that have observed an association between neurodevelopmental problems and MeHg exposure^{51,52} however, studies need to be conducted within Pacific ethnic group in NZ to verify whether sea-food and fish diets are responsible or contribute to these high rates of behavioural problems.

Other studies have concluded that beneficial influence of nutrients (such as selenium and omega-3 fatty acids) from fish may counter any adverse effects of MeHg on the developing nervous system.⁵³ Again the results are equivocal. For example, a Faroe Island birth cohort study investigated omega-3 fatty acids and selenium as a potential modifier of the effects of MeHg exposure through fish/seafood diet but found no such associations.⁵⁴ Accordingly, Choi and Grandjean (2008) suggested in their review that to assess the full impact on the toxicity of MeHg and the beneficial effects of nutrients, both the good and bad effects should be assessed at the same time in order to separate opposite impacts on the outcomes.⁵⁵

Control measures and risk communications in NZ

The NZ Food and Safety Authority (NZFSA) manages a national monitoring program for heavy metals in fish while the Food Standards Australia New Zealand (FSANZ) prescribes a maximum level of MeHg in fish and seafood (0.5ppm for most fish and 1 ppm for certain fish).⁵⁶ The Joint Food and Agricultural Organization (FAO)/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for MeHg as 1.6 µg/kg body weight (bw)/week,⁵⁷ which is the equivalent of 0.23 µg/kg bw/day. This value is considered protective of developing foetuses, the most sensitive sub-group in the population. Therefore with the evidence, NZFSA adopted the 2003, JECFA revised PTWI for MeHg.¹¹ The NZFSA estimated the average daily intake of MeHg by adults, children, toddlers and infants from food sources which ranged from approximately 0.60 to 0.74 µg/kg bw/week.¹¹

The dietary exposures in the 2003/04 NZTDS were based on average energy diets for each of the age-sex groups.¹² According to Vanover and Thomson (2005) some people might have significantly higher exposures, especially within the high exposure groups.¹¹ There is specifically recommended dietary information provided by the NZFSA on its website. However, it is unclear whether this information reaches pregnant women or at-risk sub-populations (such as Māori and Pacific people).

Investigation is needed within NZ to identify the best method to inform and educate vulnerable sub-populations on the risks and benefits of fish consumption so these people can make well informed choices on consuming fish for good health whilst avoiding fish which may be harmful due to high MeHg levels. Also, with emerging evidence that even low MeHg levels may cause adverse health effects, further studies need to be conducted to confirm the patterns and extent of fish consumption in NZ. That is, the types of fish being consumed, the levels of MeHg in specific fish, and the extent of MeHg exposure within the NZ populations.

Conclusion

Accumulating evidence suggests that potentially damaging prenatal and postnatal exposure to MeHg may occur for some children born to vulnerable population sub-groups, primarily through fish/seafood consumption. Efforts to more fully understand and evaluate the health risks associated with this neurotoxin should be undertaken.

Any experimental or epidemiological data presents some uncertainty on whether the measured effects captures the most sensitive or critical effects. Neurodevelopmental effects are the most extensively studied sensitive end-point for MeHg exposure but there are other indications of adverse effects on humans such as immune functions, cardiovascular health and developmental disorders, and the relationships with broader societal factors. In addition to neurodevelopment effects, these other indicators need to be researched further in NZ particularly in view of the emerging literature of the subclinical effects of low level MeHg exposure.

The effects of MeHg exposure can be profound and debilitating. Yet, with appropriate monitoring and health promotion, excessive MeHg exposure is largely avoidable. NZFSA, FSANZ, and NZ health authorities are making efforts to manage the risks involved with MeHg exposure but there is not enough

information about the current levels of MeHg in NZ people nor the types and amount of fish eaten to assess any causative relationship or effect. The NZFSA assessed MeHg exposure levels in NZ seafood/fish and adopted those recommended by the WHO guidelines (1.6 µg/kg body weight(bw)/week).¹¹ The NZFSA concluded, at that time, that the NZ population is highly unlikely to have any adverse health effects as a result of dietary exposure to MeHg. However, the emerging evidence suggests that NZ should perhaps reassess these levels.

The MeHg levels in high seafood consuming populations such as Māori and Pacific Island people, pregnant women, women of reproductive age, and children or adolescents who are most vulnerable is largely unknown in NZ. Unfortunately, Māori and Pacific Island communities, with a tradition of seafood eating also have risk factors which are disproportionately over-represented and could exacerbate the effects of MeHg exposure. The burden associated with the sequelae of MeHg exposure is likely to fall heaviest on these people. Māori and Pacific Island people also carry the highest oral health burden and lowest SES, which potentially exposes them to increased MeHg risk. No studies within NZ have researched the separate and synergetic effects of MeHg, elemental Hg and other neurotoxins in Māori and Pacific people. Such programs would be useful for health officials and communities to determine appropriate safety levels, and enable people to make informed dietary choices.

Acknowledgements

The authors gratefully thank Professor Elaine Rush, for directing us to the information on the omega levels in NZ fish, Professor Tord Kjellstrom and Associate Professor Geoffrey Savage for providing information related to Hg, the Ministry of Fishery for providing documents related to Hg in fish.

We would like to acknowledge the sad loss of Dr Richard Anstiss who greatly contributed to this paper. Our heartfelt condolence goes to his family.

Appendix 2: Ethics Approvals for Study 2



Centre for Pacific Health and Development Research
Auckland University of Technology

19 June 2007

To Health and Disability Ethics Committee,

NTX/07/05/050

RE: Health of Pacific Children: Environmental and Nutritional Determinants

Principal Investigator: Ms Shamshad Karatela

Supervisor: Dr Janis Paterson (AUT)
Co-supervisors: Prof. Philip Schluter (AUT)
Dr Richard Anstiss (AUT)

In response to point 3 of the previous decision made to defer approval by the ethics committee regarding the above research proposal, I am happy to inform you that we have consulted with the newly formed Pacific Advisory board to the Pacific Island Families Study (PIF) on Thursday 19th June, regarding the concern of going back to the cohort.

