An investigation of the potential association between mercury and Autism Spectrum Disorder: an interdisciplinary approach

by

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Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Deakin University

August, 2015
I am the author of the thesis entitled:

An investigation of the potential association between mercury and Autism Spectrum Disorder: an interdisciplinary approach

submitted for the degree of Doctor of Philosophy

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Acknowledgements

I am immensely fortunate to have been well supported throughout the process, dare I say journey, of completing this thesis. It has been a rewarding, challenging and educational period of my life and there are many people to which I would like to extend my gratitude.

Firstly to my supervisor David Austin, a fervently dedicated autism researcher, advocate and parent. The autism field is all the better for your involvement and willingness to challenge fiercely held beliefs. It is not easy to remain standing in the face of such resistance, but the manner in which you back your beliefs is both commendable and inspiring. We have shared many spirited discussions (and the odd coffee or two!) throughout the PhD and preceding ethereapy years. I am as grateful for the disagreements as I am for your understanding and patience, as they have helped shape a more profound independence of thought. Very simply, this thesis and PhD would not have been undertaken or completed without you.

To the (now defunct) SABRI team! It was a wonderful opportunity to have such a knowledgeable team to discuss PhD programmes and the world of autism more broadly. Thank-you to Enzo Palombo and Simon Knowles for the time and knowledge you invested into SABRI and kindly agreeing to be my former associate supervisors. To my fellow autism PhD candidates Christine Brown, Shakuntla Gondalia, and for a brief time Matt Garrecht, it makes a considerable difference working beside others who share a common goal. Shared frustrations and good humour have greatly helped lessen the burden at times. To the Friends of SABRI, much of this PhD would have been impossible without their generous donations and willingness to participate in studies. I hope this completed thesis goes some way to expressing my appreciation of their generosity and that their support has not been
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misplaced. To Jahar Bhowmik, while perhaps not a member of SABRI, your statistical expertise with the final two studies in this thesis was invaluable and I can’t help but to see you as an honorary member of the group. Your easy going manner and proficiency with stats was the balm I needed putting the final paper of the thesis together.

The Pink Disease Support Group (PDSG) played a crucial part in the final study presented in this thesis. Di Farnsworth, the moderator of the PDSG, was unfailingly kind in sharing her extensive knowledge of pink disease and enthusiasm in supporting the study. A great deal was asked of her in assisting in the recruitment process, especially given her declining health. I hope the study has brought some new found attention to the condition and the diminishing opportunity that the population of pink disease survivors provides.

To Britt Klein, along with David you gave me my first start in the academic world not too many years ago. Such strange times, but I’m so grateful to you both for involving me in many different aspects of research. I am in admiration of your drive and ambition and the importance you place on being fair-minded and in trying to create collective opportunities. I hope that I can manage to integrate some of your qualities into my own developing career. I appreciate our many impromptu chats and emails over the years (often at rather odd times!), and am grateful for your interest and support of my work in the autism field.

Finally, this thesis has been supported by the School of Psychology, Deakin University and the Faculty of Life and Social Sciences, Swinburne University of Technology. I am grateful to both universities for the support provided during my time as a PhD student.
List of peer-reviewed publications that comprise this thesis
(in order of presentation)

Shandley, K. & Austin DW. (2010). Reconceptualising Autism: Moving beyond the

Inc.

children with Autism. *Journal of Toxicology and Environmental Health, Part

7(5):535-42.

identified as a risk factor for autism spectrum disorders. *Journal of Toxicology
and Environmental Health, Part A*. 74(18):1185-94.
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“Where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause-and-effect relationships are not fully established scientifically.”

Wingspread Statement on the Precautionary Principle.
Wingspread Consensus Conference,
Wingspread Conference Center, Racine, Wisconsin.
Abstract

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder and, although the pathogenesis of ASD is yet to be established, contemporary research indicates that an environmental trigger may play a critical role. One proposed environmental trigger that has been the subject of considerable interest and controversy is that of mercury (Hg). The mounting evidence that Hg is associated with ASD is compelling, but not definitive. It was the intention of this thesis to explore the potential relationship between Hg and ASD via an interdisciplinary approach. Five published papers are presented in this thesis:

Paper 1a is a critical opinion piece (Chapter 2; Shandley & Austin, 2010). The premise of the paper is that progressive biomedical research on autism has been slow to emerge, possibly due in part to the investigative approach of Leo Kanner and Hans Asperger, and the influential role of their personal backgrounds and clinical training in psychoanalysis. Paper 1b appears as a chapter in a book (Chapter 2; Shandley & Austin, 2011). The chapter is aimed at postgraduate psychology students, encouraging them to become informed users of the Diagnostic and Statistical Manual (DSM) by critically examining the information and criteria for ASD. This chapter extends upon the arguments of paper 1a, with a greater emphasis on the appearance of ASD in the DSM, its revision over subsequent editions, and some of the associated controversies.

Paper 2 reports on the first empirical study presented in this thesis (Chapter 4; Austin & Shandley, 2008). The study analysed the urinary porphyrin profiles of a sample of Australian children diagnosed with ASD. The mean urinary porphyrin levels from this study were compared against the mean levels reported by Nataf et al.
(2006) and Geier and Geier (2007) for their ASD and control groups. In addition, the porphyrin levels from all studies were compared to an external control group of typically developing children (Minder & Schneider-Yin, 1996). The purpose of the study was to establish whether the porphyrin profiles of a sample of Australian children exhibit a pattern indicative of Hg damage. This was a critical first study as no previously published studies had investigated Hg damage among a sample of Australian children diagnosed with ASD by using porphyrin profiles (or by any other means). The hypothesis that the porphyrin levels of the Australian ASD group would be elevated in comparison to the other three control groups was supported. Although there were limitations to this study, the results indicated that a larger, controlled urinary porphyrin study was warranted.

Paper 3 reports on the second empirical study presented in this thesis (Chapter 5; Shandley, Austin & Bhowmik, 2014). This study was designed to address the limitations of the first porphyrin study (small sample size, lack of appropriate control group) and extend upon previous porphyrin studies by determining whether, (1) group membership, and (2) ASD severity could be predicted using porphyrin profiles. Porphyrin levels were compared across three groups of children: (a) children diagnosed with ASD, (b) siblings of children diagnosed with ASD (internal sibling control), and (c) children with no known blood relative diagnosed with an ASD (external control). The study failed to find a difference between urinary porphyrins across the three groups. Furthermore, neither group membership, nor ASD severity could be predicted based upon urinary porphyrins. However, the result needs to be considered in light of the potential unreliability of urinary porphyrins as a measure of Hg damage for younger cohorts.
Paper 4 reports on the final empirical study presented in this thesis (Chapter 6; Shandley & Austin, 2011). This study took a different approach to examine whether an association exists between Hg and ASD. The concept for this study was based on a component of the Hg-autism hypothesis which postulates that ASD develops due to an interaction between a genetic susceptibility and environmental trigger (Bernard et al., 2001). Therefore, this study investigated whether an individual susceptibility to Hg is heritable (and therefore genetic). A cohort of people with a known sensitivity to Hg (survivors of pink disease) was surveyed to determine the prevalence of ASD among their descendants. The ASD prevalence rate was then compared to the prevalence rates for a range of clinical conditions (matched by birth year). The results of the study found the prevalence of ASD among the grandchildren of pink disease survivors to be significantly higher (6-7 fold) than the comparable general population ASD prevalence rate. Furthermore, the results indicate that there was not an elevated risk for disease generally among the pink disease cohort, but rather a specific risk for ASD.

Based upon the studies undertaken in the present thesis it is not possible to definitively conclude whether there is an association between Hg and ASD. The apparent association between Hg and ASD appears to be considerably more complex than proposed and is likely mediated by physiological and/or Hg characteristics. It is clear that several challenging, multifactorial studies will be required to understand the nature of the association between Hg and ASD, if one indeed exists.
Chapter 1: Autism Spectrum Disorder

Overview

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with no known cause or cure. It is a devastating, life-long and often debilitating condition which manifests in the first few years of life. The most recent US figure estimates ASD prevalence to be as high as 1 in 50 among 6-17 year old children (Blumberg et al., 2013), with a male to female ratio of 4:1 (Fombonne, 2003). Although the symptoms and degree of severity vary widely, ASD is characterised by (i) impairments in reciprocal social interactions and verbal and non-verbal communication skills, and (ii) stereotyped behaviour, interests and activities (American Psychiatric Association [APA], 2013). The cost to families and society of a child diagnosed with ASD is considerable, regardless of the child’s cognitive ability (Jarbrink & Knapp, 2001). The annual cost of ASD cases in Australia has been estimated to range from AUD$4.5-$7.2 billion (Synergies Economic Consulting, 2007). The lifetime per capita incremental societal cost of an individual with ASD is estimated to range from US$3.5-$5 million, of which lost productivity and adult care comprise the largest components (Ganz, 2007). For children aged 0-14 years, ASD is the second leading cause of burden of disease/injury for males and eighth for females (Begg et al. 2007).

Diagnosis

Diagnostic and Statistical Manual of Mental Disorders

Autism: Eugen Bleuler, a Swiss psychiatrist invented the term autism in 1910-11 to provide a rich description of schizophrenia (Grinker, 2007). It is in this context
that the term autism first appeared in the Diagnostic and Statistical Manual of Mental Disorders (DSM) first edition and subsequent second edition in 1952 and 1968 respectively (APA, 1952, 1968). In contrast to our understanding today of autism as a discrete diagnostic entity, the term was used purely as a behavioural descriptive under the category of ‘schizophrenia, childhood type.’

In a paper published in 1943 in the now defunct journal *Nervous Child*, Leo Kanner, an Austrian-born, German-educated psychiatrist, published his seminal paper ‘Autistic disturbances of affective contact’ in which he describes in great detail the behavioural characteristics and ‘peculiarities’ of 11 children and their parents (Kanner, 1943). It is in this paper that Kanner first introduces a new condition which he labelled ‘early infantile autism.’ Although Kanner did not specify diagnostic criteria, he did note that “the outstanding, ‘pathognomonic,’ fundamental disorder is the children’s *inability to relate themselves* in the ordinary way to people and situations from the beginning of life (p.242, quotation and italics in the original).”

Some years later, following the release of the first edition of the DSM, Kanner and Eisenberg (1956) used Kanner’s narratives to identify six diagnostic features: (a) a profound lack of affective contact with other people, (b) an anxiously obsessive desire for the preservation of sameness in the child’s routines and environment, (c) a fascination for objects, which are handled with skill in fine motor movements, (d) mutism or a kind of language that does not seem intended for inter-personal communication, (e) good cognitive potential shown in feats of memory or skills on performance tests, and (f) onset from birth or before 30 months. Two diagnostic criteria were regarded as essential to a diagnosis of autism: (1) a profound lack of affective contact, and (2) elaborate repetitive, ritualistic behaviour. However, autism did not appear in the DSM as a clinical condition until the third edition as ‘infantile
autism’ within a new class of conditions titled Pervasive Developmental Disorders (APA, 1980).

Rutter (1978) was widely attributed for operationalizing Kanner’s narratives into the definition of infantile autism published in the DSM-III. This also marked the introduction of the ‘triad of impairments’ or ‘triad of autistic behaviours,’ whereby a diagnosis of autism was made if a child demonstrated gross deficiencies in (a) social interaction and (b) language development (relative to their age), and (c) displayed bizarre and/or repetitive behaviours (APA, 1980). The DSM-III criteria also includes: (d) pervasive lack of responsiveness to others, (e) absences of delusions, hallucinations, loosening of associations, and incoherence as in Schizophrenia, and (f) onset before 30 months of age.

Substantial revisions were made in the DSM-III-R, with the criteria broadened to reflect a developmental orientation and the heterogeneity of autism (APA, 1987). The DSM-III-R criteria comprised of 16 items, eight of which must be present, with a minimum of two items from social interaction, one from communication and one from repertoire of activities and interests required for a diagnosis to be made (see Table 1). The age of onset was also increased by 6 months (from 30 to 36 months of age). Additionally, the name was changed from early infantile autism to Autistic Disorder. The subsequent fourth edition and text revision largely retained the same definition (APA, 1994, 2000), although the number of diagnostic criteria was reduced in the DSM-IV-TR with a minimum of 6 out of 12 items required to be present for a diagnosis to be made (see Table 2).
Table 1

Diagnostic criteria for Autistic Disorder (DSM-III-R, 1987)

At least eight of the following sixteen items are present, these to include at least two items from A, one from B, and one from C.

A. Qualitative impairment in reciprocal social interaction as manifested by the following

1. Marked lack of awareness of the existence or feelings of others;
2. No or abnormal seeking of comfort at times of distress;
3. No or impaired imitation;
4. No or abnormal social play; and
5. Gross impairment in ability to make peer friendships.

B. Qualitative impairment in verbal and nonverbal communication and in imaginative activity, as manifested by the following:

1. No mode of communication, such as, communicative babbling, facial expression, gesture, mime, or spoken language;
2. Markedly abnormal nonverbal communication, as in the use of eye-to-eye gaze, facial expression, body posture, or gestures to initiate or modulate social interaction;
3. Absence of imaginative activity, such as play-acting of adult roles, fantasy character or animals; lack of interest in stories about imaginary events;
4. Marked abnormalities in the production of speech, including volume, pitch, stress, rate, rhythm, and intonation;
5. Marked abnormalities in the form or content of speech, including stereotyped and repetitive use of speech; use of "you" when "I" is meant; idiosyncratic use of words or phrases; or frequent irrelevant remarks; and
6. Marked impairment in the ability to initiate or sustain a conversation with others, despite adequate speech;

C. Markedly restricted repertoire of activities and interests as manifested by the following:

1. Stereotyped body movements;
2. Persistent preoccupation with parts of objects or attachment to unusual objects;
3. Marked distress over changes in trivial aspects of environment;
4. Unreasonable insistence on following routines in precise detail;
5. Markedly restricted range of interests and a preoccupation with one narrow interest

D. Onset during infancy or early childhood

- Specify if childhood onset (after 36 months of age)
Table 2

Diagnostic criteria for Autistic Disorder (DSM-IV-TR, 2000)

A. A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3):

1. *Qualitative impairment in social interaction, as manifested by at least two of the following:*
   
   a. Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction
   
   b. Failure to develop peer relationships appropriate to developmental level
   
   c. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people
   
   d. Lack of social or emotional reciprocity

2. *Qualitative impairments in communication as manifested by at least one of the following:*
   
   a. Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
   
   b. In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
   
   c. Stereotyped and repetitive use of language or idiosyncratic language
   
   d. Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

3. *Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:*
   
   a. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
   
   b. Apparently inflexible adherence to specific, nonfunctional routines or rituals
   
   c. Stereotyped and repetitive motor manners
   
   d. Persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play.

C. The disturbance is not better accounted for by Rett’s Disorder or Childhood Disintegrative Disorder
Asperger’s Disorder: Hans Asperger, an Austrian-born psychiatrist and paediatrician, published the first definition of Asperger’s Disorder in 1944 (Asperger, 1944). Although Asperger’s paper only described four young boys, he discerned a pattern of behaviour and abilities that he called ‘autistic psychopathology.’ While Asperger identified his self-named disorder around the same time that Kanner described autism, it was not included as a discrete clinical condition in the DSM until the fourth edition (and continued in the text revision of this edition) (APA, 1994, 2000). Asperger’s paper was not translated into English until 1981 (Frith, 1991), and it is possible that this delay affected the timing of the appearance of Asperger’s Disorder in the DSM. The primary difference between Asperger’s Disorder and Autistic Disorder in the DSM-IV and DSM-IV-TR is that there is comparatively less disability in Asperger’s. This is reflected in the diagnostic requirement in that only two of the ‘triad of autistic impairments’ be present (see Table 3).

Autism Spectrum Disorder: In May 2013, ASD was formally established as a discrete diagnostic entity in the fifth edition of the DSM, replacing the individual diagnostic labels of Autistic Disorder, Asperger’s Disorder, Pervasive Developmental Disorder–Not Otherwise Specified (PDD-NOS), and Childhood Disintegrative Disorder (APA, 2013). A second major change in the DSM-5 is the reduction of three diagnostic domains to two, with social interaction and communication collapsed into one domain (see Table 4). Formerly, the diagnostic label itself designated the clinical severity, however, the DSM-5 separates severity into three levels each for social communication and restricted, repetitive behaviors based upon level of support required with level one needing the least support to level three needing the most.
Table 3

Diagnostic criteria for Asperger’s Disorder (DSM-IV-TR, 2000)

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<td>A.</td>
<td>Qualitative impairment in social interaction, as manifested by at least two of the following:</td>
</tr>
<tr>
<td></td>
<td>1. Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction</td>
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<tr>
<td></td>
<td>2. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people</td>
</tr>
<tr>
<td></td>
<td>3. Failure to develop peer relationships appropriate to developmental level</td>
</tr>
<tr>
<td></td>
<td>4. Lack of social or emotional reciprocity</td>
</tr>
<tr>
<td>B.</td>
<td>Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:</td>
</tr>
<tr>
<td></td>
<td>1. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus</td>
</tr>
<tr>
<td></td>
<td>2. Apparently inflexible adherence to specific, nonfunctional routines or rituals</td>
</tr>
<tr>
<td></td>
<td>3. Stereotyped and repetitive motor mannerisms</td>
</tr>
<tr>
<td></td>
<td>4. Persistent preoccupation with parts of objects</td>
</tr>
<tr>
<td>C.</td>
<td>The disturbance causes clinically significant impairment in social, occupational, or other important areas of functioning.</td>
</tr>
<tr>
<td>D.</td>
<td>There is no clinically significant general delay in language.</td>
</tr>
<tr>
<td>E.</td>
<td>There is no clinically significant delay in cognitive development or in the development of age-appropriate self-help skills, adaptive behavior (other than in social interaction), and curiosity about the environment in childhood.</td>
</tr>
<tr>
<td>F.</td>
<td>Criteria are not met for another specific Pervasive Developmental Disorder or Schizophrenia</td>
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Changes in the DSM-5 have been met with a great deal of apprehension. There is concern that the new diagnostic criteria will fail to identify some individuals thereby restricting their access to services and sources of funding (Mayes, Black & Tierney, 2013). Anger has also been leveled at the decision to remove the label Asperger’s Disorder and group it with the more severe condition autism. Arguments reflect, in part, a loss of identity and fear of being stereotyped alongside autism (Giles, 2014).
The diagnostic process

The diagnosis of ASD typically relies on a behavioural assessment conducted by a trained psychologist or psychiatrist. A considerable number of screening and diagnostic instruments are available, although the Autism Diagnostic Observation Schedule (Lord, Rutter, DiLavore & Risi, 2001) is generally considered the gold standard. Of paramount importance to the long-term well-being and development of the child is a reliable and timely diagnosis. Logically the earlier an accurate diagnosis can be made, the earlier suitable interventions can commence. A report prepared by Synergies Economic Consulting (2012) for the Australian Autism Early Intervention Outcomes Unit (AEIOU) Foundation provides a comprehensive outline of studies demonstrating the beneficial impacts of early intervention strategies in relation to education (i.e., improved cognitive functioning [IQ], decreased need of special schooling), and improved employment outcomes, independence, and quality of life for the affected individual and their families.

A fundamental challenge to the diagnosis of ASD is the reliance on the observation of a set of behavioural symptoms. Essentially this means that the diagnostic process requires that the child reach an age whereby the behaviours would typically be present (at least 2-3 years). Although recent research indicates that signs of ASD may be present as young as 12 months of age (Barbaro & Dissanayake, 2010), questions of reliability arise, especially for children presenting with symptoms consistent with the lower end of the spectrum and those with comorbid conditions. Emerging research also indicates that ASD may be a biological disorder of brain development/function (Voineagu et al., 2011) suggesting the possibility of molecular testing, in conjunction with clinical evaluation, as another avenue in the diagnostic process. However, at this time, diagnosis remains an inherently subjective process.
Table 4

Diagnostic criteria for Autism Spectrum Disorder (DSM-5, 2013)

1. Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history:
   - Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interest, emotions, or affect; to failure to initiate or respond to social interactions.
   - Deficits in nonverbal communicative behaviors used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of gestures; to a total lack of facial expression and nonverbal communication.
   - Deficits in developing, maintaining, and understanding relationships, ranging, for example, from difficulties adjusting behavior to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers.

Specify current severity:
   - Severity is based on social communication impairments and restricted, repetitive patterns of behavior.

2. Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least two of the following, currently or by history:
   - Stereotyped or repetitive motor movements, use of objects, or speech.
   - Insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior.
   - Highly restricted, fixed interests that are abnormal in intensity or focus.
   - Hyper- or hypo-reactivity to sensory input or unusual interest in sensory aspects of the environment.

Specify current severity:
   - Severity is based on social communication impairments and restricted, repetitive patterns of behavior.

1. Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life).

2. Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning.

3. These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum
**Prevalence**

The first epidemiologic study of autism was conducted in 1966 in England (Wing, 1993). The study applied Kanner’s criteria to a large, population-based methodology establishing a prevalence rate of 4-5 cases per 10,000, or approximately 1 in 2500 children. Other epidemiological studies published prior to 1985 using Rutter’s criteria and variations thereof, found prevalence rates ranging from 1.9 to 6.0 cases per 10,000 children (Centers for Disease Control [CDC], 2001; Yeargin-Allsopp & Rice, 2004). Prevalence figures from published studies between 1985 and 1995 indicate that the number of autism cases doubled with the mean prevalence rate reaching 11.8 cases per 10,000 children (CDC, 2001). Similarly, Fombonne (2005) reported an increase from 4.4 to 12.7 cases per 10,000 children between studies published in 1966 to 1991 and 1992 to 2001. ASD prevalence rates reported by the Autism and Developmental Disabilities Monitoring network show an increase from 6.7 cases per 1,000 (approximately 1 in 150) in 2000 to 11.3 cases per 1000 (approximately 1 in 88) in 2008 among 8-year old children (CDC, 2007, 2012). The most recent US figure to date estimate ASD prevalence to be as high as 1 in 50 among 6-17 year old children (Blumberg et al., 2013). In Australia, a national study determined that the prevalence rate is 1 in 160 children aged 6-12 years (MacDermott, Williams, Ridley, Glasson & Wray, 2008). The data was based on ASD cases (autism and Asperger’s) known to Centrelink (an Australian federal government social service agency) for 2005. More recent estimates suggest Australian ASD prevalence may be as high as 1 in 119 children (Barbaro & Dissanayake, 2010).

It has been argued that the rise in ASD prevalence can be fully accounted for by widening diagnostic criteria, greater education and awareness, diagnostic
substitution and an increase in service availability (Fombonne, Zakarian, Bennett, Meng & McLean-Heywood, 2006). At this point in time, there is no definitive research as to whether the rise in ASD prevalence is genuine or simply an artefact of the aforementioned factors. Until such a time as a conclusion is reached, it is prudent to assume that the rise is at least partially based on a genuine increase in cases and, importantly, that ASD is a major public health issue.

**Gender and Age Features**

Males are approximately four times more likely to meet the criteria for ASD than females (Fombonne, 2003). Although the reason for the higher prevalence of ASD among males is yet to be determined (Yeargin-Allsopp & Rice, 2004), there is some suggestion that ASD may go unrecognised among females due to the manifestation of milder social and communication difficulties (Rivet & Matson, 2011). Genetic differences have also been hypothesised for the difference in diagnosis between males and females (Constantino & Charman, 2012; Sato et al., 2012)

ASD typically manifests in early infancy where signs of abnormal development are present within the first 24 months of life. ASD may also present in a regressive form whereby abnormalities in social interaction, communication and/or play behaviour appear abruptly following apparent normal development (Baron-Cohen, 2002).
Differential Diagnosis and Comorbidity

Differential diagnosis

To diagnose ASD according to the DSM-5 diagnostic criteria, the assessor must first eliminate a number of other conditions, specifically, Rett syndrome; selective mutism; language disorders and social (pragmatic) communication disorder; intellectual disability (ID) without ASD; stereotypic movement disorder; attention-deficit/hyperactivity disorder (ADHD); and schizophrenia.

Comorbidity

ASD is commonly associated with a wide range of intellectual/developmental, psychiatric, medical and genetic disorders. Approximately 70% of individuals with ASD also meet the diagnostic criteria for ID (LaMalfa, Lassi, Bertelli, Salvini & Placidi, 2004). Learning difficulties and developmental coordination disorder are also reportedly common (Baird, Douglas & Murphy, 2011). A meta-analysis of 23 studies conducted by Amiet et al. (2008) showed that the pooled prevalence rates of epilepsy ranged from 8% (for those without comorbid ID) to 21.4% (for those with comorbid ID). ASD has been associated with increased risks of autoimmune disease, specifically, asthma, allergic rhinitis, atopic dermatitis, urticarial, and type 1 diabetes (Chen et al., 2013), in addition to sleep problems (Hoffman, Sweeney, Gilliam & Lopez-Wagner, 2006), gastrointestinal symptoms (Molloy & Manning-Courtney, 2003), sensory sensitivities (Ben-Sasson et al., 2009; Bogdashina, 2003) and low muscle tone (De Jong, Punt, De Groot, Minderaa & Hadders-Algra, 2011). Approximately 70% of individuals with ASD have at least one comorbid mental disorder (Simonoff et al., 2008), such as, specific phobia, obsessive-compulsive disorder, or ADHD (Leyfer et al., 2006). Genetic disorders including Fragile X,
tuberous sclerosis, and Down syndrome have also been associated more commonly with ASD than in the general population (Fombonne, 1999).
Chapter 2: Reconceptualising autism

Autism was originally conceptualised as a psychogenic condition, with the implication being that the parents (particularly the mother) was the cause of the child’s behaviour. Known as the refrigerator mother theory of autism, it was presumed that autism was caused by emotionally cold and distant (‘frigid’) mothers. While this theory is attributed to child psychologist Bruno Bettelheim, it is apparent that he was at least partially influenced by Kanner’s observations of the parents of the afflicted children, stating that they were obsessive, cold-hearted and had limited interest in people (Bettelheim, 1967; Kanner, 1943). Rimland’s book ‘Infantile autism: the syndrome and its implications for a neural theory of behavior,’ published in 1964, was the catalyst in shifting popular opinion away from Bettelheim’s theory. The refrigerator mother theory of autism is now largely discredited, but it remains influential in some parts of the world, most notably in France, due to the ongoing and powerful influence of psychoanalytic theory (Yudell, 2012).

Contemporary research has found ASD to have a strong genetic component (Freitag, 2007), and substantial research over the past 2 decades has aimed to find a gene (or genes) consistent among children with an ASD diagnosis (e.g., Ozonoff et al., 2011; Folstein & Rosen-Sheidley, 2001; Rosenberg et al., 2009; Veenstra-Vander, Weele & Cook Jr, 2004). Nevertheless, studies seeking a single-gene cause have been largely inconclusive. A more promising approach to understanding the genetic factors involved in ASD is that of epigenetics. Epigenetics refers to non-permanent heritable changes in organisms caused by the modification of gene expression without altering the DNA sequence. Essentially, each cell turns on (expresses) only a proportion of genes, while the rest of the genes are turned off (repressed). Genes can be turned on or off through a process known as gene
An investigation of the potential association between mercury and ASD regulation. Although the underlying mechanisms are not yet fully understood, the environment is considered to have a critical role in epigenetic regulation (Feil & Fraga, 2012). A recent review by Hall and Kelley (2014) explored the key findings relating to epigenetics in ASD, concluding that “possible epigenetic processes related to autism present a new and promising way to investigate the genetic nature of autism” (p.878).

Altered glutathione metabolism has also been identified as a potential precursor to the development of ASD. Glutathione (L-g-glutamyl-L-cysteinyl-glycine) is an intracellular peptide which plays a central role in cell biology and is critical in protecting organisms against toxicity and disease (Pastore, Federici, Bertini & Piemonte, 2003). The glutathione redox ratio, a ratio of glutathione to glutathione disulphide (oxidized glutathione), is commonly used as a measure of oxidative stress. Neurological development may be affected in the early stages of life due to an imbalance in the glutathione redox ratio through a decrease in the proliferation of cells, DNA damage, and an increase in apoptosis (Main, Angley, O'Doherty, Thomas & Fenech, 2012). Furthermore, elevated oxidative stress and decreased glutathione has been identified as a possible mechanism by which Hg may induce ASD (James et al. 2004; Geier & Geier, 2006a; Youn, in, Kim & Lim, 2010). Thus, an individual low in glutathione would have both a diminished capacity to minimise oxidative damage produced by Hg and the ability to excrete the toxicant.

Although the aetiology of autism remains elusive, our understanding of the disorder has come far since the infamous days of the refrigerator mother theory. Advances in understanding have been particularly evident over the last two decades. ASD is now largely considered a whole-body condition with psychological/psychiatric, biological and medical characteristics, rather than as a
pure psychiatric manifestation (Herbert, 2010). While this emergence in understanding is obviously important, it was perhaps surprisingly slow. Arguably, the cause of this, at least in part, is due to the original writings on autism and Asperger’s Disorder by Kanner and Asperger respectively.

Kanner’s seminal paper (1943) provides a detailed observational description, in narrative form, of child and parental behavioural characteristics, but makes little reference to medical or physiological examinations or evaluations. Indeed, it would appear that he did not order any such examinations to be performed, only referencing those undertaken prior to the commencement of his sessions with each family. Asperger’s paper, published in 1944, is remarkably similar in its lack of reference to biological or medical testing. Both Kanner and Asperger independently appear to have neglected to consider (or were too ready to discount) a medical/biological basis to the behaviour they were observing, and instead chose to declare a new psychiatric condition. In the context of both authors’ psychoanalytic training and practice, this is perhaps unsurprising. In the two original papers that follow, the potential implications that Kanner and Asperger’s respective personal and professional background has had on ASD research and practice is discussed, and how this has perhaps led to an over-emphasis on the behavioural aspects of the condition, with a subsequent under-emphasis on the biological.

The first of the papers (Shandley & Austin, 2010) is a critical opinion piece. The basic premise of the paper is that progressive biomedical research on autism has been slow to emerge, possibly due in part to the investigative approach employed by Kanner and Asperger, and the influence of their personal backgrounds and clinical training in psychoanalysis. The second paper (Shandley & Austin, 2011) appears as a chapter within the book ‘A critical introduction to DSM.’ The book is aimed at
postgraduate psychology students and encourages them to become informed users of the DSM, critically examining the contents as opposed to accepting the information at face value. As such, this chapter extends upon the arguments of the first paper in the present thesis, with a greater emphasis placed on the appearance of ASD in the DSM and some of the associated controversies.
Paper 1a


Reprint of the original open access paper
### AUTHORSHIP STATEMENT

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<td>Researching Autism: Moving beyond the behavioural to address cause, care and prevention</td>
<td>Autism Insights, Volume 2, pages 25-30</td>
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<td>School of Psychology</td>
<td>An Investigation of the potential association between mercury and Autism Spectrum Disorder: An interdisciplinary approach</td>
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Conceptual development of paper, drafting of manuscript, revision, principal author.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below. Signature and date: Kerrie Shandley 30/4/14.

4. Description of all author contributions

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<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
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<td>Conceptual development of paper, drafting of manuscript, revision, principal author.</td>
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<td>David Austin</td>
<td>Review and feedback</td>
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<td>David Austin</td>
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with the publication.
Reconceptualizing Autism: Moving Beyond the Behavioral to Address Cause, Cure and Prevention

Kerrie Shandley and David W. Austin
Swinburne Autism Bio-Research Initiative (SABRI), Faculty of Life and Social Sciences, Swinburne University of Technology, Hawthorn, VIC 3122, Australia. Email: kshandley@swin.edu.au

Abstract: Since the publication of Leo Kanner’s seminal paper in 1943, there has been essentially no definitive light shed on the cause, prevention or cure of autism. It is our contention that the reason lies, at least in part, with the original psychiatric conceptualization of the condition and the subsequent acceptance of this framework by health professionals ever since. We suggest an urgent revision of autism as a disease state such that its operationalization in major diagnostic systems such as the Diagnostic and Statistical Manual of Mental Disorders and International Classification of Diseases recognizes the biological variables known to be associated with autism.

Keywords: autism, psychiatric conceptualization

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Introduction

Since the publication of Leo Kanner’s seminal paper in 1943, research into autism has resulted in the publication of over 12,000 peer-reviewed papers, yet, there has been essentially no definitive light shed on cause, prevention or cure. The question that must be asked is, “Why?” It is our contention that the reason lies, at least in part, with the original psychiatric conceptualization of the condition by Kanner and the subsequent acceptance of this framework by health professionals ever since.

As autism was first “discovered” by a psychiatrist (Kanner) and is classified in the Diagnostic and Statistical Manual of Mental Disorders (DSM), historically, research into the condition has primarily been the domain of psychologists and psychiatrists (especially developmental). These professions typically view autism as a developmental or behavioral disorder and therefore field trials designed to establish the diagnostic definition of autism, as well as other research within the broad field of autism, tends to focus on behavioral or developmental aspects of the condition. The obvious limitation of this approach is the evident internally reinforcing model whereby autism is conceptualized as a behavioral or developmental disorder and therefore research and field trials that follow are designed to examine variables of that nature almost exclusively, thus confirming the original assumptions (e.g. 2).

The majority of autism research tends to focus on assessment/diagnostic measures, behavioral/educational interventions, and incidence/prevalence rates (for a review see 3). Where biological/physiological variables are considered, it has been predominantly in the area of neurological functioning (e.g. 4). In contrast to the enduring conceptualization of autism as a behavioral or developmental disorder (or even a psychiatric or neurodevelopmental disorder), emerging evidence over the last decade has clearly identified a range of biomedical irregularities that are consistently present in individuals with autism, suggesting that there is a biological/medical component to autism that needs to be integrated into our understanding and formal conceptualizations if we are ever to gain a genuine and complete picture of the condition.

It is crucial to understand the origins of a condition such as autism as, once a disease state is defined and labeled, the way in which a condition is perceived and treated by clinicians, researchers, medical professionals and policy makers is not easily altered. Therefore, if the process of properly recognizing and naming a condition is not rigorously conducted, via thorough medical examination and differential diagnosis, the end result can be instrumentally destructive to the fundamental goal of medical research to identify the cause of a condition and the subsequent determination of prevention and cure.

Autism as a Psychiatric Disorder: The Historical Context

In his seminal paper proposing the discovery of a new condition that would come to be known as autism, Kanner,1 in a 19,000 word detailed case series analysis of 11 children, did not once mention considering a biological explanation for the “fascinating peculiarities” he observed, nor indicate that a single biological hypothesis was pursued. Kanner’s bias to a psychiatric explanation for the patient presentations he was seeing is also reflected in his journal of choice, “Nervous child”.

In his paper, the cases are presented in a narrative form with almost no references to medical examinations or evaluations and, instead, a relentless description of child and parental behavioral characteristics is provided, with the author clearly trying to draw behavioral/psychological parallels between the child and parents: “This much is certain, that there is a great deal of obsessiveness in the family background.4 (p. 230) In this perspective lies the genesis of the “refrigerator mother” theory of autism whereby autism was presumed for several decades to be caused by emotionally frigid mothers. Although the theory is attributed to another psychiatrist, Bruno Bettelheim, the theory was clearly supported by Kanner who in a 1960 interview described parents of autistic children as “just happening to defrost long enough to produce a child.”5

In medical practice, the appropriate action when presented with a difficult case is to do the most thorough exploration as possible as to the patient’s physical state (e.g. physical examination, blood, urine, imaging studies) and, if the diagnosis is still not clear from that point, to develop a list of possible diseases which might best explain the patient presentation.
Following, one then continues to think critically and conduct further testing as necessary in order to go about excluding disorders from the list of possibilities. This well known standard medical practice appears completely absent from Kanner’s professional practice. Instead of conducting the aforementioned process, Kanner instead wrote a 19,000 word case description and suggested that he had discovered a new psychiatric condition. In an attempt to understand the approach of Kanner, it is useful to understand a little of the man and his background.

Kanner was an Austrian-born psychiatrist influenced by the dominant psychiatric paradigm of the time, psychoanalysis. Hence, his personal perspective appeared to be brought to bear on the original 11 children; “We must, then, assume that these children have come into the world with innate inability to form the usual, biologically provided affective contact with people, just as other children come into the world with innate physical or intellectual handicaps.1 (p. 250)” In other words, Kanner assumed that the children were abnormal at birth. This is an extraordinary assumption, completely unsupported by any science of the time, and is curiously incongruent with his support for the refrigerator mother theory of autism which places the primary cause of autism as occurring during the first months and years after birth.

Kanner, an avid poet with a strong love of the arts, and “with no more training in pediatrics or child psychiatry than he had received as a medical student,”6 announced to the world a new psychiatric disorder without ever explaining how the symptom profiles he had observed may not already be better explained by medical or psychiatric conditions known at the time. In a startling coincidence, at around the same time, Hans Asperger, another Austrian physician with a penchant for poetry, published the first definition of Asperger’s syndrome in 1944.7 In a paper describing just four boys, he identified a pattern of behavior and abilities that he called “autistic psychopathy.” His special interest was “psychically abnormal” children.

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Table 1. Diagnostic and statistical manual edition 4 text revision for autistic disorder.

<table>
<thead>
<tr>
<th>A. A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3):</th>
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<tbody>
<tr>
<td>1. Qualitative impairment in social interaction, as manifested by at least two of the following:</td>
</tr>
<tr>
<td>a. Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction</td>
</tr>
<tr>
<td>b. Failure to develop peer relationships appropriate to developmental level</td>
</tr>
<tr>
<td>c. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. by a lack of showing, bringing, or pointing out objects of interest)</td>
</tr>
<tr>
<td>d. Lack of social or emotional reciprocity</td>
</tr>
<tr>
<td>2. Qualitative impairments in communication as manifested by at least one of the following:</td>
</tr>
<tr>
<td>a. Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)</td>
</tr>
<tr>
<td>b. In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others</td>
</tr>
<tr>
<td>c. Stereotyped and repetitive use of language or idiosyncratic language</td>
</tr>
<tr>
<td>d. Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level</td>
</tr>
<tr>
<td>3. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:</td>
</tr>
<tr>
<td>a. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus</td>
</tr>
<tr>
<td>b. Apparently inflexible adherence to specific, nonfunctional routines or rituals</td>
</tr>
<tr>
<td>c. Stereotyped and repetitive motor manners (e.g. hand or finger flapping or twisting, or complex whole-body movements)</td>
</tr>
<tr>
<td>d. Persistent preoccupation with parts of objects</td>
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</tbody>
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| B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: |
| (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play. |

| C. The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder. |
In this, we see that Asperger reveals his psychiatric bias to be similar to Kanner’s. In a further similarity, Asperger also appears to have failed to conduct any biological testing or make any meaningful attempt to determine whether the four boys had, in fact, an already known condition, rather than proposing that somehow, mysteriously, a new disease had landed at his doorstep exclusively in the guise of just four young boys (Asperger was unaware of Kanner’s paper at this time).

So, the names Kanner and Asperger are inextricably linked to the autism spectrum disorders, and many herald the two men as the fathers of child psychiatry. Several important questions remain however. How likely is it that these two men, “discovered”, by chance, a brand new disorder, never before seen in the human species, which appeared simultaneously on separate continents at around the same time? And what does it suggest that both men were Austrian in upbringing and heavily influenced by the psychoanalytic perspective of behaviour and human development, both failed to undertake testing of physiological variables or attempt medical explanations for the behaviour they were seeing, and both published articles of their “discovery” as a new psychiatric disorder?