Our advisory board were happy to approve the use of the data and opportunity to go back to the cohort as they were satisfied with the processes that make it clear that this research is a separate study from the PIF and that participants are in no way obligated to take part in the study and can opt out at any time.

These points will be made clear at initial contact via phone by a Pacific person who will insure that this is understood fully and conveyed in an appropriate manner. This will also be on the information/consent sheet and explained again in person by a Pacific interviewer.

Kind regards,

A handwritten signature in black ink that reads 'Gerhard Sundborn'. The script is cursive and elegant.

Gerhard Sundborn

Co-ordinator / Senior Research Fellow
Centre for Pacific Health and Development Research
Pacific Island Families Study
Auckland University of Technology
Phone: 921-9999 extension 7735



Northern X Regional Ethics Committee

Ministry of Health
3rd Floor, Unisys Building
650 Great South Road, Penrose
Private Bag 92 522
Wellesley Street, Auckland
Phone (09) 580 9105
Fax (09) 580 9001

Email: pat_chaine@moh.govt.nz

Please quote the above ethics committee reference number in all correspondence.

24 July 2007

Ms Shamshad Karatela
Faculty of Health & Environmental Sciences
Auckland University of Technology
PB 92 006
Auckland 1142

Dear Shamshad

NTX/07/05/050 **Health of Pacific children: environmental and nutritional determinants (Healthy End): PIS/Cons V#3, 17/07/07.**
Principal Investigator: Ms Shamshad Karatela
Supervisor: Dr Janis Paterson
Co-investigators: Prof. Philip Schluter, Dr Richard Anstiss
Auckland University of Technology

Thank you for the requested changes, received 18 July 2007. The above study has now been given ethical approval by the **Northern X Regional Ethics Committee**. A list of members of this committee is attached.

Approved Documents

- Participant Information Sheet/Consent Form V#3 dated 17 July 2007
- Child Information Sheet/Assent Form V#3 dated 17 July 2007
- Questionnaires (7 April 2007).

Certification

The Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out.

Accreditation

The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Progress Reports

The study is approved until 30 July 2009. However, the Committee will review the approved application annually and notify the Principal Investigator if it withdraws approval. It is the Principal Investigator's responsibility to forward a progress report covering all sites prior to ethical review of the project on **24 July 2008**. The report form should be sent to you 2 months prior to this date but if not, it is available on <http://www.newhealth.govt.nz/ethicscommittees> (progress reports). Please note that failure to provide a progress report may result in the withdrawal of ethical approval.

Final Report

A final report is required at the end of the study. The report form is available on <http://www.newhealth.govt.nz/ethicscommittees> (progress reports) and should be forwarded along with a summary of the results. If the study will not be completed as advised, please forward a progress report and an application for extension of ethical approval one month before the above date.

Requirements for SAE Reporting

The Principal Investigator will inform the Committee as soon as possible of the following:

- Any serious adverse events occurring during the study which are considered related to the study.

All SAE reports must be signed by the Principal Investigator and include a comment on whether he/she considers there are any ethical issues relating to this study continuing due to this adverse event. It is assumed by signing the report, the Principal Investigator has undertaken to ensure that all investigators are made aware of the event.

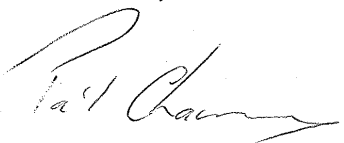
Amendments

All amendments to the study must be advised to the Committee **prior** to their implementation, except in the case where immediate implementation is required for reasons of safety. In such cases the Committee must be notified as soon as possible of the change.

The Principal Investigator is responsible for advising any other study sites of approvals and all other correspondence with the Ethics Committee.

It should be noted that Ethics Committee approval does not imply any resource commitment or administrative facilitation by any healthcare provider within whose facility the research is to be carried out. Where applicable, authority for this must be obtained separately from the appropriate manager within the organisation.

Yours sincerely



Pat Chainey
Administrator
Northern X Regional Ethics Committee

Appendix 3: Ethics Approval for Study 2 and Study 3



e-mail: pat_chailey@moh.govt.nz

Northern X Regional Ethics Committee

Ministry of Health
3rd Floor, Unisys Building
650 Great South Road, Penrose
Private Bag 92 522
Wellesley Street, Auckland
Phone (09) 580 9105
Fax (09) 580 9001

Please note postal address: Administrator, Northern X Regional Ethics Committee, PB 92 522 Wellesley St, Auckland 1141
Phone: 09 580 9105

5 May 2009

Ms Shamshad Karatela
Faculty of Health & Environmental Sciences
Auckland University of Technology
PB 92 006
Auckland 1142

Dear Shamshad

**NTX/07/05/050 Health of Pacific children: environmental and nutritional determinants
(Healthy End): PIS/Cons V#4, 29/04/09**

Principal Investigator: Ms Shamshad Karatela
Co-Investigators: Dr Janis Paterson, Prof Philip Schluter, Dr Richard Anstiss

Thank you for your letter dated 26 April 2009

The request for an amendment was reviewed by the Deputy Chairperson of the Northern X Regional Ethics Committee under delegated authority.

Ethical approval has been given for :

- Amendment to change sampling from hair samples to toe nail clippings which will be more culturally appropriate
- Participant Information Sheet V#4 dated 29 April 2009
- Child Information Sheet and Assent Form for Parent/Caregiver V#4 dated 29 April 2009

Yours sincerely,

Pat Chailey
Administrator
Northern X Regional Ethics Committee

Appendix 4: Ethics Approvals for Study 3



Northern Y Regional Ethics Committee

Ministry of Health
3rd Floor, BNZ Building
354 Victoria Street
PO Box 1031
Hamilton
Phone (07) 858 7021
Fax (07) 858 7070
Email: northerny_ethicscommittee@moh.govt.nz

23 March 2009

Dr Janis Paterson
Faculty of Health & Environmental Sciences
Auckland University of Technology
Private Bag 92006
Auckland 1020

Dear Dr Paterson

The Core Pacific Islands Families Study: Towards Adolescence (PIF: TA)
Investigators: Prof Janis Paterson, ~~Dr Teuila Percival~~, Dr Teuila Percival.
Ethics ref: NTY/08/12/119
Locations: AUT University.

The above study has been given ethical approval by the **Northern Y Regional Ethics Committee**.

Approved Documents

- Child assessment protocol – 9-year phase.
- Primary interview protocol – 9-year phase.
- 9 year info summary and the Information Sheet (child) version 3 (6/03/09)
- 9 yr consent form: primary version 3 (6/03/09)
- 9 yr consent form: 2010-11 consent to locate version 3 (6/03/09)
- 9 year info summary (Primary) version 3 (6/03/09)
- 9 year full info (Primary) version 3 (6/03/09)
- Summary 9 yr assessment Information version 3 (6/03/09)
- 9 yr child assessment and teacher assent Information version 3 (6/03/09)

Accreditation

The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Progress Reports

The study is approved until **15 June 2013**. The Committee will review the approved application annually and notify the Principal Investigator if it withdraws approval. It is the Principal Investigator's responsibility to forward a progress report covering all sites prior to ethical review of the project in **23 March 2010**. The report form is available at <http://www.ethicscommittees.health.govt.nz>. Please note that failure to provide a progress report may result in the withdrawal of ethical approval. A final report is also required at the conclusion of the study.

Amendments

It is also a condition of approval that the Committee is advised of any adverse events, if the study does not commence, or the study is altered in any way, including all documentation eg advertisements, letters to prospective participants.

Please quote the above ethics committee reference number in all correspondence.

It should be noted that Ethics Committee approval does not imply any resource commitment or administrative facilitation by any healthcare provider within whose facility the research is to

be carried out. Where applicable, authority for this must be obtained separately from the appropriate manager within the organisation.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Amrita Kuruvilla', with a horizontal line underneath.

Amrita Kuruvilla
Northern Y Ethics Committee Administrator

Email: amrita_kuruvilla@moh.govt.nz

23 March 2009

Dr Janis Paterson
Faculty of Health & Environmental Sciences
Auckland University of Technology
Private Bag 92006
Auckland 1020

Dear Dr Paterson

Pacific Island Families Study: Nutrition, Body Size & Physical Activity of 9 year old children (PIF:NBS-2).

Investigators: **Prof Janis Paterson, Prof Elaine Rush, Dr Teuila Percival, Prof Grant Schofield.**

Ethics ref: **NTY/08/12/118**

Locations: **AUT University.**

The above study has been given ethical approval by the **Northern Y Regional Ethics Committee.**

Approved Documents

- Child assessment protocol – 9-year phase.
- Primary interview protocol – 9-year phase.
- Teacher report.
- 9 year info summary and the Information Sheet (child) version 3 (6/03/09)
- 9 yr consent form: primary version 3 (6/03/09)
- 9 yr consent form: 2010-11 consent to locate version 3 (6/03/09)
- 9 year info summary (Primary) version 3 (6/03/09)
- 9 year full info (Primary) version 3 (6/03/09)
- Summary 9 yr assessment Information version 2 (23/01/09)

Accreditation

The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Progress Reports

The study is approved until **15 June 2013**. The Committee will review the approved application annually and notify the Principal Investigator if it withdraws approval. It is the Principal Investigator's responsibility to forward a progress report covering all sites prior to ethical review of the project in **23 March 2010**. The report form is available at <http://www.ethicscommittees.health.govt.nz>. Please note that failure to provide a progress report may result in the withdrawal of ethical approval. A final report is also required at the conclusion of the study.

Amendments

It is also a condition of approval that the Committee is advised of any adverse events, if the study does not commence, or the study is altered in any way, including all documentation eg advertisements, letters to prospective participants.

Please quote the above ethics committee reference number in all correspondence.

Appendix 5: Participant sheets, Consent/Assent and questionnaires for Study 1



Health of Pacific Children: Environmental and Nutritional Determinants

Adult Consent Form

English	I wish to have an interpreter.	Yes	No
Māori	E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.	Ae	Kao
Cook Island	Ka inangaro au i tetai tangata uri reo.	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu.	E	Nakai
Samoan	Ou te mana'o ia i ai se fa'amatala upu.	Ioe	Leai
Tokelaun	Ko au e fofou ki he tino ke fakaliliu te gagana Peletania ki na gagana o na motu o te Pahefika	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea.	Io	Ikai

I have read and I understand the information sheet dated 17/07/07 for volunteers taking part in the study designed to improve health of Pacific children. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

I have had the opportunity to discuss this study and I am satisfied with the answers I have been given.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect me or my family in anyway.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.

I consent to the destruction of the hair sample at the end of the study.

YES/NO

I consent to hair samples being sent to a laboratory for chemical analysis.

YES/NO

I understand that if I consent to such analysis, no rights will be created for the researcher/sponsor to my genetic information.

I consent to accessing my dental records from my dentists, dental practitioners, dental therapists, dental hygienists or school dental services.

YES/NO

I wish to receive a copy of final report on the study.

YES/NO

I _____ (full name) hereby consent to take part in this study.

Signature _____ Date _____

Researchers:

Shamshad Karatela Phone: (09) 921 9999 ext 7499
Janis Paterson Phone: (09) 921 9999 ext 7324
Philip Schluter Phone: (09) 921 9999 ext 7700
Richard Anstiss Phone: (09) 921 9999 ext 8826

Project explained by: Shamshad Karatela (Researcher)

Researcher: _____

Signature _____

Date _____

If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health & Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:

Phone (NZ wide): 0800 555 050; Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)

Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.



Health of Pacific Children:
Environmental and Nutritional Determinants

Child information sheet and assent form

(Parent/Caregiver Please Read to Child)

I would like to invite you to take part in an interesting project about food, teeth and health. I would like to visit you and your mum so that we can say hi and I can tell you about my project. We would like to invite lots of different boys and girls and their mums to be part of this project. You can ask me anything about this project whenever you want to.