**Autism Today**

Modern perspectives of autism are still clearly influenced by Kanner. This point is most clearly exemplified by the ‘evolution’ of autism in the DSM. Autism first appeared in the third edition of the DSM as ‘Infantile Autism’ in a new class of conditions titled Pervasive Developmental Disorders. Substantial revisions to the definition of autism were made in the DSM-III-R, with the criteria broadened to reflect a developmental orientation and the heterogeneity of autism. Subsequent editions of the DSM, both the fourth edition and the text revision of the fourth edition, have largely retained the same definition (see Table 1). Autistic disorder’s precursor was first included in the fourth edition of the DSM (and continued in the text revision of this edition). As with autism, the disorder is contextualized as a behavioral/developmental condition, the primary difference with autism being that there is comparatively less disability. Table 2 presents the DSM-IV-TR definition of Asperger’s disorder.

As can be clearly seen by the DSM definitions, autism spectrum disorders continue to be placed firmly in a developmental/behavioral context with the criteria

| Table 2. Diagnostic and statistical manual edition 4 text revision for asperger’s disorder. |
| A. Qualitative impairment in social interaction, as manifested by at least two of the following: |
| 1. Marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body gestures, and gestures to regulate social interaction |
| 2. Failure to develop peer relationships appropriate to developmental level |
| 3. A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. by a lack of showing, bringing, or pointing out objects of interest to other people) |
| 4. Lack of social or emotional reciprocity |
| B. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following: |
| 1. Encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus |
| 2. Apparently inflexible adherence to specific, nonfunctional routines or rituals |
| 3. Stereotyped and repetitive motor mannerisms (e.g. hand or finger flapping or twisting, or complex whole-body movements) |
| 4. Persistent preoccupation with parts of objects |
| C. The disturbance causes clinically significant impairment in social, occupational, or other important areas of functioning. |
| D. There is no clinically significant delay in language (e.g. single words used by age 2 years, communicative phrases used by age 3 years). |
| E. There is no clinically significant delay in cognitive development or in the development of age-appropriate self-help skills, adaptive behavior (other than in social interaction), and curiosity about the environment in childhood. |
| F. Criteria are not met for another specific Pervasive Developmental Disorder or Schizophrenia. |
centered upon the core triad of behavioral symptoms: impaired socialization, communication and repetitive/obsessive behaviours. This exclusive diagnostic triad is likely to be seen as increasingly out of step with modern understandings of autism that recognize biomedical irregularities such as elevated oxidative stress, mitochondrial-respiratory disorders, generalized inflammation, neuroinflammation, gastrointestinal abnormalities, fatty acid deficiencies and elevated urinary porphyrins.

Conclusion
Some 70 years have passed since autism was first identified, yet we are no closer to understanding what it is caused by, nor how the condition may be cured or prevented. We argue that this lack of progress is at least partially attributable to Kanner and the manner in which he conceptualized autism as a parenterally-mediated psychopathology. Why do we need to be concerned about this? Simply put, autism is a devastating condition, lifelong in duration, with the majority of affected individuals requiring supported living arrangements. The majority of sufferers will never engage in meaningful employment, marry nor have children, and cannot engage in meaningful conversation. Autism affects not only the individual, but the family unit and community as a whole.

To avoid simply treading the same unfruitful path of the previous 70 years, we would suggest an urgent revision of autism as a disease state such that its operationalization in major diagnostic systems such as the DSM and International Classification of Diseases recognizes biological variables known to be associated with autism. The affect of this would be to facilitate a more multi-disciplinary and inclusive range of health disciplines to research the biological bases of autism. Improving our understanding of these bases is a fundamental way of addressing the touchstones of medical research into autism; cause, cure, prevention and treatment.

Disclosures
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References
An investigation of the potential association between mercury and ASD


Paper 1b


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I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

| Signature and date | Kerrie Shandley | 30/4/14 |

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Chapter 7

AUTISM SPECTRUM DISORDERS

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INTRODUCTION

Autism is one of the most hotly debated disorders listed in the current Diagnostic and Statistical Manual (DSM), eliciting passionate and often conflicting opinions among health professionals, medical practitioners, parents and researchers. Despite moving on from the early and notorious "refrigerator mother" pathogenic theories (where autism was said to be caused by emotionally distant parents) to more modern epigenetic conceptualisations (where autism is viewed as being caused by an interaction between a genetic susceptibility and an environmental trigger), surprisingly little has changed in regards to autism as a diagnostic construct. The exclusive use of a triad of behavioural indicators (impaired social interaction and communication, and restricted and repetitive behaviour) to diagnose autism appears to be increasingly out of step with contemporary research into "biomarkers" or biomedical aspects of the condition. An understanding of the tensions and conflicts surrounding autism is critical in order to fully appreciate the conservative nature of information provided in the DSM. This chapter will touch on some of the controversies as they apply to the inclusion of autism in the DSM, ultimately, leading us to consider the most controversial question of all: Does autism belong in the DSM at all?

AUTISM AS A PSYCHIATRIC DISORDER: THE HISTORICAL CONTEXT

To understand how autism is conceptualised in the DSM, it is important to understand a little of the history of the men who provided the first comprehensive clinical descriptions of autism (Leo Kanner) and Asperger's disorder (Hans Asperger), and whose clinical opinions and biases continue to have an influence on our current understanding of Autism Spectrum Disorder.
An investigation of the potential association between mercury and ASD

Kanner, a psychiatrist and physician, first proposed the discovery of a new condition, which would later become known as autism (or classical autism), in his seminal paper of 1943 (Kanner, 1943). His paper, which would fail to meet the standards of most of today’s leading scientific journals, is a 19,000 word detailed case series analysis of 11 children. The cases are presented in a narrative form with almost no references to medical examinations or evaluations and, instead, a relentless description of child and parental behavioral characteristics is provided, with Kanner repeatedly attempting to draw behavioral/psychological parallels between the child and parents: “This much is certain, that there is a great deal of obsessiveness in the family background. (Kanner, 1943, p. 250)” In this perspective lies the genesis of the refrigerator mother theory of autism, whereby autism was presumed for several decades to be caused by what was termed, emotionally “rigid” mothers; that is, mothers who were considered to be devoid of any warmth or affection towards their child. Although the theory is attributed to another psychiatrist (Bruno Bettelheim), the refrigerator mother theory was clearly supported by Kanner who in a 1960 interview described parents of autistic children as “just happening to defrost long enough to produce a child” (Tice, 1988). Incredibly, not one word of this lengthy paper is devoted to the consideration of any alternative explanations for the “fascinating peculiarities” Kanner observed, nor does Kanner provide evidence that a single medical hypothesis was investigated. It is also noteworthy that Kanner’s apparent bias towards conceptualizing autism as a psychiatric anomaly resulting from a deficient relationship between mother and child, rather than a medical, physical, or neurological condition was reflected in his journal of choice, “Nervous child”.

Austrian-born, Kanner was influenced by the dominant psychiatric paradigm of the time; psychoanalysis. Hence, his personal perspective appeared to be brought to bear on the original 11 children; “We must, then, assume that these children have come into the world with innate inability to form the usual, biologically provided affective contact with people, just as other children come into the world with innate physical or intellectual handicaps. (Kanner, 1943, p. 250)” In other words, Kanner assumed that the children were abnormal from birth. Not only is this curiously inconsistent with his support of the refrigerator mother theory (which posits the exposure to the causal trigger as occurring after birth), but there is no indication that any medical examination or differential diagnostic process was undertaken to rule out other plausible explanations for the behaviors observed in these children. Furthermore, Kanner was not able to personally observe any of the autistic children from birth as he commenced contact with the children from the ages of 4 and above.

In a startling coincidence, only a year later, Hans Asperger, another Austrian-born psychiatrist and pediatrician, published the first definition of Asperger’s syndrome in 1944 (Asperger, 1944). In a paper describing just 4 young boys, he identified a pattern of behavior and abilities that he called “autistic psychopathy.” His special interest was “psychically abnormal” children. In this, we see that Asperger reveals his bias to be similar to that of Kanner’s in that Asperger’s disorder was viewed as an impairment in the child’s ability to relate to, or interact with the outside world. Unlike Kanner, Asperger does not speculate as to the cause of this impairment, however, his conclusions were based purely on observation, failing to carry out any medical testing or differential diagnosis to rule out known conditions of the time. So, the names Kanner and Asperger are inextricably linked to the autism spectrum. Several important questions remain, however. How likely is it that these two men discovered, by chance, a brand new psychiatric disorder never before seen in the immun
species, which appeared simultaneously on separate continents at around the same time? And what does it suggest that both men were Austrian-born and heavily influenced by the psychoanalytic perspective of behaviour and human development, both failed to undertake testing of physiological variables or attempt medical explanations for the behaviour they were seeing, and both published articles of their discovery as a new psychiatric disorder?

**AUTISM IN THE DSM**

Modern perspectives of autism continue to be influenced by Kanner and Asperger. This point is most clearly exemplified by the evolution of autism in the DSM. Autism first appeared in the third edition of the DSM as “Infantile Autism” in a new class of conditions titled “Pervasive Developmental Disorders” almost four decades after it was first identified (American Psychiatric Association [APA], 1980). At this time autism was considered to be an unusually early form of schizophrenia caused by maternal deprivation (the refrigerator mother theory) and other psychogenic causes, and it was Rutter (1978), who was widely attributed for operationalising Kanner’s narratives into the definition of autism published in the DSM-III. This also marks the introduction of the “tried of impairments” or “tried of autistic behaviour” now considered synonymous with autism, whereby a diagnosis of autism is made if a child demonstrates gross deficiencies in social interaction and language development (relative to their age), and displays bizarre and/or repetitive behaviours.

Substantial revisions were made in the DSM-III-R, with the criteria broadened to reflect a developmental orientation and the heterogeneity of autism (APA, 1987). The DSM-III-R criteria comprised of 16 items, eight of which must be present, with a minimum of two items from social interaction, one from communication and one from repertoire of activities and interests required for a diagnosis to be made. The age of onset was also increased by 6 months (from 30 to 36 months of age). Subsequent editions of the DSM, both the fourth edition and the text revision of the fourth edition, have largely retained the same definition (APA, 1994, 2000). In the DSM-IV-TR, the number of items was reduced with a minimum of six of 12 items required to be present for a diagnosis to be made. Interestingly, while Asperger’s disorder was described around the same time as autism, it was not included in the DSM until the fourth edition (and continued in the text revision of this edition) (APA, 1994, 2000). As with autism, the disorder is contextualized as a behavioural/developmental condition, the primary difference to autism being that there is comparatively less disability with only two of the three categories critical to a diagnosis of autism (social interaction deficits and restricted repertoire of interests and activities) required to be present.

Two substantial changes have been proposed for the DSM-V, scheduled for publication in 2013. The first proposed change recommends collapsing two of the three diagnostic domains, communication and social interaction, into one. The second proposed change recommends the introduction of a new disorder category “Autism Spectrum Disorder”, which will supersede current identifiers including autistic disorder, Asperger’s disorder, childhood disintegrative disorder, and pervasive developmental disorder – not otherwise specified. As can clearly be seen, the DSM-IV-TR criteria, including the criteria proposed for the DSM-V, continue to place autism in a developmental/behavioural context. And as an interesting
An investigation of the potential association between mercury and ASD

Competing Frameworks

With regard to thinking critically about autism and the DSM, the most fundamental question is whether it should be included in the publication at all. The answer to this question is deeply connected to the etiology of autism, which remains elusive and contentious. To illustrate, let's consider an alternative framework that has come to prominence in the past decade: autism as a biomedical condition.

This framework proposes that autism develops from the combination of an individual (perhaps genetic) susceptibility and exposure to an environmental insult at a critical time point, either in utero or early infancy (Bernard et al., 2001). Promising research implicates heavy metals, mercury in particular, as an environmental trigger. For example, behavioral changes, hyperactivity, and alterations in spousaneous and learned behaviors have been observed in animals exposed to mercury during the prenatal or early postnatal period (Agency for Toxic Substances and Disease Registry, 1999) and this response to mercury exposure has been noted in the Japanese prefectures of Kumamoto and Niigata where mercury poisoning outbreaks (Minamata disease and Niigata Minamata disease) occurred in the 1950s and 60s (Masazumi, 1972). In comparison to controls, significant elevations of mercury in the urine of children with autism has been reported as well as elevations in urinary markers of mercury damage (Austen and Shandley, 2008; Bradstreet, Geier, Kertzner, Adams and Geier, 2003; Geier and Geier, 2007; Nutt, Skorupka, Auer, Last, Sprangbrett and Latlie, 2006). Other studies have found the severity of autism to positively correlate with the child's body burden of toxic metals (Adams et al., 2009; Geier et al., 2009; Holmes, Blaxill and Haley, 2001). Additionally, some epidemiological studies have found an association between mercury exposure and ASD prevalence (e.g., Gallagher and Goodman, 2009; Geier and Geier, 2006; Palmer, Blanchard, Stein, Mandell and Miller, 2006; Palmer, Blanchard and Wood, 2009; Windham, Zhang, Gunier, Croen and Grether, 2006), but not all (e.g., Hvid, Stellfeld, Wohlforth and Melbye, 2003). Recent research provides further support for this framework showing the prevalence of autism to be 8-fold higher than the general population among the offspring of a population known to have an idiosyncratic sensitivity to mercury (Shandley and Austin, under review).

A further argument in considering the manner in which autism appears in the DSM relates to the range of biological irregularities consistently found in individuals diagnosed with autism. These irregularities include (but are not limited to): elevated oxidative stress (James et al., 2004; Kern and Jones, 2006), mitochondrial-respiratory disorders (Oliveira et al., 2007; Rosemberg and Bradstreet, 2008), generalized inflammation (Zimmerman et al., 2005), neurotransmitter abnormalities (Herbert, 2003; Pedro, Vargas, Zimmerman, 2005), gastrointestinal abnormalities (Hovarth and Pemman, 2002; Parachao, Bingham, Gibson and McCartney, 2005), and fatty acid deficiencies (Anunniner et al., 2007; Vancas et al., 2001). While the DSM includes a small paragraph on associated laboratory findings, only abnormalities in serotonin and brain activity are mentioned. In fairness, it should be noted that much of the
research identifying biomedical irregularities in autism was conducted in the years subsequent to the release of the DSM-IV-TR. Nevertheless, if autism is, in fact, an organic biomedical condition, it is worth pondering what affects its classification as a mental disorder in the DSM has had on how we research and treat this condition and, more importantly, the impact this has had on the tens of millions of children who have suffered this condition and their families.

Looking to the future, what research, and/or political process within the APA and the international scientific community would be required before autism was fundamentally changed within the DSM (to reflect a biomedical conceptualization and etiology), or delisted altogether?

MENTAL DISORDERS VERSUS MEDICAL CONDITIONS

Ironically, while the purpose of the DSM is to present a classification of mental or psychiatric disorders, the authors acknowledge that “no definition adequately specifies precise boundaries for the concept of ‘mental disorder’.” Although the DSM committee have attempted to create a clear working definition of mental disorder (see: Stein et al., 2010), the point of pathological behaviour is still somewhat arbitrary. Furthermore, the authors of the DSM contend that there is a false dichotomy between distinguishing a disorder as either “mental” or “physical” in nature. That is, that no condition is solely of the mind or body, therefore, no such dichotomy between the two domains exist (Kendell, 2001). Yet, in reality, by the very nature of the publication, this is precisely what the authors of the DSM have set out to do, create a classification system that separates disorders from mind from those of the body. While this is certainly reasonable, the mind and the body are well-established as inter-related independent functioning parts. The question is, without a concrete operational definition of a mental disorder, where do we draw the line, and has the line been drawn in the correct places for all DSM disorders?

Consider the case of Alzheimer’s disease. Alzheimer’s is commonly considered a neurodegenerative disease with associated psychiatric symptoms including loss of memory and cognitive ability plus agitation, mood disturbance and marked personality change. Nevertheless, Alzheimer’s is considered a medical condition and therefore does not appear in the DSM as a mental disorder except to have its psychiatric features noted as part of the general category of dementia. This is an important distinction as it provides a marker to the health professions that Alzheimer’s should primarily be the responsibility and interest of medical practitioners and researchers. By contrast, conditions in the DSM are held to be the primary responsibility and interest of psychiatrists and psychologists.

So, if we get the categorization of a condition (wrong, we potentially facilitate a mis-fit between condition and health professional. For example, if autism is indeed better categorized in the DSM as Alzheimer’s is (i.e., as an organic medical condition with concomitant behavioral/cognitive deficits), then for over 70 years since autism was first identified, essentially the wrong health professionals (psychologists and psychiatrists) have been on the case.
MORE CONTROVERSY?

There is another perspective radically at odds to those covered thus far that is important to consider if one is to have a broad understanding of autism and its relationship to the DSM. Above, we have discussed the possibility that autism may not appropriately belong in the DSM based on the possibility that it is an organic biomedical condition (like, for example, Alzheimer’s disease), yet there are those who suggest a radically different rationale for the deletion of autism from the DSM: the notion of “neurodiversity”. The neurodiversity movement has its roots in autism (and is often led by individuals with a diagnosis of high functioning autism or Asperger’s disorder themselves), but in recent years has spread to other conditions in the DSM (e.g., Attention Deficit Hyperactivity Disorder and schizophrenia). These individuals propose that autism is not a disorder of any type and, in fact, the behaviors associated with autism are just natural variants that need not be pathologized. Their argument is often paralleled to that of homosexuality whereby a constellation of behaviors summarized as homosexuality was originally pathological through DSM criteria, but was de-pathologized in 1973 through its delisting from the DSM-II. Proponents of a neurodiversity perspective argue that people exhibiting autistic behaviors are merely different to the norm (like homosexuality) but that this fact does not justify it being pathologized.

It is an intensely passionate debate, but tellingly, it is not one that is able to be undertaken by any individual with moderate to severe autism who is unable to speak, work, maintain significant relationships, feed or look after themselves. Such individuals require lifelong high intensity care. This latter point is of critical consideration when making a determination as to whether or not a behaviour is pathological. This is where the analogy to homosexuality breaks down as homosexuality does not lead to the kind of severe and global dysfunction that is often the case with autism.

ALL DIAGNOSES ARE NOT CREATED EQUAL

Another issue to be cognizant of is the difference between a substantive diagnosis and merely a syndrome. Cancer is a substantive diagnosis because it directly speaks to the mechanistic driving dysfunction (i.e., the abnormal growth of cells proliferating in an uncontrolled way and, in some cases, metastasizing). Autism, on the other hand, is not a substantive diagnosis and merely represents a summary word for the myriad of behaviors that identify the condition.

To put this in perspective, consider the tautology whereby someone might say, “Oh, that boy doesn’t speak and behaves strangely because he has autism.” This is a tautology because to not speak and act strangely is autism. For example, consider Sudden Infant Death Syndrome (SIDS), the label given to the sudden and unexpected death of a baby with no known illness. We may hear that a particular baby died of SIDS. This is a tautology as SIDS is not a disease state. The baby died not of SIDS, but of an undetermined cause. SIDS is merely a summary term used for this phenomenon. To reiterate, it is important when thinking critically about the DSM and autism not to be lulled into an unfounded sense of certainty as to precisely what it is that we are dealing with. Analogous to SIDS, children who have language and behavioral problems do not act that way because they have autism, they act that way for
reasons we simply do not yet understand. Autism is merely a summary word for the tried of
impairments, not the cause of them.