If you are not sure about anything, please ask your mum or dad about this or you can also talk to me.

I am finding out about food that we eat; about our teeth and how it affects our health and behaviour– you might like to find out about this as well.

If you are happy for me to see you, then I will come to your home and we will go through some interesting food and teeth questions and talk about some of the things you eat. I will also collect some hair that has already been cut by your mum, dad or a hair dresser. I will take it with me to do the project. That's all there is to it!

Please circle **YES** if you would like to take part in the project.

Please circle **NO** if you do not want to take part

I hope we can do this together. It will be great to meet you and to talk to you about my project.

Thank you for completing this form – Please will you ask your mum, dad or caregiver to sign here:

.....(Signature)

.....(Date)

Chapter 10

Chapter 11 *Contact details are as follows:*

Researchers:

Shamshad Karatela	Phone: (09) 921 9999 ext 7499
Janis Paterson	Phone: (09) 921 9999 ext 7324
Philip Schluter	Phone: (09) 921 9999 ext 7700
Richard Anstiss	Phone: (09) 921 9999 ext 8826

**If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health & Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Phone (NZ wide): 0800 555 050; Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz**

This study has received ethical approval from the Northern X Regional Ethics Committee.



Health of Pacific Children:

Environmental and Nutritional Determinants

Participant Information Sheet

Talofa lava, Malo e lelei, Bula vinaka, Fakalofa lahi atu, Kia orana, Kia ora, Welcome

Invitation

You are invited to take part in the “Health of Pacific Children” study which will look at mercury measured in mothers and children’s hair and its relationship to children’s health and behaviour. Mercury enters all our bodies through what we eat or drink, the air we breathe, through our skin or even from teeth fillings. Please remember that:

- Your participation in this study is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part this will in no way affect your future health care.
- If you do agree to take part you are free to withdraw at any time, without having to give a reason. This will in no way affect your future health care.

This information sheet will explain the research study. Please feel free to ask about anything you do not understand or if you have questions at anytime.

What is the purpose of the study?

We are trying to find out how much mercury Pacific children and their mothers have in their bodies and if children have any problems with their health or behaviour because of this mercury.

How are people chosen to be asked to be part of this study?

You have been chosen because you are part of the Pacific Island Family (PIF) study which started when your child was born. This study is inviting a small group of mothers and children from the PIF study to take part.

What will happen during the study?

If you agree to take part in this study, you will be asked to sign a consent form. Then we will ask you to:

- Complete a dietary and dental questionnaire which will take approximately 5 minutes.

- Give permission to access you and your child's dental records from your dentists, dental practitioners, dental therapists, dental hygienists or school dental services.
- Provide a small sample of scalp hair (as in the picture below) as using hair is a scientifically valid way of measuring mercury. This will not affect your hair style nor will it be noticeable where it has been cut. This will take 5 minutes and we aim to collect it when it is convenient for you (now or when you have a hair-cut).



How will this study help?

By taking part in this research you will help us know how much mercury is in Pacific children's bodies and if this level of mercury is bad for their health and if it effects the behaviour of children. Also, we want to find which food and environments might be best avoided to reduce mercury exposure.

What are the costs of taking part?

Taking part in this research will NOT cost you anything except about 15 minutes of your time.

How will your privacy be protected?

All information you give will be kept confidential and your name will not be known to anyone but the researcher. Your questionnaire and hair samples will be given a code and the results will only use this code. We will keep the consent forms, questionnaires and hair samples locked in a cabinet, in separate locations. Any reports, we'll make sure that you cannot be identified. On completion of the hair analysis – all hair samples will be destroyed (burnt) at the end of the study.

What will happen with the results?

The results of this study will be published in a medical journal. The results of this study will also inform the Ministry of Health and other public health organisations.

Will you be able to have a copy of the results?

The results of our research are not likely to be available in the near future but after 2-3 years. However, we are happy to keep you updated with the project if you so wish.

If you have any concerns or questions?

If you have any questions please feel free to contact one of the researchers:

Shamshad Karatela Phone: (09) 921 9999 ext 7499 Email: skaratel@aut.ac.nz

Janis Paterson Phone: (09) 921 9999 ext 7324 Email: JPATERSO@aut.ac.nz

Philip Schluter Phone: (09) 921 9999 ext 7700 Email: philip.schluter@aut.ac.nz

Richard Anstiss Phone: (09) 921 9999 ext 8826 Email: Ranstiss@aut.ac.nz

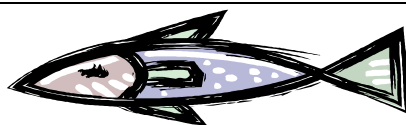
If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health & Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:

Phone (NZ wide): 0800 555 050 ; Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)

Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.

Mother's Seafood Consumption & Dental Health Questionnaire



All answers are confidential

Instructions

This questionnaire contains two parts:

Part A:

Seafood consumption questions are divided into 4 sections:

1. Fresh or frozen fish (fish fillets, fish steaks or whole fish) consumption
2. Canned, processed or pre-prepared fish food consumption
3. Shellfish/crustaceans consumption, and
4. Other fish/fish-product information.

These questions will take you 5 minutes to complete.

Part B:

Dental health part consists of 6 questions and will take you 2 minutes to complete.

Please answer all questions if you can from parts A and B. If at anytime you are not sure of what to do, or cannot answer a question, please feel free to contact any of the study team below (or anyone listed in the information sheet).

Contact details:

Project Investigator:

Ms Shamshad Karatela

Tel: 09 921 9999 x 7499

Project Supervisors:

Assoc Professor Janis Paterson Tel: 09 921 9999 x 7324

Professor Philip Schluter Tel: 09 921 9999 x 7700

Dr Richard Anstiss Tel: 09 921 9999 x 8826

Thank you for your help!

Please write the date you filled the questionnaire in.

--	--	--

PART A: SEAFOOD CONSUMPTION



Section A1.