The DSM does not claim to provide a historical and/or exhaustive account of all variables
considered relevant to a disorder listed within its pages. Nor is its focus one of dividing the
cause of a disorder, but, this issue (whether the APA choose to address it or not) remains
pivotal to the fundamental issue of what a disorder is, and therefore, whether or not it is
appropriately classified as psychiatric in nature. The cause of a condition is instrumental in
determining whether it is classified as a mental disorder, medical condition or medical
condition with psychological/psychiatric features. This is of critical concern as it is important
to understand that the DSM contributes to an internally reinforcing model whereby, because
autism is conceptualized as a behavioural or developmental disorder, research and field trials
that follow are designed to examine variables of that nature almost exclusively, thus
confirming the original assumptions and in turn informing the next version of the DSM
(Volkmar et al., 1994). In other words, if we define autism as being represented by a specific
set of behaviours, then design studies to see if those behaviours exist in a certain population
we have already identified as demonstrating those behaviours (and unsurprisingly find that
they are), we conclude that autism exists and is validly indicated by the original criteria: An
internally reinforcing model.

CONCLUSION

Autism as a diagnostic entity has changed very little since it was first identified in 1943.
This is at least partially attributable to Kanner and the manner in which he conceptualized
autism as a parentally-mediated psychopathology. Understanding the origins of autism, or any
disorder for that matter, is crucial, as once a disease state is defined and labelled, the way in
which it is perceived, treated and researched is not easily altered.

- The DSM serves an important purpose to both medical and health professionals, but
  it is not without its drawbacks and should not be taken as representing absolute truth.
  Some key points to consider when critically examining its content include, but are by
  no means limited to the following: Who discovered the disorder? What personal
  biases might they have brought to bear on the disorder through their education,
  training and/or culture?
- The DSM claims to be a scientifically-based publication, but is it the science that
  drives the inclusion of a disorder within the manual or is it cultural, historical, and/or
  political factors?
- What nature of investigation has been undertaken on the disorder? Is the research
  narrow in focus only considering limited areas without any consideration or regard
  for others?
- Many conditions have both a medical and psychological/psychiatric component.
  How has it been determined in which category the condition belongs? Consider how
  rigorously the disorder was examined before its inclusion in the DSM. Was a
  thorough medical examination and differential diagnosis process undertaken to rule
  out other explanations?
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REFERENCES


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Chapter 3: Mercury and Autism Spectrum Disorder

Overview

ASD has a strong genetic component with heritability estimated to be as high as 90% (Freitag, 2007). Concordance rates of 60-91% have been demonstrated in monozygotic twins in contrast to dizygotic twins where concordance rates are considerably lower at 0-10% (Folstein & Rosen-Sheidley, 2001; Rosenberg et al., 2009; Veenstra-Vander, Weele & Cook Jr, 2004). In addition, sibling recurrence risk has been estimated to range from 2% to 19% (Ozonoff et al., 2011). Nevertheless, heritability alone cannot account for the escalation in ASD prevalence over recent decades; the human genome simply cannot evolve that quickly.

While it has been argued that the rise in prevalence is due to the broadening of diagnostic criteria, increased awareness, diagnostic substitution and increased service availability (Fombonne et al., 2006), contemporary research suggests that an environment trigger, perhaps in conjunction with genetics, is likely to play a role in the development of ASD (Herbert, 2010; Hallmayer et al., 2011). In this context, environmental triggers encompass many possibilities including pesticides, pollution, chemicals, parental age, and maternal or neonatal infection. It is beyond the scope and capacity of this thesis to effectively investigate all possible environmental factors, however, there is one factor that has been the subject of great controversy and debate in recent times, that of the heavy metal mercury (Hg) (Bernard, Enayati, Redwood, Roger & Binstock, 2001; Mutter, Naumann, Schneider, Walach & Hayley, 2005). As yet, no definitive science has established whether Hg plays a role in the development of ASD, however, a recent review of the area indicated the existence of a link of some sort (DeSoto & Hitlan, 2010).
An investigation of the potential association between mercury and ASD

Mercury is a naturally occurring element that is ubiquitous in the environment (Goldman & Shannon, 2001). It is released into the environment through both natural (e.g., volcanoes erupting, soil decomposing) and anthropogenic (e.g., mining, coal-fired power plant emissions) means. Once released into the environment, Hg continuously cycles between the atmosphere, ocean and soil; it does not breakdown and cannot be degraded into a harmless substance. Mercury is a known neurotoxin and is especially dangerous to the foetal and infant brain and central nervous system (CNS) (Geidd, 2004; Lebel, Walker, Leemans, Phillips & Beaulieu, 2008; Rodier, 1995; Thompson et al., 2000). Humans are exposed to Hg through a number of sources, primarily, seafood, dental amalgam, and vaccines. The following chapter will discuss Hg in further detail and its impact on human health, particularly that of neonates and infants, and the association between Hg and ASD.

Chemistry

Mercury is identified by three main species: elemental, inorganic, and organic forms (Franzblau, 1994). Elemental Hg, also known as metallic or liquid Hg, forms a shiny, silver-white liquid at room temperature. It is created by smelting cinnabar ore and is highly volatile, expanding and contracting at a constant rate as the temperature rises and falls. Uncontained in the environment, elemental Hg will evaporate into a colourless and odourless vapour that can be both inhaled and absorbed through the skin. It is most commonly released into the atmosphere through anthropogenic means such as the combustion of fossil fuels. Today, it is most commonly used in dental amalgam (silver fillings), thermometers, fluorescent light bulbs, mining, some electrical switches (in the workplace, home and car), and the glass-blowing industry.
Inorganic Hg compounds are formed from the univalent and bivalent cations of Hg which include mercurous chloride, mercuric chloride, mercuric acetate and mercuric sulphide. Compounds typically appear as white powders or crystals, with the exception of mercuric sulphide which is red in its natural state and turns black following exposure to light. Inorganic Hg is used in batteries, disinfectants, and skin bleaching creams, and is still used today in cultural, ceremonial and religious practices as it has been throughout history (Wendroff, 1997; Zayas & Ozuah, 1996).

Organic Hg forms when carbon combines with Hg. Organic Hg compounds include dimethylmercury, phenylmercury, and ethylmercury, although the predominant and most toxic compound in the environment is methylmercury (MeHg). Humans are primarily exposed to MeHg through the consumption of fish and shellfish. Both elemental and inorganic Hg can be transformed into MeHg via bacteria that live in sedimentary layers of lakes and other bodies of water which is then bioaccumulated and biomagnified upwards through the aquatic food-chain. That is, bottom-dwelling fish feed upon contaminated plant life which are in turn eaten by larger fish and so on, concentrating up the food-chain in large predatory fish (e.g., king mackerel, shark, swordfish, tuna), freshwater fish (e.g., bass, eels, perch) and mammals (e.g., seals, whales) (Dietz et al. 2000; Gilmour & Riedel, 2000; Neumann & Ward 1999). Additionally, MeHg contained in landfills and sewerage treatment plants can be released directly into sources of water (Lindberg et al. 2001; Sommar, Feng & Lindqvist, 1999).

**Toxicology**

All forms of Hg are hazardous to human health, especially to the developing brain and CNS as it can cross the placental and blood-brain barrier (Campbell et al.
1992; Clarkson, Vyas & Ballatori, 2007; Rodier, 1995). Both the blood-brain barrier
and CNS continue to develop beyond birth and as such, remain vulnerable to
environmental insult both in utero and during infancy. Indeed, some regions of the
brain continue to develop and mature until early adulthood (Geidd, 2004; Lebel et
al., 2008; Rodier, 1995; Thompson et al., 2000). The toxicology of Hg is complex
and dependent on a number of factors including form, source, route and duration of
exposure, and genetic susceptibility to Hg. These factors as they relate to humans,
and more importantly neonatal and infant health are discussed below.

Elemental Hg

Elemental Hg is highly lipophilic in nature, meaning that it can readily enter
the bloodstream, allowing it to be distributed throughout the body and cross the
placental and blood-brain barriers (Magos, 1967; Clarkson & Magos, 2006).
Elemental Hg primarily enters the body via inhalation, with the vapour preferentially
depositing in the kidneys and brain (Barregård, Sällsten & Conradi, 1999; Berlin,
Jerksell & von Ubisch, 1966; Cherian & Clarkson, 1976; Florentine & Sanfilippo,
1991). Acute exposure most commonly leads to respiratory distress, including
pneumonia, bronchiolitis, and oedema (Clarkson & Magos, 2006). The Minimal Risk
Level for chronic Hg inhalation set by the Agency for Toxic Substances and Disease
Registry (ATSDR) is 0.2μg/m³ (ATSDR, 1999). Chronic exposure exceeding
10μg/m³ can affect the CNS resulting in neurological disturbances, tremors, and
memory loss (Clarkson & Magos, 2006). Additionally, low concentrations of vapour
may cause skin rashes and kidney abnormalities, while high concentrations have
resulted in death. Typically, the toxic effects of elemental Hg gradually disappear
once the individual is removed from the source of exposure. Although, once in the
brain, elemental Hg vapour can remain at high residual levels for an extensive length
of time (Takahata, Hayashi, Watanabe & Anso, 1970). Furthermore, in some cases, symptoms have persisted for as long as 30 years following removal from occupational sources where exposure to Hg was chronic (Albers et al., 1988). Indoor areas contaminated by accidental Hg spills can be extremely difficult to address, exposing occupants for months if not years, and in some cases, results in buildings being necessarily demolished (Carpi & Chen, 2001; Orloff et al., 1997).

Mercury poisoning is especially problematic among school populations in countries such as Turkey. For example, Carman et al. (2013) examined the effects of elemental Hg poisoning among 179 school children in the Turkish provinces of Kilis and Kahramanmaraş. The children were exposed to Hg via broken jars containing Hg in their school chemistry laboratories. The majority of children were reported to have touched or played with Hg in addition to inhaling its vapours. In some cases, children took Hg home where the entire family was subsequently poisoned due to Hg being boiled, heated and tasted. The most common complaints reported upon admission to hospital were headache, visual problems, rashes on the trunk and extremities, nausea, abdominal pain and peripheral neuropathy. Akyıldız, Kondolot, Kurtoglu and Konuskan (2012) likewise reported headache as the most common symptom among 26 paediatric cases of Hg poisoning exposed through inhalation or dermal absorption resulting from a broken thermometer at school.

Inorganic Hg

Inorganic Hg preferentially deposits in the kidneys, followed by the liver. Unlike elementary Hg, inorganic Hg has poor lipid solubility, decreasing its ability to cross the blood-brain and placental barriers and subsequently enter the brain (Kuhnert, Kuhnert & Erhard, 1981). However, maternal levels of inorganic Hg can be damaging to the foetus. For example, Brender et al. (2006) found mothers of
children with a neural tube defect in comparison to controls were nine times more likely to have inorganic urinary Hg levels that exceeded $>5.61\mu g/L$. Inorganic poisoning occurs primarily through ingestion; however, animal and human case studies have shown that both mercurous and mercuric salts can also be absorbed by the skin (Barr, Woodger & Rees, 1973; Bourgeois et al., 1986; De Bont, Lauwerys, Govaerts & Moulin, 1986).

Famously, Lewis Carroll’s character, the Mad Hatter, is linked to the toxic effects of inorganic Hg. In the 17th and 18th century, mercuric nitrate was used to treat animal fur in the felting process to make top hats. The ‘eccentric behaviour’ of the hatters, due to the chronic inhalation of Hg vapour, led to the expressions ‘hatter’s shakes’ and ‘mad as a hatter’ and resulted in behaviours that included irritability, anxiety, shyness, moodiness, and unpredictability, ataxia, and memory loss, in addition to other problems such as the loosening of teeth (Lishman, 1987).

Mercurous chloride, also known as calomel (sweet Hg), has a long history of use in medicinal products for both children and adults. Although calomel was considered non-hazardous, ingested over an extended period of time it can affect the kidneys, gastrointestinal tract and CNS (Wands, Weiss, Yardley & Maddrey, 1974). Calomel was once the treatment of choice for syphilis, typhus and yellow fever (Ozuah, 2000). For syphilis, calomel was administered via a range of common routes (oral, dermal, injection) and more extreme and unusual methods such as fumigation. This involved placing the afflicted individual into a box containing Hg and only a small hole for the individual to put their head through. A fire was lit underneath the box causing Hg to vaporise and slowly be absorbed by the skin over a period of hours. Death was not an uncommon result of treatment, while others suffered neurological damage, severe teeth loss and ulcerations. Hg remained the primary
treatment for conditions like syphilis from the 17th century until the discovery of penicillin (Parascandola, 2009). Indeed, the saying ‘a night with Venus, a lifetime with mercury’ originated from the connection between syphilis and Hg as a treatment.

Calomel was extensively used in children’s teething powders during the 19th and 20th century resulting in a condition that became widely known as pink disease or acrodynia. Approximately 1 in 500 exposed children experienced some degree of Hg poisoning with death resulting in 10-33% of cases (Rocaz, 1933). Although now rare, pink disease is still diagnosed on occasion, such as the cases of two brothers who played with a broken sphygmomanometer (Horowitz, Greenberg, Ling & Lifshitz, 2002) and twin girls who were diagnosed after their parents used a teething powder they purchased online that still contained Hg (Weinstein & Berstein, 2003). Pink disease is characterised by a wide range of symptoms including, irritability, neurosis, photophobia, hyperhidrosis, hypotonia, ataxia, digestive problems, excessive salivation, respiratory problems, lethargy, slurring/loss of speech, loosening/loss of teeth, and a marked reddening of the extremities usually accompanied by swelling and peeling of the skin (Rocaz 1933; Wood & Wood 1935; Leys 1950). Warkany and Hubbard (1953) reported similar effects (i.e., irritability, fretfulness and sleeplessness) on children exposed to calomel through a diaper rinse solution as it did to those exposed via teething powders. Research has revealed that medical issues are common among adult survivors of pink disease, including Young’s syndrome and bronchiectasis (Hendry, A’Hern & Cole, 1993; Williams & O’Reilly, 1959).

Two similar case reports identified neurological side-effects from the use of Chinese herbal medicines containing mercurous chloride. A 4-year-old boy given a
3-month course of Chinese medicine developed drooling, dysphagia, irregular arm movements, and impaired gait (Kang-Yum & Oransky, 1992). In the second case report, a 5-year-old boy presenting with mouth ulcers was administered a Chinese herbal spray containing 98% inorganic Hg up to 20 times a day over a period of 4-weeks. The child subsequently become irritable and developed motor and vocal tics and rashes on the trunk of his body (Li et al., 2000).

Mercuric chloride, considered more toxic than mercurous chloride, primarily affects the kidneys and gastrointestinal tract (Piotrowski, Trojanowska, Wiśniewska-Knypl & Bolanowska, 1974). Exposure to mercuric chloride (approximately 1 to 4 grams) has led to death which was attributed to renal failure, cardiovascular collapse, and severe gastrointestinal damage (Beasley, Schep, Slaughter, Temple & Michel, 2014). Acute renal failure in addition to vomiting, blisters and ulcers on the lips and tongue has been reported in the case of a 19-month old boy who accidentally ingested an unknown amount of mercuric chloride powder (Samuels et al., 1982).

Organic Hg

In Iraq, two incidents of ethylmercury poisoning occurred in 1956 and 1959/60. Both outbreaks were traced to the consumption of wheat which had been dressed with the mercurial compound Granosan M (ethylmercury-p-toluene sulfonanilide). Although the wheat came with the warning that it was not to be consumed, many families chose to ground it into flour to make bread after first testing it on fowls. A latent period ranging from days to weeks was observed before either fowl or human began showing any ill effects of Hg poisoning, giving the false impression that the wheat was safe to consume. By the time symptoms appeared among the first families that had commenced consuming the wheat, it was too late for many other families to avoid the same fate. In excess of 100 people were
hospitalised and a dozen fatalities recorded following the 1956 incident (Jalili & Abbasi, 1961). At least 1000 patients were reported to have been affected by the later incident in 1959/60 (Takizawa, 2009). Multiple bodily systems were affected by the poisoning: the kidneys were the most commonly affected organ (e.g., polyuria and polydipsia), the CNS was damaged irrespective of poisoning severity (e.g., coordination disturbances, visual impairments, disturbance of speech and tremors), generalised deep skeletal pain (including fasciculation and coarse twitching of muscles and myoidema), skin lesions (pruritus, exfoliative dermatitis in severe cases), and mild gastrointestinal symptoms were also commonly reported symptoms. In severe poisoning cases there was cardiac involvement (Jalili & Abbasi, 1961).

While children were among those harmed during these two incidents, the literature does not identify in what ways, if any, that children were differentially affected to adults. Nor does the literature discuss whether there were implications to children in utero who were exposed through the consumption of wheat by the pregnant mother.

Three events, occurring within two decades of each other, were critical in shaping our understanding of the impact that MeHg has on human health. Two outbreaks of MeHg poisoning caused by anthropogenic means occurred in the Kumamoto and Niigata Prefectures of Japan in 1956 and 1965 respectively. Minamata Bay, in the Kumamoto Prefecture, was contaminated by Hg released in the wastewater from an acetaldehyde chemical plant factory owned and operated by the Chisso Corporation. During this period of time, Minamata City was an area largely populated by relatively poor fishing villages and it was common practice for the fishermen supplement the diet of their family with fish and shellfish caught from Minamata Bay. In 1956, a 5-year old girl presenting to the Chisso Corporation’s Minamata factory hospital became the first recognized case of MeHg poisoning.
although it was not proven until 1959 (Harada, 1997). The Minamata Bay disaster gave rise to two conditions, Minamata disease and Congenital Minamata disease.

Minamata disease (essentially MeHg poisoning) caused severe damage to the CNS, resulting in a range of neurological symptoms of which the most common were disturbance of sensation and coordination, visual and hearing impairments, tremor, dysarthria and mental disturbances (Harada, 1997, Uchino et al., 1995). Congenital Minamata disease was the result of MeHg crossing the placental barrier in pregnant women who had consumed the contaminated seafood. Many of these children were born with cerebral palsy, cerebral palsy-like features (e.g., microcephaly, hyperreflexia, gross motor and mental impairment) or other deformities, while in most cases, the mother remained largely unaffected from the toxic effects of Hg (Harada, 1968, 1995). A similar episode of MeHg poisoning occurred shortly after in 1965 in the Niigata Prefecture of Japan where again Hg was released in the wastewater of an acetaldehyde chemical plant this time owned by the Showa Electrical Company (Lofroth, 1978).

In Iraq, a further mass MeHg poisoning outbreak occurred in 1971/2 similar in nature to the ethylmercury incidents witnessed only two decades prior. Following poor wheat harvests in Iraq, wheat was imported from Mexico and in order to protect it during the long transport, MeHg was used as a form of fungicide. While the wheat was distributed to farmers for planting (as opposed to consumption), the cause of the outbreak was traced to the ingestion of seed wheat that had been ground into flour to make bread. Reportedly over 6,500 people were hospitalised, of which 459 died (Bakir et al., 1973). These figures are considered conservative as many families chose not to take their sick family members to the hospital (or discharged them from hospital) upon learning that there was no cure. As with the incident in Minamata,
children exposed during infancy and in utero were profoundly affected with highly
similar symptoms including cerebral palsy, altered muscle tone and deep tendon
reflexes, and delayed developmental milestones (Amin-Zaki et al., 1974, 1979; Bakir
et al. 1973; Rustam & Hamdi, 1974).

The financial consequences of MeHg toxicity are considerable. It has been
calculated that the costs to children exposed to MeHg in utero, based on cord-blood
concentrations ≥5.8µg/L, equates to approximately US$8.7 billion per annum. The
cost is estimated on the basis of loss of productivity as a direct consequence of loss
of IQ due to MeHg toxicity (Trasande, Landrigan & Schechter, 2005)

Diet

Fish and shellfish are a rich source of protein, omega-3 fatty acids, vitamins A
and D and iodine and are therefore highly recommended as part of a healthy well-
balanced diet (Weichselbaum, Coe, Buttriss & Stanner, 2013). The safe level for
seafood consumption (2-3 serves, approximately 75-150 grams per serve) was
established from a study of children in the Seychelle Islands (Myers et al., 2003). As
no adverse neurodevelopmental effects from prenatal MeHg exposure were detected,
the mean exposure in the study was selected as the no-observed-adverse-effect level
(NOAEL). Although it has been argued that the methodology employed to determine
the safe level may not provide the best indicator of a no-effect exposure level
(Crump et al., 2000). Furthermore, the safe level recommended for women who are
pregnant or nursing may be set too high (Brown & Austin, 2012).