Do you eat fresh or frozen fish (fish fillet, fish steak or whole fish)?

Yes

☐

No

☐

If you do, how often do you eat fresh or frozen fish (fillets, fish steak or whole fish)?

Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.

Please choose the main type of fish you usually eat. (If you eat more than 1 type, can you rank the type you eat most with '1', the next most with '2').

Salmon		Gem fish	
Cream fish		Orange Roughy	
Snapper		King fish	
Skate wing		Albacure/Yellowfin	
Hake		Skippy Jack	
Ruby		Pilchards	
Gurnard		Sardine	
Cardinal		Mackerel	
Tuna		Herring	
Monk fish		Trout	
Frost fish		Kippers	
Mullet		Hoki	
Kahwai		Dory	
Trumpeter/Tropical fish		Marlin	
Lemon fish/Shark		Gem Fish	
Hapuka/Grouper/Cod		Flounder	

Tarakihi		OTHER (name):
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Section A2.

Do you eat canned, processed or pre-prepared fish foods?

Yes ☐ No ☐

If you do, how often do you eat these fish foods?

	Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.
Fish and chips						
Fish cakes/Fish sticks						
Dried or salted fish						
Canned Tuna						
Canned Sardines						
Canned Mackerel						
Canned Oysters						
Canned Mussels						
Canned crustaceans (prawn, crab meat)						
Fish roe/caviar						
Raw fish (tuna, salmon)						
OTHER (name):						

Section A3.

Do you eat shellfish/crustaceans?

Yes ☐ No ☐

If you do, how many times do you eat the following shellfish/crustaceans?

	Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.
Shrimps/prawns						
Lobster/crayfish						
Crab						
Scallops						
Mussels						
Oysters						
Squid/octopus (Calamari)						
Pippis						
Cockles						
OTHER (name):						

Section A4.

Do you eat any other seafood not mentioned above?

Yes

☐

No

☐

If you do, please specify this seafood and how regularly this was eaten:

Do you (or someone) in your family catch fish that you eat?

Yes

☐

No

☐

If yes, please specify how often this was done and the main type of fish caught?

Do you/someone in your family gather shellfish to eat?

Yes

☐

No

☐

If yes, please specify how often this was done and the main type of shellfish gathered?

Do you take fish oil supplements ?

Yes ☐

No ☐

If you take these supplements, how often?

Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Please specify name and brand: _____

How much seafood did you eat when you were pregnant with your 7-year old child?

Less than now	About the same as now	More than now
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

PART B: DENTAL HEALTH



1) Have you ever been to a dentist?

Yes ☐

No ☐

If yes:

- Did you see a dentist in the last year for:**

General checkup ☐

Yes ☐

No ☐

Other reason ☐

Yes ☐ No ☐

Please specify: _____

- **Have you ever had any fillings?**

Yes ☐ No ☐

If yes, how many silver (amalgam) fillings do you have now?

☐

2) How many times did you brush your teeth yesterday?

☐

Child Seafood Consumption & Dental Health Questionnaire



All answers are confidential

Instructions

Parent/s or care givers please help your child answer this questionnaire.

This questionnaire contains two parts:

Part A:

Seafood consumption questions are divided into 4 sections:

5. Fresh or frozen fish (fish fillets, fish steaks or whole fish) consumption
6. Canned, processed or pre-prepared fish food consumption
7. Shellfish/crustaceans consumption, and
8. Other fish/fish-product information.

These questions will take you 5 minutes to complete.

Part B:

Dental health part consists of 6 questions and will take you 2 minutes to complete.

Please answer all questions if you can from parts A and B. If at anytime you are not sure of what to do, or cannot answer a question, please feel free to contact any of the study team below (or anyone listed in the information sheet).

Contact details:

Project Investigator:

Ms Shamshad Karatela

Tel: 09 921 9999 x 7499

Project Supervisors:

Assoc Professor Janis Paterson Tel: 09 921 9999 x 7324

Professor Philip Schluter Tel: 09 921 9999 x 7700

Dr Richard Anstiss Tel: 09 921 9999 x 8826

Thank you for your help!

Please write the date you filled the questionnaire in.

—	—	—
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PART A: SEAFOOD CONSUMPTION



Section A1.

Do you eat fresh or frozen fish (fish fillet, fish steak or whole fish)?

Yes

☐

No

☐

If you do, how often do you eat fresh or frozen fish (fillets, fish steak or whole fish)?

Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.

Please choose the main type of fish you usually eat. (If you eat more than 1 type, can you rank the type you eat most with '1', the next most with '2').

Salmon		Gem fish	
Cream fish		Orange Roughy	
Snapper		King fish	
Skate wing		Albacure/Yellowfin	
Hake		Skippy Jack	
Ruby		Pilchards	
Gurnard		Sardine	
Cardinal		Mackerel	
Tuna		Herring	
Monk fish		Trout	
Frost fish		Kippers	
Mullet		Hoki	

Kahwai		Dory	
Trumpeter/Tropical fish		Marlin	
Lemon fish/Shark		Gem Fish	
Hapuka/Grouper/Cod		Flounder	
Tarakihi		OTHER (name):	

Section A2.

Do you eat canned, processed or pre-prepared fish foods?

Yes

☐

No

☐

If you do, how often do you eat these fish foods?

	Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.
Fish and chips						
Fish cakes/Fish sticks						
Dried or salted fish						
Canned Tuna						
Canned Sardines						
Canned Mackerel						
Canned Oysters						
Canned Mussels						
Canned crustaceans (prawn, crab meat)						

Fish roe/caviar						
Raw fish (tuna, salmon)						
OTHER (name):						

Section A3.

Do you eat shellfish/crustaceans?

Yes

☐

No

☐

If you do, how many times do you eat the following shellfish/crustaceans?

	Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.
Shrimps/prawns						
Lobster/crayfish						
Crab						
Scallops						
Mussels						
Oysters						

Squid/octopus (Calamari)						
Pippis						
Cockles						
OTHER (name):						

Section A4.