The effects of a diet high in concentrations of MeHg (due to seafood
consumption) to the unborn or newborn child via the placenta and breast milk have
been well-documented, indeed it has been reported by the National Academy of
Sciences that seafood consumption during pregnancy is the most important and best-
studied end-point for MeHg poisoning (National Research Council, 2000). For example, the concentration of Hg in the pregnant mother’s cord blood has been shown to positively increase with the amount of seafood consumed during pregnancy (Bjornberg et al., 2003). Likewise, studies have found the concentration of Hg in breast milk to have a significant positive correlation with the amount of fish and shellfish consumed during lactation (Chien et al., 2006; Drasch, Schupp, Hofl, Reinke & Roider, 1994; Drexler & Schaller, 1998).

Neurodevelopmental problems associated with seafood consumption have also been identified. A prospective study on a cohort of Faroese children exposed to MeHg in utero through their mother’s consumption of pilot whale meat identified neuropsychological deficits at 7-years of age (Grandjean et al., 1997) and irreversible impairment to areas of brain function at 14-years (Murata, Weihe, Budtz-Jørgensen, Jørgensen, & Grandjean, 2004). Furthermore, for pregnant women within the low exposure range, a positive association was identified between exposure level and developmental delay. Specifically, the developmental delay was extended by 1-2 months for each doubling of prenatal MeHg exposure level (Grandjean et al., 1997). A limitation that needs to be taken into consideration is the use of hair to provide a measure of Hg concentration in the Faroese studies. As identified by the authors, hair colour, treatment and growth rate, for example, may have potentially contaminated the results. A possible relationship between neurodevelopmental problems and infants fed fish congee as a weaning food (a common behaviour among some coastal communities in China and South East Asia) was also found among a small number of children presenting to a clinic in Sydney, Australia (Corbett & Poon, 2008).
**Dental amalgam**

Dental amalgam is a major source of elemental Hg exposure. Known also as silver fillings, dental amalgam have a long use in western dentistry, dating back to the 1830s, although its use in recent years has been declining in some counties such as the UK and US, whereas in others (e.g., Norway, Sweden, Denmark and Russia) its use has been banned altogether (United States Department of Health and Human Services [US DHHS], 1993). An amalgam is a metal alloy and is comprised of elemental Hg in the range of 45-55% (Ferracane, 2001). Once an amalgam is placed, Hg is continually released in the form of vapour, ions and particles throughout the life of the filling. Furthermore, the release of vapour is stimulated by ordinary, everyday activities, such as chewing gum, eating, brushing teeth and drinking hot fluids, in addition to habitual behaviours like bruxism (teeth grinding) (Svare et al, 1981, Barregård, Sällsten & Jarvholm, 1995). For example, there was a 3.5-fold increase (11μg/litre to 38μg/litre) before and after chewing in the average salivary Hg level of 108 people with dental amalgam (Bjorkman, Sandborgh-Englund & Ekstrand, 1997).

In the general, non-occupationally exposed population, dental amalgam is the principal source of Hg exposure, exceeding that of seafood or industrial-related processes (Skare, 1995). Approximately 70-80% of inhaled Hg vapour is absorbed in the blood through the lungs, however, Hg vapour entering through the nose following exhalation can also be found in sections of the brain (e.g., olfactory lobe and tract, pituitary gland) (Stock, 1935). Both human and animal studies have demonstrated that dental amalgam makes a significant contribution to Hg body burden (World Health Organization [WHO], 1991; US DHHS, 1993; Weiner & Nylander, 1995).
Foetuses and infants are also at risk of elemental Hg exposure through maternal amalgam. Hg released from dental amalgam fillings can pass through the placenta and breast milk accumulating in the child’s tissues (Vimy, Hooper, King & Lorscheider, 1997). Animal studies have shown that Hg from amalgam fillings can impact the foetal liver, kidney and brain and transfer to breast milk in detectable amounts (Takahashi, Tsuruta, Hasegawa, Kameyama & Yoshida, 2001; Vimy, Takahashi & Lorscheider, 1990). Concentrations of Hg in the colostrum and breast milk of women collected within four weeks following birth have been found to significantly correlate with the number of amalgam fillings (da Costa, Malm & Dórea, 2005; Drasch, Aigner, Roider, Staiger & Lipowsky, 1998; Drexler & Schaller, 1998). While it appears that the concentration of Hg in breast milk declines over time (Drexler & Schaller, 1998), da Costa et al. reported that the amount of Hg transferred to the infant via breast milk was above the WHO reference for more than half of the women in their sample. Cord blood concentrations of Hg have also shown a positive correlation with the number of maternal amalgams (Björnberg et al., 2003). Furthermore, Hg concentrations in the kidneys of infants reportedly increase according to the number of maternal fillings (Drasch et al., 1994; Drasch & Roider, 1995). Interestingly, a study by Drasch et al. (1998) found the Hg concentration of formula milk samples (reconstituted with Hg-free water) were equivalent to mothers who fell in the mid-range of amalgam fillings (1-7 fillings), suggesting, based on Hg exposure alone, that it is safer for a child to be formula fed if a mother has more than seven dental fillings. Nevertheless, not all studies have found a significant correlation between maternal amalgam surfaces and concentration of Hg in the breast milk (Klemann et al., 1990).
There is a paucity of studies investigating the long-term effects of exposure to maternal amalgam *in utero* and during infancy. Some studies suggest there are no ensuing neurodevelopmental or neuropsychological effects (Bellinger, Daniel, Trachtenberg, Tavares & McKinlay, 2007; Watson et al., 2013), although a case-control study conducted in Norway indicates that dental work may be associated with cleft palate. Preliminary results of the study, presented at the University of Southern Denmark, found that the odds of giving birth to a child with cleft palate quadrupled for women who had restorative work in the first two months of pregnancy. Furthermore, greater odds of cleft palate were associated with undergoing multiple procedures in the first trimester (DeRoos, 2011).

**Vaccinations**

The reduction of once prevalent childhood diseases such as measles, mumps, rubella, diphtheria and polio are attributed to the development of vaccines and nationalised vaccine programs (Ehreth, 2003). Nonetheless, the safety of vaccines remains a keenly debated issue particularly in relation to ASD.

Two divergent theories suggesting an association between vaccines and ASD have been especially prominent in the past couple of decades. The first of the two came to prominence following the now retracted publication of a paper by Wakefield et al. (1998) in the eminent journal *The Lancet*. The authors found intestinal abnormalities in a small group of children (*n*=12) who had been diagnosed with a regressive form of ASD, that is, the children had developed normally before suddenly losing acquired skills, including communication. The cause of the intense scrutiny of both the paper and authors was the inclusion in the findings that the onset of behavioural symptoms for 8 of the 12 children was reported by parents to be associated with the measles-mumps-rubella (MMR) vaccine.
The second of the two theories, which is pertinent to this thesis, suggests that there is an association between the preservative thiomersal (also known as thimerosal and merthiolate) and ASD. Developed in 1937, thiomersal (sodium ethylmercurithiosalicylate) is 49.55% ethylmercury by weight and has been used as a preservative in vaccines since the 1930s to maintain sterility and extend shelf-life, particularly in multi-dose vials (Tan & Parkin, 2000). Upon entering the body, thiomersal is metabolised to ethylmercury and then into inorganic Hg compounds (Qvarnström, Lambertsson, Havarinasab, Hultman & Frech, 2003). Concern has been raised that infants are receiving doses of Hg that may exceed safe levels due to the amount of thiomersal-containing vaccines (TCVs) received in the first few years of life. These concerns are not without justification. It is stated in the Australian Immunisation Handbook (Australian Technical Advisory Group on Immunisation [ATAGI], 2008) that “the possibility existed that vaccination of newborn babies, particularly those of very low birth weight, with repeated doses of thiomersal-containing vaccines, might have resulted in levels of mercury above the recommended guidelines” (p.355). Indeed, following the administration of TCVs, significant amounts of Hg has been measured in the blood and hair of infants (Stajich, Lopez, Harry & Sexson, 2000; Pichichero et al., 2008, 2009; Marques, Dórea, Bastos & Malm, 2007). Furthermore, body mass and developmental maturity was critical with premature infants reported to accumulate >3 times the level of blood Hg in contrast to other infants. For example, Stajich et al. (2000) found mean levels of Hg among pre-term babies to increase from .54μg/L to 7.36μg/L post-vaccination. Mean Hg levels among at-term babies increased .04μg/L to 2.24μg/L.

A number of recent epidemiological and ecological studies have reported a significant association between TCVs and neurodevelopmental disorders, including
An investigation of the potential association between mercury and ASD

autism, speech disorders, mental retardation, thinking abnormalities, deficits in psychomotor development and infantile spasms (Gallagher & Goodman, 2008, 2010; Geier & Geier, 2003a, 2003b, 2003c, 2004, 2005, 2006b, 2006c, 2006d, 2006e, 2006f; Mrozek-Budzyn, Majewska, Kieltyka & Augustyniak, 2012; Young, Geier, & Geier, 2008). Adverse neurodevelopmental consequences have also been found in a number of animal studies on rats, mice and monkeys designed to mirror the paediatric US vaccination schedule (Hornig, Chian & Lipkin, 2004; Ida-Eto et al., 2013; Olczak, Duszczyk, Mierzejewski, Meyza & Majewska, 2011; Sulkowski, Chen, Midha, Zavacki & Sajdel-Sulkowska, 2012; Hewitson et al., 2010; Hewitson, Lopresti, Stott, Mason & Tomko, 2010). Furthermore, Burbacher et al. (2005) found monkeys injected with TCVs to have a higher average brain-to-blood concentration ratio than MeHg-exposed monkeys, 3.5 ± 0.5 in comparison to 2.5 ± 0.3. However, it is important to take into consideration researcher bias, whether intentional or unintentional, on study outcomes. For example, the Geiers’ have published many studies finding evidence against the use of vaccinations and Hg, but they are also well-known advocates against the use of Hg and are funded by like-minded individuals and groups with strong political activity in this area.

Not all studies have found an association between TCVs and neurodevelopmental disorders, in particular ASD (Andrews, Miller, Grant, Stowe, Osborne & Taylor, 2004; Fombonne, Zakarian, Bennett, Meng & McLean-Heywood, 2006; Hviid, Stellfeld, Wohlfahrt & Melbye, 2003; Madsen et al. 2003; Stehr-Green, Tull, Stellfeld, Mortenson & Simpson, 2003). A further study by Verstraeten et al. (2003) was inconclusive. More than 5000 cases have been filed for autism-related injuries as a result of vaccination to the US Court of Federal Claims, typically referred to as the Vaccine Court (Keelan & Wilson, 2011). Of these, six test cases
that purported a link between autism and vaccines (three of which specifically cited Hg as the causative agent) were heard by the Vaccine Court to ascertain whether there was any validity to the claim of a link, and ultimately whether any further cases would be heard. In 2010, the final of the six cases (Cedillo v. DHHS) came to a conclusion, the ruling stated that the claimant had failed to present a viable theory of how autism could be caused by vaccines on a personal or more general level (Haertlein, 2012).

Currently there is not enough evidence to support or refute a link between vaccines and ASD. While thiomersal was phased out from 1999 in the US and 2000 in Australia (National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, 2007; ATAGI, 2008), it is still used in developing countries (Dórea, Marques & Brandao, 2009), in inactivated influenza vaccines in the US (which may be administered to pregnant women and infants), and in multi-dose vials in Canada and the US (CDC, 2012; Public Health Agency of Canada, 2011). Indeed, surprisingly in the face of existing evidence, the United Nations Minamata Convention on Hg, whose primary objective is to protect human health from exposure to Hg and Hg compounds, exempted TCVs from regulation (Sykes et al., 2014)’.

Pollution

According to the United Nations Environmental Programme (UNEP) (2002), approximately 70% of elemental Hg is released into the atmosphere through anthropogenic means, such as the combustion of fossil fuels via power plants, chloralkali production, and waste incineration. Through combustion, Hg present in fossil fuels is thermally converted into elemental Hg vapour which readily becomes airborne, travelling long distances until the particles ultimately deposit in the soil and
An investigation of the potential association between mercury and ASD (Galbreath & Zygarlicke, 1996). In recent years, there has been a growing body of research investigating possible associations in pre- and postnatal exposure to ambient levels of air pollution (due to power plants, traffic and urbanisation) with adverse neurodevelopmental outcomes.

An ecological study by Palmer, Blanchard, Stein, Mandell and Miller (2006) investigated whether environmentally released Hg (based on data from the US Environmental Protection Agency [EPA] Toxic Release Inventory [TRI]) was associated with autism prevalence and special education services among 254 counties and 1184 school districts in Texas. The authors reported a 61% increase in autism prevalence and 43% increase in special education services for every 1000 pounds of environmentally released Hg. A further ecological study by Palmer, Blanchard and Wood (2009) found Hg emissions from coal-burning power plants in 1998 to be significantly associated with autism prevalence in 2002. In addition, a decrease in autism cases was correlated with increased distance from the source of the emissions. However, the use of TRI data in both Palmer et al. studies has been criticised. Lewandowski, Bartell, Yager and Levin (2009) suggest that TRI data may not reflect concentrations of toxic substances in the immediate environment. Furthermore they question whether the use of TRI data allows a causal relationship to be established, that is, whether it can be proven that exposure to Hg preceded a diagnosis of ASD. Consequently, Lewandowski et al. conducted a replication study to assess the robustness of TRI. The study investigated TRI Hg emissions data in the same Texan counties and school districts reported by Palmer et al. (2006); however the emissions data was analysed for different years. The study failed to replicate the findings reported in the Palmer et al. studies.
Several studies have reported a positive correlation between airborne Hg and ASD prevalence rates and ASD risk. For example, in San Francisco Bay, areas with concentrations of atmospheric Hg in the highest quartile (based on data from the US EPA) were found to have a significantly higher ASD prevalence rate than areas with concentrations of atmospheric Hg in the lowest quartile (Windham, Zhang, Gunier, Croen & Grether, 2006). The study was strengthened by its ability to identify and confirm cases. However, a notable limitation to this study was the failure to take into consideration other sources of toxic exposure, including diet, occupation and smoking.

Roberts et al. (2013) found perinatal exposure to air pollutants had a significant positive linear trend with risk of ASD for Hg (in addition to other heavy metals). Guxen et al. (2012) also found residential air pollution in Spain to adversely affect infant mental development. Interestingly, antioxidant intake (e.g., maternal consumption of fruits and vegetables) appeared to mediate the adverse effect of air pollutants on neurodevelopment, but not in Gipuzkoa, a highly industrialised region of Spain.

In addition to power plants, traffic-heavy locations appear to make a substantial contribution to air pollution. Volk, Hertz-Picciotto, Delwiche, Lurmann and McConnell (2011) found autism to be associated with pregnant women residing within 310 meters of heavy traffic (via freeways and major roadways) during their third trimester (OR = 2.22; 95% CI, 1.16–4.42) and at the time of delivery (OR = 1.86; 95% CI, 1.04–3.45). Becerra, Wilhelm, Olsen, Cockburn and Ritz (2013) also found an association between exposure to traffic-related air pollution during pregnancy and autism. The authors employed a land use regression model based on data from air monitoring stations to estimate Hg exposure. Lai, Tseng, Hou and Guo
(2012) found ASD prevalence rates to be higher in urban areas of Taiwan than rural areas. One reason suggested by the authors for the difference in prevalence rates was that urbanisation may be associated with increased levels of hazardous air pollutants. It is also likely that urbanisation is associated with increased levels of traffic, which as noted above, has been associated with autism.

Not all studies have found Hg air pollutants to be associated with deficits in neurodevelopment. Tang et al. (2008) investigated whether child development was adversely affected by prenatal exposure to pollutants from a seasonally operated coal power plant in Tongliang, a county of Chongqing, China. The power plant is responsible for approximately 46% of Chongqing’s total Hg emissions. Tang et al. measured levels of polycyclic aromatic hydrocarbons (PAH) – DNA adducts and concentrations of lead and Hg in umbilical cord blood. The results of the study showed that children were adversely affected by exposure to pollutants at 2 years of age as measured by motor, adaptive, language, and social development quotients. However, while a reduction in one or more of the developmental quotients was significantly associated with PAH-DNA adducts and concentrations of lead in the cord blood, no such association was found with Hg. Some limitations with the study were noted by the authors, with perhaps the most critical limitation being that they did not collect postnatal blood PAH-DNA, lead or Hg levels. Therefore, postnatal exposure could not be compared against the cognitive development of the sample at two years of age. Dórea (2008) identified a possible methodological flaw beyond those discussed by Tang et al.’s study, raising the issue that uncontrolled sources of Hg, namely TCV and MeHg in food sources other than fish (particularly rice) were not accounted for in the analysis.
Genetics

Which genes an individual inherits plays a crucial role in detoxifying the body of Hg. Apolipoprotein E (APOE) is a major protein transporter expressed in the brain and is a known mediating factor in neuronal repair (Buttini et al., 1999; Mahley, 1988). There are three genetic variants of APOE: APOE2, APOE3 and APOE4. APOE consists of 299 amino acids with different ratios of cysteine and arginine at positions 112 and 158. Cysteine, unlike arginine, contains a sulfhydryl group which can bind, and importantly detoxify heavy metals (including Hg) in the nerve cell. APOE2 contains two cysteines and APOE3 contains one cysteine and one arginine, whereas APOE4 contains two arginines (Pendergrass, Hayley, Vimy, Winfield & Lorscheider, 1997; Mahley, 1988). Therefore, individuals who inherit at least one APOE4 are likely at greater risk of heavy metal accumulation in brain tissue and as such at greater risk of developing heavy metal induced neurological diseases and neurodevelopmental behaviours (Godfrey, Wojcik & Krone, 2003; Ng et al., 2013; Stewart, Schwartz, Simon, Kelsey & Todd, 2002). There is some suggestion that individuals diagnosed with autism may have a reduced capacity to excrete Hg due to inheriting at least one APOE3 or APOE4. For example, Giunco et al (2009) found APOE2 and APOE4 to be more prevalent among a sample of individuals with autism. Other studies however, have failed to find an association between APOE and autism (e.g., Raiford et al., 2004).

Mercury and Autism

Mutter et al. (2005) reported that in the US, infants have been exposed to levels of Hg substantially higher than US federal safety guidelines. In 2001, a paper published in the journal Medical Hypotheses by Bernard et al. drew parallels between
the symptomatology of Hg toxicity and ASD (e.g., mental retardation, loss of speech, social withdrawal) suggesting that ASD is a form of Hg poisoning. Later studies appear to support this assertion identifying a number of biological irregularities common among children with ASD that are consistent with Hg toxicity, such as, elevated oxidative stress (e.g., James, et al., 2004; Kern & Jones, 2006), immune dysfunction (e.g., Cohly & Panja, 2005), neural and generalised inflammation (e.g., Herbert, 2005; Pardo, Vargas & Zimmerman, 2005; Vargas, Nascimbene, Krishnan, Zimmerman & Pardo, 2005; Zimmerman et al., 2005) and depleted glutathione levels (e.g., James et al., 2004).

The paper by Bernard et al. has become the cornerstone of the Hg-autism hypothesis which postulates that ASD develops due to an interaction between a genetic susceptibility and environmental trigger. Essentially, the Hg-autism hypothesis is a two-part hypothesis that states that a child needs to have a predisposing genetic sensitivity to Hg in conjunction with being exposed to Hg for ASD to develop. Exposure may be chronic or acute and occur either in utero or during infancy.

Mercury can be measured in a number of different ways, most commonly in the hair, blood or urine, although Hg has also been measured in the teeth, finger/toe nails, faeces, and brain. Before considering ASD studies specifically, it is perhaps first important to note that the method of measuring Hg greatly influences the interpretation of results. For example, the half-life of Hg in the blood is relatively short with an initial half-life of 1-3 days followed by a slower phase half-life of 1-3 weeks (Barregård et al., 1992), or perhaps several weeks longer in the case of chronic exposure (Sallsten, Barregård & Schütz, 1993). As such, blood Hg can be a useful measure of recent or ongoing exposure but is not an ideal measure of past exposure.
In contrast, concentrations of Hg in the hair provide a better measure of past exposure than blood Hg. Depending on the biological test conducted, measuring Hg in urine can provide: (1) a measure of total urinary Hg concentration, (2) a biological marker of Hg damage (via porphyrins), or (3) a measure of Hg body burden (via provoked urine challenge). Elevations in porphyrin levels indicate that enzymes in the biosynthesis pathway have been impaired due to the toxic effects of heavy metals. Elevations in the urinary levels of pentacoproporphyrin (5CP), precoproporphyrin (PrCP), and coproporphyrin (CP) are specifically associated with Hg exposure (Heyer, Bittner, Echeverria & Woods, 2006; Woods et al., 2005). A provoked urine challenge involves administering a chelating agent (e.g., 2,3-dimercapto-1-propanesulfonic acid [DMPS]; dimercaptosuccinic acid [DMSA] or ethylenediaminetetraacetic acid [EDTA]) orally, intravenously or as a suppository. The chelating agent binds to Hg (and other metals) circulating in the blood and organs, particularly the kidneys, which is then rapidly excreted in the urine, from where Hg concentration is measured (Ruha, 2013).

The number of studies investigating Hg and ASD has steadily grown since the paper by Bernard et al., however, the results have been inconsistent. Table 5 presents a list of Hg and ASD studies conducted in recent years reporting measurements of Hg in the blood, hair or urine.

Whether an end-point of Hg exposure includes ASD remains a topic of intense debate. While the mounting evidence that Hg is associated with ASD is compelling, the evidence is not definitive. More research addressing this potential association is critical. It is therefore the intention of this thesis to apply an interdisciplinary approach to explore the question of whether a relationship exists between Hg and ASD. Specifically, a biological and hereditary approach will be taken. Urinary
porphyrins have been selected as the primary biological outcome measure. There are two key reasons for this decision. First, the process of collecting urine for porphyrin analysis is non-invasive and reasonably low-stress, which is essential for vulnerable populations, such as children with ASD. Urine collection is conducted at home, which can be easier on the child and allows the parent to have some control over the process. They can choose which day collection will occur and prepare their child over a period of days if necessary. Second, porphyrins provide a measure of Hg damage as opposed to the current level or concentration of Hg. Therefore, when the exposure occurred is not as critical as it would be should Hg be measured in the blood for example.

In the following chapters, three published studies will be presented. The first study (Chapter 4) will determine whether the porphyrin profiles of an Australian sample of children with a diagnosis of ASD exhibit elevated urinary porphyrin levels that reflect a pattern of Hg damage. This will be a pilot study, the results of which will determine whether a further study is warranted. The second study (Chapter 5) will address the limitations of the pilot study and expand upon previously published porphyrin studies. A larger sample will be recruited, in addition to recruiting two normally developing, healthy control groups (sibling control and external control). Furthermore, the study will determine whether group membership and ASD severity can be predicted using porphyrin profiles. The final study (Chapter 6) will examine individual susceptibility to Hg by determining the ASD prevalence among the descendants of a cohort of the general population with a known sensitivity to Hg (pink disease survivors).
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<thead>
<tr>
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<th>Publication year</th>
<th>Results</th>
</tr>
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<tr>
<td>Blood</td>
<td>Adams et al.</td>
<td>2013</td>
<td>Failed to find a significant difference in Hg levels in whole blood or red blood cells between ASD and control groups. However, regression analysis showed Hg levels in whole blood and red blood cells to be consistently significant with autism severity</td>
</tr>
<tr>
<td></td>
<td>Albizzati et al.</td>
<td>2012</td>
<td>Failed to find a significant difference in whole blood Hg concentration between ASD and controls</td>
</tr>
<tr>
<td></td>
<td>DeSoto &amp; Hitlan</td>
<td>2007</td>
<td>Significant relationship between Hg whole blood levels and diagnosis of ASD</td>
</tr>
<tr>
<td></td>
<td>Geier et al.</td>
<td>2010</td>
<td>Significantly higher Hg levels in red blood cells among ASD group compared to controls</td>
</tr>
<tr>
<td></td>
<td>Hertz-Picciotto et al.</td>
<td>2010</td>
<td>Failed to find a significant difference in whole blood Hg levels between ASD and controls</td>
</tr>
<tr>
<td></td>
<td>Rahbar et al.</td>
<td>2012</td>
<td>Failed to find a significant difference in whole blood Hg concentrations between ASD and controls</td>
</tr>
<tr>
<td></td>
<td>Stamova et al.</td>
<td>2011</td>
<td>Failed to find a significant difference in whole blood Hg between boys with ASD and control</td>
</tr>
<tr>
<td>Hair</td>
<td>Adams et al.</td>
<td>2008</td>
<td>ASD group 2.5-fold more likely to have lower hair Hg levels compared to matched controls</td>
</tr>
<tr>
<td></td>
<td>Adams et al.</td>
<td>2006</td>
<td>Failed to find a significant difference in hair Hg levels between ASD and controls</td>
</tr>
<tr>
<td></td>
<td>Al-Ayadhi</td>
<td>2005</td>
<td>Significantly higher hair Hg levels among ASD group compared to controls</td>
</tr>
<tr>
<td></td>
<td>Albizzati et al.</td>
<td>2012</td>
<td>Failed to find a significant difference in hair Hg levels between ASD and controls</td>
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<tr>
<td></td>
<td>Blaurock-Busch et al.</td>
<td>2011</td>
<td>Failed to find a significant difference in hair Hg levels between ASD and controls</td>
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<tr>
<td></td>
<td>Blaurock-Busch et al.</td>
<td>2012</td>
<td>Hair Hg levels significantly higher among ASD group compared to controls. Significant positive correlation between Hg and object use and auditory response</td>
</tr>
<tr>
<td></td>
<td>De Palma et al.</td>
<td>2012</td>
<td>Failed to find a significant difference in hair Hg levels between ASD and controls</td>
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<tr>
<td></td>
<td>El-baz et al.</td>
<td>2010</td>
<td>Significantly higher hair Hg levels among ASD group compared to controls. Failed to find significant difference in hair Hg levels across IQ groups, although Hg levels highest in most disabled group</td>
</tr>
<tr>
<td></td>
<td>Fido &amp; Al-Saad</td>
<td>2005</td>
<td>Boys with ASD had significantly higher hair Hg concentrations in comparison to matched controls</td>
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<tr>
<td></td>
<td>Geier et al.</td>
<td>2012</td>
<td>Significant positive correlation between hair Hg concentrations and ASD severity</td>
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<tr>
<td></td>
<td>Holmes et al.</td>
<td>2003</td>
<td>Hair Hg levels significantly lower in ASD group compared to controls. Negative correlation between hair Hg level and ASD severity</td>
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<tr>
<td></td>
<td>Kern et al.</td>
<td>2007</td>
<td>Failed to find a significant difference in hair Hg levels between ASD and matched controls</td>
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An investigation of the potential association between mercury and ASD

<table>
<thead>
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<th>Results</th>
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</tr>
<tr>
<td>Hair</td>
<td>Priya &amp; Geetha</td>
<td>2011</td>
<td>Significantly higher hair Hg levels in ASD group compared to controls. Elevation of Hg more pronounced among low functioning autism group in contrast to moderate and high functioning autism groups</td>
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<tr>
<td>Hair</td>
<td>Williams et al.</td>
<td>2008</td>
<td>Failed to find a significant difference in hair Hg levels between ASD group and controls</td>
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<td>Teeth</td>
<td>Adams et al.</td>
<td>2007</td>
<td>Significant difference between ASD and control group. Hg levels in the baby teeth of the ASD group higher than the control group. Use of oral antibiotics discussed as a potential confounder</td>
</tr>
<tr>
<td>Urine</td>
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</tr>
<tr>
<td>Total Hg</td>
<td>Adams et al.</td>
<td>2013</td>
<td>Failed to find a significant difference in urinary Hg concentration between ASD and controls</td>
</tr>
<tr>
<td>Total Hg</td>
<td>Albizzati et al.</td>
<td>2012</td>
<td>Failed to find a significant difference in urinary Hg concentration between ASD and controls</td>
</tr>
<tr>
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<td>Blaurock-Busch et al.</td>
<td>2011</td>
<td>Significant difference in mean urinary Hg levels between ASD and controls</td>
</tr>
<tr>
<td>Total Hg</td>
<td>Wright et al.</td>
<td>2012</td>
<td>Failed to find significant difference in urinary Hg (corrected for creatinine) levels between children with ASD and 3 control groups (mainstream schoolchildren, children from special schools &amp; ASD siblings)</td>
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<td>Porphyrins</td>
<td>Geier &amp; Geier</td>
<td>2006b</td>
<td>Median CP levels of unchelated ASD group significantly higher than sibling control. No significant difference found between chelated ASD group and sibling control</td>
</tr>
<tr>
<td>Porphyrins</td>
<td>Geier &amp; Geier</td>
<td>2007</td>
<td>Supported results of 2006 study (above). Unchelated ASD group significantly higher urinary 5CP levels in comparison to chelated group and sibling control. Unchelated ASD group had higher elevations in PrCP and CP relative to general population control.</td>
</tr>
<tr>
<td>Porphyrins</td>
<td>Nataf et al.</td>
<td>2006</td>
<td>Elevated levels of 7CP, 6CP, 5CP, PrCP and CP among children diagnosed with autistic disorder relative to controls. Positive correlation between porphyrin levels and ASD severity.</td>
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<tr>
<td>Chelation</td>
<td>Bradstreet et al.</td>
<td>2003</td>
<td>(DMSA administered) ASD group excreted Hg at 3-6 times the level of neurotypical children</td>
</tr>
<tr>
<td>Chelation</td>
<td>Soden et al.</td>
<td>2007</td>
<td>(DMSA administered) Failed to find increased urinary Hg levels between ASD group and controls</td>
</tr>
</tbody>
</table>

1Reanalysis of dataset analysed by Ip et al. (2004); 2Hair taken from infants (12-24mths). All other studies analysed hair from young children and in some cases teenagers.
Chapter 4: An investigation of porphyrinuria in Australian children with Autism.

In order to establish whether a relationship between Hg and ASD potentially exists, it was first necessary to determine whether there was evidence of Hg toxicity among a small convenience sample of Australian children diagnosed with ASD. The results of urinary porphyrin profiles were analysed to establish whether this was the case.

Urinary porphyrins are a non-invasive method of measuring general xenobiotic exposure (Brewster, 1988) and Hg exposure specifically (Heyer et al., 2006; Woods et al., 2005). Porphyrinuria, the excessive excretion of porphyrins, develops due to a disruption of enzymes in the heme biosynthesis pathway (see Figure 1). The steps in the pathway most vulnerable to heavy metal disruption involve uroporphyrin decarboxylase (UROD) (Woods & Kardish, 1983) and coproporphyrinogen oxidase (CPOX) (Woods et al., 2005). Inhibition to the CPOX and UROD enzymes cause elevations of urinary coproporphyrin (CP) and pentacarboxyporphyrin (5CP). The atypical porphyrin keto-isocoproporphyrin, more commonly known as precoproporphyrin (PrCP), produced by in vivo conversion of 5CP in the presence of heavy metal, has been specifically associated with Hg exposure (Heyer et al., 2006; Woods et al., 2005).

Urinary porphyrins have been recognized as an essential measure of Hg toxicity. In 2006 the first biennial meeting of the Hg bioindicators roundtable was held with the purpose of identifying a set of reliable tools for measuring Hg toxicity over a period of time for developed and developing countries and across species (humans, wildlife, and rodents). A key recommendation from the committee
was that further porphyrin studies be conducted so as to evaluate PrCP (Henshel et al., 2007).

Three studies, one conducted in France (Nataf et al., 2006) and two in the US (Geier & Geier, 2006d, 2007), set the precedence for this study. Nataf et al., the first team to investigate porphyrins and autism, observed a porphyrin pattern among French children with Autistic Disorder (AUT) that implicated Hg toxicity. In a sample of 269 French children aged 2-15 years, the researchers found elevated levels of heptacoproporphyrin (7CP), hexacoproporphyrin (6CP), 5CP, PrCP, and CP among children diagnosed with AUT ($n=106$) (and AUT + epilepsy, $n=9$) relative to an internal and external control group. It is important to be cautious interpreting the results in light of the limitations of the study, namely, the small internal control group ($n=12$) and the comparison to an external group from another country (Switzerland). Subsequently Geier and Geier (2006d, 2007) replicated these findings in two small studies in the US. The researchers first investigated the difference in
An investigation of the potential association between mercury and ASD urinary CP levels among chelated and unchelated individuals diagnosed with ASD ($n=37$, 7-22 years of age) in comparison to a neurotypical sibling control group ($n=7$, 7-20 years of age). They found that the median CP levels of unchelated individuals with ASD were significantly higher than that of the control group, whereas no significant difference was found between chelated individuals with ASD and the control group (Geier & Geier, 2006d). An extension of this study by the same investigators (Geier & Geier, 2007) supported the results. Additionally, they found that unchelated individuals with ASD had significantly higher urinary 5CP levels in comparison to chelated individuals with ASD and to chelated individuals + sibling controls. Unchelated individuals with ASD also had higher elevations in both PrCP and CP (normalized to uroporphyrin) relative to the general population control.

The following paper reports on the first empirical study presented in this thesis. A convenience sample of Australian children ($N=41$) with a diagnosis of ASD presenting to a clinical practice were recruited into the study. A pre-requisite of entry into the study was that the child had formerly undergone a urinary porphyrin test. The mean urinary porphyrin levels of this ASD group were then compared against the mean urinary porphyrin levels reported by Nataf et al. (2006) and Geier and Geier (2007) for their ASD and control groups. In addition, the porphyrin levels from all studies were compared to an external control group of normally developing children (Minder & Schneider-Yin, 1996).

The purpose of the study was to establish whether the porphyrin profiles of a sample of Australian children exhibit a pattern indicative of Hg damage. This is a critical first study as no previously published studies have investigated Hg toxicity among a sample of Australian children diagnosed with ASD by using porphyrin
profiles (or by any other means). Furthermore, this study was designed as a pilot trial in order to determine whether a larger case-control study was warranted.
Paper 2


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<th>School/Institute/Division if based at Deakin; Organization and address if non-Deakin</th>
<th>Email or phone</th>
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<tbody>
<tr>
<td>David Austin</td>
<td>School of Psychology</td>
<td><a href="mailto:david.austin@deakin.edu.au">david.austin@deakin.edu.au</a></td>
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2. Inclusion of publication in a thesis

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<td>Kerrie Shandley</td>
<td>School of Psychology</td>
<td>An investigation of the potential association between mercury and Autism Spectrum Disorder: An Interdisciplinary approach</td>
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Conceptual development of study, ethics application, data management and analysis, drafting of manuscript, manuscript revision.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

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<td>Kerrie Shandley</td>
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4. Description of all author contributions

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<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>David Austin</td>
<td>Recruitment, review and feedback of manuscript, principal author</td>
</tr>
<tr>
<td>Kerrie Shandley</td>
<td>Conceptual development of study, data management and analysis, drafting of manuscript, manuscript revision.</td>
</tr>
</tbody>
</table>
5. Author Declarations
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6. Other contributor declarations
I agree to be named as a non-author contributor to this work.

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An Investigation of Porphyrinuria in Australian Children with Autism

David W. Austin and Kerrie Shandley
Swinburne Autism Bio-Research Initiative (SABRI), Faculty of Life and Social Sciences, Swinburne University of Technology, Melbourne, Australia

Two recent studies, from France (Nataf et al., 2006) and the United States (Geier & Geier, 2007), identified atypical urinary porphyrin profiles in children with an autism spectrum disorder (ASD). These profiles serve as an indirect measure of environmental toxicity generally, and mercury (Hg) toxicity specifically, with the latter being a variable proposed as a causal mechanism of ASD (Bernard et al., 2001; Mutter et al., 2005). To examine whether this phenomenon occurred in a sample of Australian children with ASD, an analysis of urinary porphyrin profiles was conducted. A consistent trend in abnormal porphyrin levels was evidenced when data was compared with those previously reported in the literature. The results are suggestive of environmental toxic exposure impairing heme synthesis. Three independent studies from three continents have now demonstrated that porphyrinuria is consonant with ASD, and that Hg may be a likely xenobiotic to produce porphyrin profiles of this nature.

Autism is a neurodevelopmental disorder presenting in childhood that affects up to 1 in 150 children in the United States (Centers for Disease Control, 2006) and 1 in 160 in Australia (Wray & Williams, 2007). Autism is characterized by severe impairments in socialization, communication, and behavior (American Psychiatric Association, 1994). The prevalence of autism is increasing at epidemic rates (Yuzbasi, 2003) that cannot be accounted for by changing diagnostic criteria or improved diagnostic systems (Bloxill et al., 2003; Croen et al., 2002).

Mercury (Hg) toxicity has been proposed as a causal mechanism whereby a small subset of children are uniquely sensitive to Hg and, in such individuals, exposure triggers a cascade of events leading to autism (Bernard et al., 2001; Mutter et al., 2005; Kern & Jones, 2006). Urinary porphyrins provide a convenient and non-invasive measure of xenobiotic exposure generally (Brewster, 1988) and of Hg specifically (Wood et al., 2005; Heyer et al., 2006).

Excess urinary porphyrin excretion (porphyrinuria) results from the inhibition of enzymatic steps in conditions including genetic deficiencies in heme production enzymes, hepatitis, renal disease, and erythroid disease (Gross et al., 2000), as well as by heavy metal inhibition (Bowers et al., 1992; Woods, 1996). The causal relationship between Hg and porphyrinuria has been demonstrated both in rats (Pingree et al., 2001) and in humans (Woods et al., 1993).

The steps in the heme pathway most vulnerable to heavy metal inhibition are those that involve uroporphyrin decarboxylase (Woods & Kardish, 1983) and coproporphyrinogen oxidase (Woods et al., 2005). The result of these inhibitions is specific elevations of urinary coproporphyrin and pentacarboxyproporphyrin levels. Although nonmetal agents targeting the heme pathway also elevate urinary porphyrin levels (Daniel et al., 1997), precoproporphyrin (also known as keto-isocoproporphyrin) is produced by in vivo conversion of pentacarboxyproporphyrin in the presence of heavy metal, providing a specific porphyrin marker for Hg exposure (Woods et al., 2005; Heyer et al., 2006).

Two previous studies reported porphyrinuria among autistic subjects consistent with elevated body burden of Hg (Nataf et al., 2006; Geier & Geier, 2007). The pattern is one of generalized porphyrinuria with marked elevation of coproporphyrin and of uroporphyrin and of the ratio of coproporphyrin to uroporphyrin. This study aimed to examine this phenomenon among a group of Australian autistic children.

METHODS

Subjects

Urinary porphyrin profiles were obtained from 41 consecutive patients with an ASD presenting to the first author’s psychology clinic from October 2006 through March 2008. Each patient was previously diagnosed with an ASD, by a health professional, based upon accepted international...
An investigation of the potential association between mercury and ASD

D. W. Austin and K. Shandley

Standards (American Psychiatric Association, 1994). Table I provides a summary of patient sample characteristics. Subjects were excluded if they had ever undergone any specific therapy to remove Hg (chelation) or if the child had confirmed fragile X, Prader-Willi/Angelman, or Rett syndrome.

Measures

All patients were tested by the ISO-certified Laboratoire Philippe Auguste for urinary porphyrins using high-pressure liquid chromatography with fluorometric detection, including uroporphyrin (UP), heptacarboxylyeloporphyrin (7cP), hexacarboxyporphyrin (6cP), penta- and tetraacetylporphyrin (5cP, 4cP), and coproporphyrin (CP; types I and II).