Do you eat any other seafood not mentioned above?

Yes

☐

No

☐

If you do, please specify this seafood and how regularly this was eaten:

Do you take fish oil supplements?

Yes

☐

No

☐

If you take these supplements, how often?

Never or rarely	Once in the last four weeks	Once in two weeks	1-3 times a week	4-7 times a week	More than 7 times a week.
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Please specify name and brand: _____

PART B: DENTAL HEALTH



1) Have you ever been to a dentist?

Yes

☐

No

☐

If yes:

- Did you see a dentist in the last year for:

General checkup

☐

Yes

☐

No

☐

Other reason

☐

Yes

☐

No

☐

Please specify: _____

- **Have you ever had any fillings?**

Yes

No

If yes, how many silver (amalgam) fillings do you have now?

2) How many times did you brush your teeth yesterday?

Appendix 6: Participant sheets, Consent/Assent for Study 2



Health of Pacific Children: Environmental and Nutritional Determinants

Adult Consent Form

English	I wish to have an interpreter.	Yes	No
Māori	E hiahia ana ahau ki tetahi kaiwhakamaori/kaiwhaka pakeha korero.	Ae	Kao
Cook Island	Ka inangaro au i tetahi tangata uri reo.	Ae	Kare
Fijian	Au gadreva me dua e vakadewa vosa vei au	Io	Sega
Niuean	Fia manako au ke fakaaoga e taha tagata fakahokohoko kupu.	E	Nakai
Samoan	Ou te mana'o ia i ai se fa'amatala upu.	Ioe	Leai
Tokelaun	Ko au e fofou ki he tino ke fakaliliu te gagana Peletania ki na gagana o na motu o te Pahefika	Ioe	Leai
Tongan	Oku ou fiema'u ha fakatonulea.	Io	Ikai

I have read and I understand the information sheet dated 29/04/2009 for volunteers taking part in the study designed to improve health of Pacific children. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

I have had the opportunity to discuss this study and I am satisfied with the answers I have been given.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect me or my family in anyway.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.

I consent to the destruction of the nail sample at the end of the study.

YES/NO

I consent to nail samples being sent to a laboratory for chemical analysis.

YES/NO

I understand that if I consent to such analysis, no rights will be created for the researcher/sponsor to my genetic information.

I consent to accessing my dental records from my dentists, dental practitioners, dental therapists, dental hygienists or school dental services.

YES/NO

I wish to receive a copy of final report on the study.

YES/NO

I _____ (full name) hereby consent to take part in this study.

Signature _____ Date _____

Researchers:

Shamshad Karatela	Phone: (09) 921 9999 ext 7499
Janis Paterson	Phone: (09) 921 9999 ext 7324
Philip Schluter	Phone: (09) 921 9999 ext 7700
Richard Anstiss	Phone: (09) 921 9999 ext 8826

Project explained by: Shamshad Karatela (Researcher)

Researcher: _____

Signature _____

Date _____

If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health & Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Phone (NZ wide): 0800 555 050 ; Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.



Health of Pacific Children:
Environmental and Nutritional Determinants

Participant Information Sheet

Talofa lava, Malo e lelei, Bula vinaka, Fakalofa lahi atu, Kia orana, Kia ora, Welcome

Invitation

You are invited to take part in the “Health of Pacific Children” study which will look at mercury measured in mothers and children’s hair and its relationship to children’s health and behaviour. Mercury enters all our bodies through what we eat or drink, the air we breathe, through our skin or even from teeth fillings. Please remember that:

- Your participation in this study is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part this will in no way affect your future health care.
- If you do agree to take part you are free to withdraw at any time, without having to give a reason. This will in no way affect your future health care.

This information sheet will explain the research study. Please feel free to ask about anything you do not understand or if you have questions at anytime.

What is the purpose of the study?

We are trying to find out how much mercury Pacific children and their mothers have in their bodies and if children have any problems with their health or behaviour because of this mercury.

How are people chosen to be asked to be part of this study?

You have been chosen because you are part of the Pacific Island Family (PIF) study which started when your child was born. This study is inviting a small group of mothers and children from the PIF study to take part.

What will happen during the study?

If you agree to take part in this study, you will be asked to sign a consent form. Then we will ask you to:

- Complete a dietary and dental questionnaire which will take approximately 5 minutes.
- Give permission to access you and your child’s dental records from your dentists,

dental practitioners, dental therapists, dental hygienists or school dental services.

- Provide a small sample of toenails as using toenails is a scientifically valid way of measuring mercury. This will take 5 minutes and we aim to collect it when it is convenient for you.

How will this study help?

By taking part in this research you will help us know how much mercury is in Pacific children's bodies and if this level of mercury is bad for their health and if it effects the behaviour of children. Also, we want to find which food and environments might be best avoided to reduce mercury exposure.

What are the costs of taking part?

Taking part in this research will NOT cost you anything except about 15 minutes of your time.

How will your privacy be protected?

All information you give will be kept confidential and your name will not be known to anyone but the researcher. Your questionnaire and hair samples will be given a code and the results will only use this code. We will keep the consent forms, questionnaires and toenail samples locked in a cabinet, in separate locations. Any reports, we'll make sure that you cannot be identified. On completion of the hair analysis – all toenail samples will be destroyed (burnt) at the end of the study.

What will happen with the results?

The results of this study will be published in a medical journal. The results of this study will also inform the Ministry of Health and other public health organisations.

Will you be able to have a copy of the results?

The results of our research are not likely to be available in the near future but after 2-3 years. However, we are happy to keep you updated with the project if you so wish.

If you have any concerns or questions?

If you have any questions please feel free to contact one of the researchers:

Shamshad Karatela Phone: (09) 921 9999 ext 7499 Email: skaratel@aut.ac.nz

Janis Paterson Phone: (09) 921 9999 ext 7324 Email: JPATERSO@aut.ac.nz

Philip Schluter Phone: (09) 921 9999 ext 7700 Email: philip.schluter@aut.ac.nz

Richard Anstiss Phone: (09) 921 9999 ext 8826 Email: Ranstiss@aut.ac.nz

If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health & Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Phone (NZ wide): 0800 555 050 ; Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.