Statistical Analysis

Raw data (ASD and control) from the Nataf et al. (2006) study and normal control data from the Minder and Schneider-Yin (1996) study were obtained. Consequently, mean porphyrin levels (standardized to creatinine levels) and the CP/UP ratio was calculated for the Australian sample and compared to these data sets. Porphyrin levels and ratios for the Geier and Geier study (2007) were ascertained from their published paper, as the raw data were not available. A one-way-between-groups analysis of variance (ANOVA) was conducted to compare ASD and control group CP/UP ratios. This ratio is an important measure, as coproporphyrin is postulated to be directly affected by Hg, with uroporphyrin to a lesser extent. Tukey HSD post hoc comparisons determined differences between pairs of means.

Results

Table 1 shows the mean differences between the ASD patients and the non-ASD controls. Elevations on all porphyrins for the ASD groups in comparison to both control and laboratory reference range are evident, with the greatest discrepancies evident for penta- and tetraacetylporphyrin.

A statistically significant difference was found in the CP/UP ratio between the present study ASD data, Nataf et al. (2006)

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<td>7cP</td>
<td>5.06 (3.12)</td>
<td>3.70 (7.16)</td>
<td>2.89 (3.18)</td>
<td>2.94 (1.86)</td>
<td>2.32 (0.92)</td>
</tr>
<tr>
<td>6cP</td>
<td>1.03 (0.97)</td>
<td>1.97 (4.56)</td>
<td>0.44 (0.53)</td>
<td>0.93 (0.79)</td>
<td>0.56 (0.28)</td>
</tr>
<tr>
<td>5cP</td>
<td>5.19 (2.33)</td>
<td>1.57 (1.04)</td>
<td>1.00 (0.50)</td>
<td>4.02 (2.46)</td>
<td>1.99 (1.23)</td>
</tr>
<tr>
<td>PrCP</td>
<td>20.34 (10.69)</td>
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<td></td>
<td>18.13 (13.07)</td>
<td>7.95 (5.41)</td>
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<tr>
<td>CP</td>
<td>289.61 (175.75)</td>
<td>299.70 (1910)</td>
<td>162.20 (43.20)</td>
<td>185.80 (159.14)</td>
<td>71.25 (49.45)</td>
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<td>Porphyrin ratios</td>
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<tr>
<td>PrCP/UP</td>
<td>1.17 (0.72)</td>
<td>1.67* (2.80)</td>
<td>0.36* (0.09)</td>
<td>1.20 (0.72)</td>
<td>0.60 (0.38)</td>
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<tr>
<td>CP/UP</td>
<td>16.31 (9.37)</td>
<td>26.66* (49.19)</td>
<td>5.56* (1.48)</td>
<td>12.11 (8.99)</td>
<td>5.35 (3.56)</td>
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Note. Data are given as means with SD in parentheses. Porphyrin levels are expressed as nmol/g CRT. Abbreviations: CRT = creatinine; UP = uroporphyrin I and II; 7cP = heptacarboxylyeloporphyrin; 6cP = hexacarboxyporphyrin; 5cP = pentaacetylporphyrin; 4cP = tetraacetylporphyrin; CP = coproporphyrin I and II.

*The laboratory reference range was obtained from Laboratoire Philippe Auguste, Paris, France.

n = 11.

n = 5.
An investigation of the potential association between mercury and ASD

FIG. 1. Cyporphyrinuria (CPU)/urine porphyrin (UP) ratio means. Asterisk denotes significant difference in the Minder and Schneider-Yin (1996) control group at $p < .05$. ns = not significant. Gieler and Gierer (2007) means not included in statistical comparisons as raw data were unavailable.

ASD and control data, and Minder and Schneider-Yin (1996) control data. Post hoc comparisons indicated that the CP/UP ratio for the two ASD groups (Austin and Shandalley, and Natuf et al., 2006) differed significantly from both control groups (Natuf et al., 2006, and Minder & Schneider-Yin, 1996). The two ASD groups did not differ significantly from each other, and likewise, the two control groups did not differ significantly. Figure 1 graphically represents the differences in CP/UP ratio across groups.

DISCUSSION

The present study provides further support from an independent cohort that porphyriuria is a concomitant biological occurrence in ASD. Furthermore, this study provides further evidence suggestive of an environmental toxicant variable, consistent with Hg, contributing to the maintenance, and possibly development, of ASD. Given the consistency of the emerging research, health authorities worldwide need to move without delay to further elucidate the specific nature of the toxic insult. Research needs to focus on the form, source, dose, and timing of the toxic exposure and, importantly, appropriate treatment for those already exposed.

REFERENCES

Chapter 5: Are urinary porphyrins a valid diagnostic biomarker of Autism Spectrum Disorder?

The first empirical study presented in this thesis (Austin & Shandley, 2008) found support for the notion that there is a relationship between Hg and ASD. Therefore, a larger study using urinary porphyrin as the primary outcome measure was conducted. Critically, the study sought to address the limitations of the first study (small sample size, lack of appropriate comparison group) and extend upon existing porphyrin and ASD studies.

As discussed in Chapter 4, UROD and CPOX (steps in the heme biosynthesis pathway) are vulnerable to heavy metal disruption (Woods & Kardish, 1983; Woods et al., 2005). An elevation in three urinary porphyrins (5CP, CP and PrCP) have been associated with Hg toxicity, two of which are caused by an inhibition to the UROD and CPOX enzymes (5CP and CP) and the other produced by in vivo conversion of 5CP in the presence of heavy metal (PrCP) (Heyer et al., 2006; Woods et al., 2005). In addition to providing a measure of Hg damage, a recent study by Heyer, Echeverria and Woods (2012) demonstrated that urinary porphyrins (5CP and CP) have excellent predictive values in the detection of AUT and PDD-NOS. Furthermore, there is some evidence to suggest that urinary porphyrins may provide a marker of ASD severity (Nataf et al., 2006; Geier et al., 2009).

The following paper investigated whether urinary porphyrin profiles could be used to: (1) detect ASD cases, and (2) predict ASD severity. Three groups of children aged 2-6 years were recruited: (a) children diagnosed with ASD, (b) siblings of children diagnosed with ASD (sibling control), and (c) children with no known blood relative diagnosed with ASD (external control). Participating families were
provided with a urinary collection kit (one per participating child) which included information on how to collect, store and ship the samples. The limitations of the first porphyrin study were addressed by recruiting a substantially larger sample and including two distinct healthy controls. Furthermore, this study extends upon previously published porphyrin studies by aligning with the DSM-5 nosology. That is, ASD was treated as a single dimension as opposed to using the former individual diagnostic groupings of Autistic Disorder, Asperger’s Disorder and PDD-NOS. In addition, a considerably younger and stricter age group was recruited for this study (2-6 years).
**Paper 3**


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<td>Autism Research (in press)</td>
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<td>School of Psychology</td>
<td>An investigation of the potential association between mercury and Autism Spectrum Disorder: An interdisciplinary approach</td>
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I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: Kerrie Swardley 30/11/14

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</tr>
<tr>
<td>David Austin</td>
<td>Feedback on project development and questionnaire, review and feedback on manuscript drafts</td>
</tr>
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<td>Jahan Bhowmik</td>
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RESEARCH ARTICLE

Are Urinary Porphyrens a Valid Diagnostic Biomarker of Autism Spectrum Disorder?

Kerrie Shandley, David W. Austin, and Jahar L. Bhowmik

A fundamental challenge to the timely diagnosis of Autism Spectrum Disorder (ASD) is the reliance on the observation of a set of aberrant behavior. Consequently, the diagnostic process requires that the child reach an age where the behaviors would typically be exhibited. The identification of a reliable biological marker (biomarker) could be of considerable benefit to the diagnostic process. As a diagnostic biomarker, porphyrins present an attractive prospect as previous studies have reported consistent findings of children with ASD showing significant elevations in porphyrin levels in contrast to controls. Furthermore, there is some evidence that ASD severity may be associated with porphyrins, which would be a valuable characteristic of any ASD biomarker. Importantly, for practical use, porphyrins can be measured non-invasively via a sample of urine. The present study sought to investigate whether porphyrin profiles can reliably be used to (a) differentiate ASD cases from healthy controls and (b) predict ASD severity. The study compared the porphyrin levels of three groups of children aged 2-6 years: Group 1—children diagnosed with ASD (n = 36); Group 2—healthy, normally developing siblings of children diagnosed with ASD (n = 36); and Group 3—healthy, normally developing children with no known blood relative diagnosed with ASD (n = 54). The results of logistic regression analyses failed to find support for the hypothesis that porphyrin levels could be used as a valid tool to detect ASD cases or predict severity.


Keywords: porphyrins; biomarker; ASD diagnosis; ASD severity; heavy metal mercury

Introduction

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with no known cause or cure. Until May 2013, ASD was used as an unofficial collective term encompassing Autistic Disorder (AUT), Asperger’s Disorder (AD), and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). With the release of the fifth edition of the Diagnostic and Statistical Manual (DSM) [American Psychiatric Association, 2013], ASD has been formally established in DSM nomenclature replacing the individual diagnostic labels of AUT, AD, and PDD-NOS. ASD manifests in the first few years of life, and although the symptoms and degree of severity vary widely, behavioral impairments in social and communicative interaction and play activities are the core features [American Psychiatric Association, 2013]. The cost to families and the wider community of a child diagnosed with ASD is substantial. The annual cost of all ASD cases in Australia is estimated to range from $4.5-7.2 billion [Synergies Economic Consulting, 2007], and the estimated lifetime cost for an individual with ASD US$3.5-$5 million [Ganz, 2007].

The National Center for Health Statistics estimates that ASD now affects 1 in 59 US children 6-17 years of age [Skirven et al., 2013]. In Australia, the most widely accepted ASD prevalence rate is considerably lower at 1 in 160 children aged 6-12 years [Williams, 2009, MacDermott, 2009, Wray, 2008]. However, more recent estimates suggest ASD prevalence may be as high as 1 in 119 children [Barbaro & Disanayake, 2010]. Regardless of the ongoing debate as to whether the observed growth in prevalence reflects a genuine increase or is an artifact of broadening diagnostic criteria, greater awareness, and diagnostic substitution, ASD has become a major public health issue. Of paramount importance to the long-term well-being and development of the child is the capability to conduct reliable and timely ASD diagnoses. Logically, the earlier an accurate diagnosis can be made, the earlier suitable interventions can commence. A report prepared by Synergies Economic Consulting [2012] for the Australian Autism Early Intervention Outcomes Unit Foundation provides a comprehensive outline of studies demonstrating the beneficial impacts of early intervention strategies in relation to education (i.e., improved cognitive functioning/dIQ, increased success of special schooling) and improved employment outcomes, independence, and improved quality of life for the affected individual and their families.

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Received October 11, 2013; accepted for publication March 25, 2014.

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Published online in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/aur.1385

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A fundamental challenge to the timely diagnosis of ASD is the reliance on the observation of a set of aberrant behavior. Consequently, the diagnostic process requires that the child reach an age where the behaviors would typically be exhibited. Furthermore, diagnosis is an inherently subjective process leading to questions of reliability, especially for children presenting with symptoms consistent with the milder end of the spectrum and those with comorbid conditions. Nevertheless, contemporary research has broadened our understanding of ASD from one of a purely behavioral or psychiatric condition to that of a whole-body condition. For example, research has identified a number of biomedical irregularities common in ASD including elevated oxidative stress [e.g., James et al., 2004; Kern & Jones, 2006], mitochondrial respiratory disorders [e.g., Oliveira et al., 2007; Rosignol & Bradstreet, 2008], generalized inflammation [e.g., Zimmerman et al., 2005], neuroinflammation [e.g., Herbert, 2005; Pardo, Vargas, & Zimmerman, 2005], gastrointestinal abnormalities [e.g., Pardo, Vargas, & Zimmerman, 2005], and fatty acid deficiencies [e.g., Aumann et al., 2007; Vancaill et al., 2001]. Other potential biomarkers for ASD—and the focus of this paper—are urinary porphyrins.

Urinary porphyrins can provide a non-invasive measure of xenobiotic exposure generally [Brewster, 1998] and, of particular interest in recent years, mercury exposure specifically [Heyer, Bittner, Echeverria, & Woods, 2006; Woods et al., 2005]. A series of experimental studies, reviewed by Woods [1995], demonstrate that urinary porphyrin excretion patterns in animals and humans change as a consequence of prolonged exposure to low levels of heavy metals. Porphyrinuria, the excessive excretion of urinary porphyrins, develops due to a disruption of enzymes in the heme biosynthesis pathway (see Fig. 1). The steps in the pathway most vulnerable to heavy metal disruption involve uroporphyrinogen decarboxylase (UROD) [Woods & Leardini, 1983] and coproporphyrinogen oxidase (CPOX) [Woods et al., 2005]. Inhibition of the CPOX and UROD enzymes cause elevations of urinary coproporphyrin (COP) and pentacarboxyproporphyrin (SCP). The azytroph porphyrin ketoisocoproporphyrin, more commonly known as precoproporphyrin (PcP), produced by in vivo conversion of SCP in the presence of heavy metal, has been specifically associated with mercury exposure [Heyer et al., 2006; Woods et al., 2005].

Independent of the interest in porphyrins as an indirect measure of mercury damage is the potential usefulness of porphyrins as an early biomarker of ASD. Indeed, several researchers have found an association between elevated urinary porphyrins and ASD. Nataf et al. [2006] were the first team to investigate whether a relationship exists between urinary porphyrins and ASD. In a sample of 269 French children (2–15 years), they found elevated levels of

Figure 1. Pathway of heme biosynthesis, major urinary metabolites, and inhibition by heavy metals. Porphyrins appear in urine as porphyrin derivatives (right); urinary SCP, PcP, and COP are indicators of inhibition of UROD and/or CPOX; urinary UP is not reported to alter with inhibition of these enzymatic steps. 7-carboxy, 6-carboxy, 7CP, 6CP; hepta- and hexa-
carboxyproporphyrins and proporphyrins, respectively.

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heptacarboxyproporphrin (TCP), hexacarboxyproporphrin (HCP), SCP, PcP, and COP among children diagnosed with AUT (and AUT + epilepsy) relative to a control group. Furthermore, while the urinary porphyrin metabolite markers associated with mercury (SCP, PcP, and COP) were significantly elevated among the AUT group in comparison to the control group, they were either unchanged, or not significantly elevated, in children with ASD or PDD-NOS. Importantly, the results were indicative of a dose-response relationship with urinary porphyrin levels showing a positive association with autism severity. Studies seeking to replicate and expand upon this seminal study have been conducted in the America, Australia, and Korea.

In America, Geier and Geier [2006] investigated the difference in urinary CP levels among children on a pharmacoeutical treatment to bind and excrete mercury and unchelated individuals diagnosed with ASD ($\alpha = 37$, 7–22 years of age) in comparison to a neurotypical (NT) sibling control group ($\alpha = 7$, 7–20 years of age). They found that the median CP levels of unchelated individuals with ASD were significantly higher than that of the control group, whereas no significant difference was found between chelated individuals with ASD and the control group. An extension of this study by the same investigators [Geier & Geier, 2007] supported the results. Additionally, they found that unchelated individuals with ASD had significa-
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cantly higher urinary SCP levels in comparison to chelated individuals with ASD and to chelated individuals plus sibling controls. Unchelated individuals with ASD also had higher elevations in both VCP and CP (normalised to uroporphyrin) relative to the general population control. A study by Austin and Shandley [2008] compared the porphyrin levels of a sample of Australian children diagnosed with ASD and control groups of the French [Natal et al., 2006] and American [Geier & Geier, 2007] studies. In addition to an external control group [Minder & Schneider-Fin, 1996] and laboratory reference range. The results were consistent with the previous studies, finding a significant elevation in the C9/UP ratio in the ASD group. Indeed the mean for all urinary porphyrin metabolites (U, 7CP, 6CP, SCP, DCIP, CP) [Natal et al., 2006, only], and CP were higher than the control groups in both the comparison studies. Nevertheless, a clear limitation of the study was the lack of an Australian control group.

Youn, Jin, Kim, and Lim [2010] sought to determine whether urinary porphyrin levels could provide a diagnostic indicator for heavy metal body burden among a sample of Korean children diagnosed with ASD and the mechanism by which this may occur. The investigators found significant elevations in the urinary porphyrin levels (U, 7CP, 6CP, SCP, CP, C9/UP, and total porphyrins) of children with ASD (n = 65, 2–16 years of age) relative to a control group (n = 7, 2–6 years of age). A significant correlation was also found between folic acid deficiency and an increase in oxidative stress, with the authors suggesting that elevated mercuic body burden among children with ASD may be due to an increase in oxidative stress as a consequence of decreased hepatic detoxication. This finding was consistent with an earlier study by Geier et al. [2009] who demonstrated that children with ASD had significant transulfuration abnormalities as indicated by a decrease in plasma reduced glutathione and increased oxidised glutathione, relative to NT controls. Furthermore, plasma oxidized glutathione levels were significantly increased among children with elevated levels of mercuic and/or porphyrins. SCP (CP) in comparison to those with low levels.

As identified by Natal et al. [2006], urinary porphyrins not only appear to provide a diagnostic marker of ASD, but also a marker of ASD severity. Geier et al. [2009] investigated the possibility of porphyrins as a marker of ASD severity by categorizing a sample of 28 children diagnosed with ASD (2–16 years of age) into a mild and severe group via a median split of Childhood Autism Rating Scale (CARS) scores. The severe group showed significantly higher levels of SCP and P4CP in comparison to the mild group, whereas no significant differences were observed for other urinary porphyrins measured. In a recent study using receiver operating characteristic curve analysis, Hoyer, Echeverria, and Woods [2012] demonstrated that urinary porphyrins (SCP and CP) had excellent predictive values in the detection of ASD in children. With 94% specificity, 30% ASD cases, and 36% control cases were detectable based on urinary SCP (≥ 2.1 nmol/gCr), and 70% ASD cases, and 26% control cases were detectable based on urinary CP (≥ 108 nmol/gCr). The investigators found that the best combination of sensitivity and specificity was achieved by converting porphyrin levels to z-scores to create a combined z-SCP and z-CP score (≥ 1.13). Using this combined measure, 53% ASD cases and 21% control cases were detectable with 100% specificity.

Although most investigators of the early porphyrin studies concluded that the results supported a relationship between mercury and autism, not all studies have supported this conclusion. In a sample of male children aged 2–12 years, Woods et al. [2010] found comparable urinary P4CP levels between NT children and children with ASD, although all other urinary porphyrin levels were significantly higher among ASD children in comparison to NT children. Logistic regression analysis also failed to find an association between diagnosis (NT, ASD, or ASD) and sources of exposure to mercury based on self-reports of mother and child dental amalgams, number of vaccines administered to the child, number of fish meals per month, and mercuic urinary concentrations. The investigators were critical of studies using older children or adults as controls suggesting the results could be misleading due to natural age-related variation in creatinine-adjusted urinary porphyrin concentrations [Woods et al., 2009]. In a study by Woods et al. [2010] study, levels of 7CP and CP were higher among younger children, declining by 2.5 times from 2 to 12 years of age. The variance in urinary porphyrin levels was also illustrated by Bloom, Zaldivar, Morledge, and Pohl-Fitzpatrick [1999] who found urinary porphyrin levels to be significantly lower among older children in contrast to younger children with porphyrin levels among older children approaching adult values.