Health of Pacific Children:
Environmental and Nutritional Determinants

Child information sheet and assent form

(Parent/Caregiver Please Read to Child)

I would like to invite you to take part in an interesting project about food, teeth and health. I would like to visit you and your mum so that we can say hi and I can tell you about my project. We would like to invite lots of different boys and girls and their mums to be part of this project. You can ask me anything about this project whenever you want to.

If you are not sure about anything, please ask your mum or dad about this or you can also talk to me.

I am finding out about food that we eat, about our teeth and how it affects our health and behaviour— you might like to find out about this as well.

If you are happy for me to see you, then I will come to your home and we will go through some interesting food and teeth questions and talk about some of the things you eat. I will also collect some toenails. I will take it with me to do the project. That's all there is to it!

Please circle **YES** if you would like to take part in the project.

Please circle **NO** if you do not want to take part

I hope we can do this together. It will be great to meet you and to talk to you about my project.

Thank you for completing this form – Please will you ask your mum, dad or caregiver to sign here:

.....(Signature)

.....(Date)

Chapter 12

Chapter 13 *Contact details are as follows:*

Researchers:

Shamshad Karatela	Phone: (09) 921 9999 ext 7499
Janis Paterson	Phone: (09) 921 9999 ext 7324
Philip Schluter	Phone: (09) 921 9999 ext 7700
Richard Anstiss	Phone: (09) 921 9999 ext 8826

**If you have any queries or concerns regarding your rights as a participant in this research study, you can contact an independent Health & Disability Advocate. This is a free service provided under the Health & Disability Commissioner Act:
Phone (NZ wide): 0800 555 050; Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz**

This study has received ethical approval from the Northern X Regional Ethics Committee.

Appendix 7: Interviewer information, participant sheets, consent/assent for Study 2 and Study 3



Health of Pacific Children Sub-Study



Interviewer Information Sheet

Beginning in August, we intend to seek permission from approximately 200 mothers to collect toenail samples from their children during the child assessment to investigate levels of the chemical mercury in their bodies. Consent to do this will be sought at the same time as consent for everything else; there will be a **separate section added to the regular consent form** with a 'yes/no' tick box for the collection of toenails.

We need to make it clear to mothers that this is an additional, separate consent which is completely optional. That is, they can agree to the primary interview and the regular child assessment and **not** agree to the extra request for toenails if they choose. Given the potentially sensitive nature of the request, it should be asked without any duress (pressure) and with appropriate explanation if required.

The packs with red stickers on them will be packs containing the versions of the information sheets and consent form with the toenail collection information added in. **Only at interviews conducted with a red-stickered pack should you mention, and request consent for, toenail samples.** If you have a regular interview pack with no red sticker on it, **do not** ask for consent to collect toenail samples.

Below is some information on this sub-study, including information about mercury and our rationale for collecting toenails. Please read it carefully as it will be useful in answering any queries that mothers may have.

What is the chemical mercury?

- It is normal in the body.
- Too much is not good.
- It can affect behaviour/learning.

How do we get mercury in our bodies?

- Mainly from eating seafood, but also through the air we breathe and through teeth fillings.

Why collect toenails?

- We can measure mercury in toenails.

Who will cut the toenails?

- The child will first be asked to cut their own toenails.
- If the child feels they cannot cut their own toenails, the assessor will offer to do it.
- The child does not have to take part if they don't want to.

Why are we doing the study?

- We are not sure if Pacific children have high or low mercury levels, particularly since seafood is a significant part of some Pacific peoples' diets. This study will help us find out whether levels are high or low in Pacific kids and whether it may be affecting their behaviour, learning, and school performance.



Pacific Islands Families (PIF) Study: Towards Adolescence
• ***Nine Year Summary Information for Participants: Primary***

Talofa lava, Malo e lelei, Bula vinaka, Fakalofa lahi atu,
Kia orana, Kia ora, Welcome

• You are invited to take part again in the *Pacific Islands Families (PIF) Study* which will look at the health and development of Pacific children within their family until the children are aged nine. The main benefit of the PIF Study will be to provide information about Pacific family life. Communities can then use this information to provide better health and social services for Pacific families.

• **Participants**

1400 babies born in Middlemore Hospital in the year 2000 who have a permanent resident parent (either mother or father), and who see themselves as being of a Pacific Islands descent were eligible for the Study.

• **What will happen during the study?**

• When your child is **9 years old** you will be visited by a Pacific interviewer to explain more about this phase of the Study and see if you wish to take part.

• Taking part in the 9-year phase will involve agreeing to an interview, at a time suitable for you. If you prefer, this 1-hour interview can take place straight away. The child assessment will take place at their school, or at home if you prefer. A questionnaire will be given to the child's main teacher about their friendships, school work, homework, and behaviour at school.

• You are also invited to take part in the "Health of Pacific Children" study which will look at mercury measured in children's toe nails and its relationship to children's health and behaviour. Mercury enters all our bodies through what we eat or drink (especially seafood), the air we breathe, through our skin, and even from teeth fillings. We are trying to find out how much mercury Pacific children have in their bodies and if they have any problems with their health or behaviour because of this mercury.

• **Important points about the interviews and confidentiality**

- If you agree to take part, you will be asked to sign a consent form.
- You do not have to take part and you can say no to any question or further interviews.
- If you would like to be interviewed using a Pacific language, we will organise an interviewer who speaks your language.
- At the interview you will be asked a wide variety of questions which have been developed in consultation with Pacific communities.
- All information collected will be coded with a number and kept with no names on it in locked storage.
- Reports from this study will never have your name on it.

If you have any questions about this Study please contact Leon Iusitini on (09) 921 9999 extn 7933, or one of the lead researchers below:

Researchers

- Janis Paterson, Co-Director, Faculty of Health & Environmental Sciences, Auckland University of Technology, Private Bag 92006, Auckland 1020, Ph (09) 921 9999 extn 7324
- Gerhard Sundborn, Senior Research Fellow, AUT University, Ph (09) 921 9999 extn 7735

The study has received ethical approval from the Northern Y Regional Ethics Committee

If you have any questions or concerns about your rights as a participant in this research study you can contact an independent health and disability advocate Act. **Telephone 0800 555 050 (NZ wide) Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)** Email: advocacy@hdc.org.nz



Pacific Islands Families (PIF) Study: Towards Adolescence

9 Year Phase

CONSENT FORM: PRIMARY

I have read and have had explained the information sheet dated 14 December 2009 for volunteers taking part in the Study to explore the ways in which Pacific children develop within their family, school and wider environment. I understand the information that has been given to me.

I have had the opportunity to discuss this study and I am satisfied with the answers I have been given. I also understand that taking part in this study is voluntary (my choice) and that I can withdraw my child from the study at any time.

I understand that this will include taking part in an interview and my child taking part in some health and development assessments which include language, vision, height, weight, cognitive development and child experiences (e.g. school life and friendships) when my child is approximately 9 years old. In addition my child's teacher will be asked some questions about my child in the school environment.

I understand that if I join the study no-one else will know except study staff and those health professionals directly involved in the study. The records containing all the answers I give will not have my name on it. I have had time to think about whether to take part and I know to contact the study staff if I have any questions about the study.

I agree to my child providing toe nail clippings to study the relationship between mercury levels in the body with learning and behaviour. I agree to allow study staff access to my child's dental records from his/her dentist, dental practitioner, dental therapist, dental hygienist or school dental service.

Yes

☐

No

☐

I _____ (full name) hereby consent/agree to take part in this study.

I consent to _____ (insert name of interviewer) interviewing me.

Signature: _____

Date: _____

Researchers: Janis Paterson and Gerhard Sundborn

Phone: (09) 921 9999 extn 7324 (Janis Paterson) or extn 7735 (Gerhard Sundborn)

PIF Pacific Project Manager: Junior Tutagalevao

Phone: (09) 921 9999 extn 7260

Interviewer signature:

Date:

Pacific Islands Families (PIF) Study: Towards Adolescence

2010-11 CONSENT TO LOCATE FORM

Over the past 8 years of the PIF Study, we have found that many of our families move on to other homes, making it difficult for our interviewers to find them each year.

We would like your permission to use a number of different ways to help find you and your child to do another interview in the future, if we get continued funding. The only information we would be seeking from the following agencies will be your current address or phone number.

Many parents will move house over the next few years so we will need some extra help to locate you. Please circle "yes" or "no" to each of the ways we have suggested below.

Middlemore Hospital	Yes	No
South Sea Kids	Yes	No
Local Schools	Yes	No
Housing New Zealand	Yes	No

I _____(full name) hereby consent/agree to the PIF Study using the options marked "yes" (above) to help in locating me for the 2011 phase of the study.

Signature: _____

Date: _____

The study has received ethical approval from the Northern Y Regional Ethics Committee.

If you have any questions or concerns about your rights as a participant in this research study you can contact an independent health and disability advocate Act.

Telephone 0800 555 050 (NZ wide) Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)

Email: advocacy@hdc.org.nz



Talofa lava, Malo e lelei, Bula vinaka, Fakalofa lahi atu, Welcome

The PIF Study 9-Year Child Assessment is being carried out in the child's school. This assessment will include the following:

1. A physical growth assessment. These will include simple measures of your child's weight, height, waist, and arms. We will be using a small instrument powered by a small torch battery to measure the amount of water in your child's body (to calculate body fat). This involves putting two small stickers on your child's head and two on their foot. This is not invasive (harmful) in any way and only takes a few minutes.
2. A language development assessment. This involves testing your child's language understanding and expression. The interviewer will do this testing directly with the child.
3. A vision assessment. Your child's vision will be checked using a simple pen and paper vision test. If this test shows that there are possible vision problems we will refer you on to a specialist for further assessments. Children with vision problems will quite often be unaware of their reduced standard of vision and this may affect their learning and behaviour.
4. A cognitive development assessment. This involves testing your child's knowledge and general abilities using a standard measure which has been used in many other studies.
5. Child experiences. Your child will also be asked a few simple questions about things such as their experiences at school, their friendships, their feelings, and leisure activities.
6. Teacher questionnaire. A small questionnaire will be given to the child's main teacher and will ask questions about their friendships, school work, homework, and behaviour at school.
7. Toenail clippings. Your child will be asked to provide toe nail clippings to be able to do chemical (particularly mercury) analysis in the laboratory at no cost. This will tell us if they have any chemicals in their body which may be affecting their school performance. We will also seek access to the child's dental records from his/her dentist, dental practitioner, dental therapist, dental hygienist, or school dental service.

We ask permission for your child to participate in the child assessment that will be carried out by the PIF team at their school, or at home if you prefer. Once again, it should be emphasised that taking part in the PIF Study is entirely voluntary (your choice). If you do agree to take part you are free to withdraw from the study at any time, without having to give a reason. If you have any questions about this Study please contact Leon Lusitini on (09) 921 9999 extn 7933 or one of the researchers below:

Researchers

Janis Paterson, Co-Director, Faculty of Health and Environmental Sciences, AUT University, Private Bag 92006, Auckland 1020, Ph (09) 921 9999 extn 7324

Chapter 14 Gerhard Sundborn, Senior Research Fellow, AUT University, Ph (09) 921 9999 extn 7735

This study has been funded by the Foundation for Research, Science and Technology with support from the Maurice and Phyllis Paykel Trust. The study has received ethical approval from the Northern Y Regional Ethics Committee.

If you have any questions or concerns about your rights as a participant in this research study you can contact an independent health and disability advocate Act.

Telephone 0800 555 050 (NZ wide) Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT)

Email: advocacy@hdc.org.nz

Appendix 8: Results for Study 3- Association between BMI z scores and elements