To address the issue of age-appropriate control groups raised by Woods et al. [2010], Korn et al. [2013] conducted a small age- and gender-matched study with 20 children with ASD and 20 controls (2–13 years). The results showed that urinary porphyrins (CP, P4CP) were elevated among children with ASD relative to their age- and gender-matched controls. Although the investigators concurred with the conclusions of previous studies suggesting a relationship between mercury and autism, it was acknowledged that the influence of other factors could not be ruled out. For example, other heavy metals such as lead are known to increase CP. The issue of genetics also needs to be considered as children with ASD may experience problems with heavy metal detoxication due to problems in the transulfuration pathway and glutathione synthesis [for a review, see Deth, Muratore,
An investigation of the potential association between mercury and ASD

Bencey, Power-Charnitsky, & Waly, 2008). Furthermore, a recent study by Shandley and Austin [2011] demonstrated that the grandchildren of Pink Disease survivors, a cohort with an identified idiosyncratic sensitivity to mercury, were at considerably greater risk (6–7 fold) of being diagnosed with ASD. Although the study did not investigate a genetic mechanism for the outcome, the results are suggestive of a heritable component and therefore a possible genetic basis to mercury sensitivity.

The purpose of the present study is to investigate whether porphyrin profiles can reliably be used to: (a) detect ASD cases and (b) ASD severity. The study extends upon earlier porphyrin studies by recruiting children who fall within the typical diagnostic age range (2-6 years). Furthermore, the study compares the porphyrin levels of children diagnosed with an ASD against two neurotypical control groups: (a) siblings of children diagnosed with ASD (sibling control); and (b) children with no known blood relative diagnosed with an ASD (external control).

Method

Participants

The study sample consisted of 160 children (118 males, 42 females) aged 2-6 years of age. The birth mother was primarily the consenting party (n = 105, 94.6%), with the majority of participants located in Victoria (67.3%), followed by New South Wales (14.5%), and Queensland (8.2%). Of the sample, 64 consenting parties had one child participating, 42 had two, and four had three children participating.

Measures

Participation in the study involved the completion of two components, an online survey, and the collection and submission of a first morning urine sample for porphyrin analysis. The online survey consisted of five sections: (a) sociodemographic details and basic health history; information of the birth mother and biological father; (b) sociodemographic and diagnostic information of the participating child; (c) conception, pregnancy, and birth information; (d) information regarding the participating child’s first week of life; and (e) information on the current status of the participating child.

The urinary porphyrin analysis was conducted by an independent ISO-certified laboratory, Philippe Auguste (France). The urinary porphyrins measured were: uroporphyrin (U), 7CP, 6CP, 5CP, 1CP, and 1IP.

Procedure

Ethical approval to conduct the present study was granted by the Swinburne University Human Research Ethics Committee. Families with at least one child 2-6 years of age were sought to participate in the study. Inclusion criteria included that the child was based in Australia, not currently taking any prescription medications had never undergone chelation therapy; or been diagnosed with a comorbid condition/disorder, for example, Down syndrome, epilepsy, Fragile X syndrome, attention (deficit) hyperactivity disorder, Tourette’s syndrome, cerebral palsy, or any other neurological disorder. Participants were recruited through newsletters, online autism forums, autism community groups, university media channels, and word-of-mouth.

Recruitment notices directed interested individuals to the online survey to read the information statement detailing the requirements of participation, eligibility criteria, and consent procedure. Upon completion of the survey, responses were given a preliminary check for accuracy and the consenting party contacted as necessary. Following this, a urinary collection kit was posted (one per participating child). Urine samples were sent by the parent/guardian to ISO-certified Laboratoire Philippe Auguste where they were analyzed using high-pressure liquid chromatography with fluorometric detection. This methodology has been previously described [see Natoff et al., 2006]. Each consenting party received a $50 voucher (one per participating child) at the conclusion of the study.

Statistical Analysis

Consistent with the new DSM-5 nosology, AUT, AD, and PDD-NOS were merged into the single dimension of ASD for the purpose of analysis. The present study compares three groups: Group 1 (ASD)—children with a confirmed diagnosis of ASD (n = 70), 64 male, 6 female, 2-6 years of age), Group 2 (sibling control)—children who have a sibling with ASD (n = 36; 20 male, 16 female, 2-6 years of age); and Group 3 (external control)—children with no known blood relation diagnosed with ASD (n = 54; 34 male, 20 female, 2-6 years of age).

Two logistic regressions (multinomial and binary) were conducted to: (a) determine whether group membership (ASD, sibling control, external control) could be predicted from porphyrin levels; and (b) determine whether ASD severity level (mild—moderate, severe) could be predicted from porphyrin levels. Multicollinearity among the porphyrins was checked through correlation coefficients and tolerance figures. No strong correlations between the predictors (porphyrins) were identified and all tolerance figures were larger than 0.4. Both regressions controlled for age.

Data were analyzed using IBM SPSS Version 21 (IBM Corp., NY). Nominal level of statistical significance was set at 0.05 for all tests, unless otherwise specified.
Results

Porphyrin levels by group and age are presented in Table 1. Consistent with previous studies, there was a reduction in porphyrin levels across all three groups from the youngest to oldest age group, making it necessary for age to be controlled in the ensuing analyses.

Group Membership and Porphyrin Levels

Goodness of fit results indicated a reasonable fit for the data, \( \chi^2(9) = 321.56, P = 0.098 \). The Pseudo R-square were low (Cox and Snell = 0.086, Nagelkerke = 0.098). However, the model correctly classified 57.5% of the cases overall. The model correctly classify 84.3% of the ASD group and 33.3% of the sibling control group. Overall analysis revealed that porphyrin level was not related to group membership.

The results presented in Table 2 suggest that there was no significant effect for any of the porphyrins on the odds ratio for the ASD group in contrast to the external control group when age was controlled. In addition, there was no significant difference in porphyrin levels between the sibling control and external control group.

ASD Severity and Porphyrin Levels

CAIRS scores were provided by parents for 29 children. To establish whether there was a dose-response relationship between ASD severity and porphyrin levels, this group of children were separated into two groups based on published norms [Schepler, Reichenb, DeVeItis, & Daly, 1980]: mild-moderate \( n = 15 \), 14 male, 1 female, CAIRS score 30–37) and severe \( n = 14 \), 12 male, 2 female, CAIRS score \( > 37 \).

Table 1. Mean ± SD for Porphyrin Levels by Group and Age

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age</th>
<th>UP</th>
<th>TCF</th>
<th>6CP</th>
<th>SCF</th>
<th>PCF</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD</td>
<td>7</td>
<td>2-0.5</td>
<td>27.03 ± 8.48</td>
<td>6.14 ± 2.74</td>
<td>0.79 ± 0.33</td>
<td>6.35 ± 1.29</td>
<td>20.01 ± 31.45</td>
<td>297.67 ± 91.14</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0-4.5</td>
<td>22.30 ± 2.70</td>
<td>4.83 ± 1.61</td>
<td>0.72 ± 0.26</td>
<td>6.30 ± 1.81</td>
<td>22.16 ± 2.58</td>
<td>210.21 ± 136.87</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>0-4.5</td>
<td>21.26 ± 2.86</td>
<td>5.05 ± 1.17</td>
<td>0.73 ± 0.38</td>
<td>5.28 ± 1.47</td>
<td>18.05 ± 5.00</td>
<td>211.78 ± 84.22</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>3-6.5</td>
<td>21.52 ± 2.40</td>
<td>4.43 ± 1.10</td>
<td>0.67 ± 0.26</td>
<td>5.23 ± 1.43</td>
<td>17.53 ± 8.08</td>
<td>210.80 ± 92.96</td>
</tr>
<tr>
<td>Sibling control</td>
<td>70</td>
<td>All</td>
<td>22.28 ± 2.87</td>
<td>4.96 ± 1.74</td>
<td>0.72 ± 0.33</td>
<td>5.87 ± 1.62</td>
<td>19.67 ± 2.93</td>
<td>210.42 ± 94.14</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0-3.5</td>
<td>24.66 ± 2.98</td>
<td>6.17 ± 1.82</td>
<td>0.88 ± 0.36</td>
<td>5.98 ± 2.07</td>
<td>24.72 ± 11.90</td>
<td>280.10 ± 117.78</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4-6.5</td>
<td>23.40 ± 2.58</td>
<td>4.73 ± 1.33</td>
<td>0.94 ± 0.22</td>
<td>6.70 ± 2.02</td>
<td>26.39 ± 7.79</td>
<td>277.61 ± 93.89</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6-8.5</td>
<td>26.21 ± 3.77</td>
<td>5.28 ± 1.10</td>
<td>0.90 ± 0.32</td>
<td>6.43 ± 1.37</td>
<td>16.35 ± 4.75</td>
<td>155.30 ± 13.21</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8-10.5</td>
<td>18.84 ± 3.16</td>
<td>3.98 ± 0.84</td>
<td>0.59 ± 0.05</td>
<td>4.38 ± 1.57</td>
<td>16.05 ± 5.95</td>
<td>127.88 ± 62.41</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>All</td>
<td>34.29 ± 2.78</td>
<td>5.31 ± 1.69</td>
<td>0.85 ± 0.36</td>
<td>6.38 ± 2.38</td>
<td>22.74 ± 6.81</td>
<td>252.14 ± 138.59</td>
</tr>
<tr>
<td>External control</td>
<td>23</td>
<td>0-3.5</td>
<td>27.38 ± 2.07</td>
<td>5.43 ± 1.58</td>
<td>0.79 ± 0.36</td>
<td>6.14 ± 1.88</td>
<td>22.13 ± 5.69</td>
<td>277.17 ± 120.99</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>4-6.5</td>
<td>22.64 ± 2.07</td>
<td>4.60 ± 1.32</td>
<td>0.62 ± 0.26</td>
<td>5.37 ± 1.26</td>
<td>17.58 ± 3.69</td>
<td>191.40 ± 53.39</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6-8.5</td>
<td>22.52 ± 3.33</td>
<td>4.93 ± 0.69</td>
<td>0.66 ± 0.27</td>
<td>6.43 ± 2.16</td>
<td>19.71 ± 10.43</td>
<td>209.24 ± 86.42</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8-10.5</td>
<td>20.03 ± 3.13</td>
<td>3.79 ± 0.70</td>
<td>0.72 ± 0.37</td>
<td>4.85 ± 1.28</td>
<td>17.33 ± 7.04</td>
<td>165.71 ± 56.21</td>
</tr>
</tbody>
</table>

ASD, autism spectrum disorder; UP, unporphyrin; TCF, tetraproporphyrin; 6CP, hexacoproporphyrin; SCF, pentacoproporphyrin; PCF, propocoproporphyrin; CP, coproporphyrin I and II.

Goodness of fit results indicated a good fit for the data, \( \chi^2(9) = 19.61, P = 0.020 \), and the results from the Hosmer and Lemeshow Test supported the model, \( \chi^2(8) = 9.50, P = 0.302 \). The Pseudo R-square (Cox and Snell = 0.491; Nagelkerke = 0.656) were reasonably high. The model correctly classified 93.1% of cases overall with 86.7% of the children in the mild-moderate range of the CAIRS correctly classified and 100% of the children in the severe range on the CAIRS correctly classified. Table 3 shows the regression coefficients, Wald Statistics, odds ratios, and 95% confidence intervals for odds ratios for each of the six predictors (3P, 7CD, 6CP, SCF, PCF, and CP).

The results presented in Table 3 suggest that the effect of UP and 7CP were significant at 5% level of significance. The odds of severe ASD compared to mild-moderate ASD decreased significantly by 39% on average for each unit increase of UP when age and other porphyrin levels were controlled. For each additional unit of 7CP, the odds of severe ASD in contrast to mild-moderate ASD are expected to be 14 times higher when age and other porphyrin levels are controlled. However, there was not a significant effect on odds ratio for any of the other porphyrin levels in relation to ASD severity.

Discussion

The purpose of the present study was to determine whether urinary porphyrins could be used as a diagnostic tool for ASD. Specifically, could porphyrin levels predict group membership and/or ASD? A multinomial logistic regression analysis showed that group membership (ASD, sibling control, external control) could not be predicted on the basis of porphyrin levels. Furthermore, a binomial logistic regression analysis showed that ASD severity...
Table 2. Results of Multinomial Logistic Regression Analysis for Variables Predicting ASD with Respect to the Six Porphyrin Levels (Covariates) with External Control as the Reference Category

<table>
<thead>
<tr>
<th>Predictors</th>
<th>β</th>
<th>SE (β)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper</th>
<th>Wald statistic</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASI</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UP</td>
<td>-0.600</td>
<td>0.041</td>
<td>0.414</td>
<td></td>
<td>0.209</td>
<td>1.953</td>
<td>2.181</td>
<td>1</td>
<td>0.140</td>
</tr>
<tr>
<td>6CP</td>
<td>0.109</td>
<td>0.206</td>
<td>1.216</td>
<td></td>
<td>0.893</td>
<td>1.579</td>
<td>1.859</td>
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<tr>
<td>6CP</td>
<td>0.341</td>
<td>0.722</td>
<td>1.047</td>
<td></td>
<td>0.342</td>
<td>5.789</td>
<td>0.216</td>
<td>1</td>
<td>0.686</td>
</tr>
<tr>
<td>5CP</td>
<td>-0.175</td>
<td>0.172</td>
<td>0.840</td>
<td></td>
<td>0.599</td>
<td>1.217</td>
<td>1.029</td>
<td>1</td>
<td>0.310</td>
</tr>
<tr>
<td>5CP</td>
<td>-0.230</td>
<td>0.015</td>
<td>0.800</td>
<td></td>
<td>0.198</td>
<td>3.260</td>
<td>0.330</td>
<td>1</td>
<td>0.686</td>
</tr>
<tr>
<td>5CP</td>
<td>0.006</td>
<td>0.004</td>
<td>1.006</td>
<td></td>
<td>0.599</td>
<td>1.003</td>
<td>2.809</td>
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<td>0.094</td>
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<tr>
<td>Constant</td>
<td>0.254</td>
<td>0.016</td>
<td></td>
<td></td>
<td>0.110</td>
<td>0.740</td>
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<td></td>
<td></td>
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<tr>
<td>Sibling</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UP</td>
<td>-0.065</td>
<td>0.042</td>
<td>0.937</td>
<td></td>
<td>0.862</td>
<td>1.018</td>
<td>2.156</td>
<td>1</td>
<td>0.125</td>
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<tr>
<td>6CP</td>
<td>0.279</td>
<td>0.235</td>
<td>1.275</td>
<td></td>
<td>1.154</td>
<td>1.440</td>
<td>1.113</td>
<td>1</td>
<td>0.287</td>
</tr>
<tr>
<td>5CP</td>
<td>1.404</td>
<td>0.757</td>
<td>4.101</td>
<td></td>
<td>0.500</td>
<td>10.081</td>
<td>3.473</td>
<td>1</td>
<td>0.062</td>
</tr>
<tr>
<td>5CP</td>
<td>-0.051</td>
<td>0.179</td>
<td>0.944</td>
<td></td>
<td>0.669</td>
<td>1.339</td>
<td>0.181</td>
<td>1</td>
<td>0.675</td>
</tr>
<tr>
<td>5CP</td>
<td>0.037</td>
<td>0.038</td>
<td>1.047</td>
<td></td>
<td>0.945</td>
<td>1.059</td>
<td>0.208</td>
<td>1</td>
<td>0.649</td>
</tr>
<tr>
<td>5CP</td>
<td>0.0001</td>
<td>0.004</td>
<td>1.000</td>
<td></td>
<td>0.599</td>
<td>1.000</td>
<td>0.003</td>
<td>1</td>
<td>0.976</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.494</td>
<td>0.993</td>
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<td></td>
<td>0.167</td>
<td>9.766</td>
<td></td>
<td></td>
<td>0.158</td>
</tr>
</tbody>
</table>

The external control group is the reference category for both ASD and sibling control groups.

ASI, autism spectrum disorder; UP, uroporphyrin; 7CP, heptacarboxylic porphyrin; 6CP, hexacarboxylic porphyrin; 5CP, pentacarboxylic porphyrin; 4CP, tetracarboxylic porphyrin; CF, coproporphyrin II and III.

Table 3. Results of Binary Logistic Regression Analysis for Variables Predicting Severity of ASD with Respect to the Six Porphyrin Levels (Covariates)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>β</th>
<th>SE (β)</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper</th>
<th>Wald statistic</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UP</td>
<td>-0.094</td>
<td>0.215</td>
<td>0.610</td>
<td></td>
<td>0.378</td>
<td>0.906</td>
<td>4.068</td>
<td>1</td>
<td>0.046</td>
</tr>
<tr>
<td>7CP</td>
<td>2.649</td>
<td>1.271</td>
<td>14.089</td>
<td></td>
<td>1.159</td>
<td>170.26</td>
<td>4.327</td>
<td>1</td>
<td>0.038</td>
</tr>
<tr>
<td>6CP</td>
<td>-5.081</td>
<td>4.812</td>
<td>0.003</td>
<td></td>
<td>0.000</td>
<td>31.489</td>
<td>1.545</td>
<td>1</td>
<td>0.214</td>
</tr>
<tr>
<td>5CP</td>
<td>0.550</td>
<td>0.271</td>
<td>1.760</td>
<td></td>
<td>0.447</td>
<td>6.298</td>
<td>0.576</td>
<td>1</td>
<td>0.448</td>
</tr>
<tr>
<td>5CP</td>
<td>0.116</td>
<td>0.133</td>
<td>1.122</td>
<td></td>
<td>0.806</td>
<td>1.549</td>
<td>0.759</td>
<td>1</td>
<td>0.381</td>
</tr>
<tr>
<td>5CP</td>
<td>-0.037</td>
<td>0.013</td>
<td>0.968</td>
<td></td>
<td>0.954</td>
<td>1.032</td>
<td>3.355</td>
<td>1</td>
<td>0.067</td>
</tr>
<tr>
<td>Constant</td>
<td>-2.983</td>
<td>2.062</td>
<td></td>
<td></td>
<td>0.097</td>
<td>5.922</td>
<td></td>
<td></td>
<td>0.244</td>
</tr>
</tbody>
</table>

UP, uroporphyrin; 7CP, heptacarboxylic porphyrin; 6CP, hexacarboxylic porphyrin; 5CP, pentacarboxylic porphyrin; 4CP, tetracarboxylic porphyrin; CF, coproporphyrin II and III.

(mild-moderate, severe) also could not be predicted on the basis of most porphyrin levels with the exception of 7CP. The analysis revealed that ASD severity can be increased significantly on average for each additional unit of 7CP when age and other porphyrin levels were controlled. Contrary to expectation, the odds of severe ASD in contrast to mild-moderate ASD are expected to decrease significantly by 39% for each unit increase of UP when age and other porphyrin levels were controlled. Therefore, the results indicate that urinary porphyrins appear not to be a valid biomarker of ASD.

With the identification of a range of biomedical issues common in children with ASD, finding a reliable biomarker could be of considerable benefit to the diagnostic process. It would add an objective element to what is currently a purely subjective process and potentially aid in lowering the diagnostic age. Porphyrins present an attractive prospect as a diagnostic biomarker. Porphyrins are a naturally occurring substance in the body and importantly, can be tested non-invasively via a sample of urine. As outlined in the introduction, previous studies have found largely consistent results with children with ASD demonstrating significant elevations in urinary porphyrins in comparison to a range of control groups. However, the present study is the first to our knowledge in the published literature to not to show such an outcome. Therefore, the question that needs to be considered is why the results of this study appear so fundamentally different to those before it. There are a number of factors to consider. The present study is the first ASD-porphyrin study to recruit children within the age range typically associated with age of diagnosis (2-6 years). Previous studies used

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much wider age ranges, at least double that of the present study with participants at a minimum of 2-12 years of age. This is an important consideration as porphyrins are notoriously unstable and typically decrease throughout childhood. Studies including older participants where porphyrin levels approach adult values may show a more consistent porphyrin pattern and therefore greater stability. Body mass may also need to be considered. Neither the present study, nor any of those before it, has taken into account the body mass of the participants. This is despite this and Page (1954) identifying that weight has a greater correlation with CP excretion than age or body surface.

This is also the first porphyrin study to align with the new DSM-5 nosology by merging children with a diagnosis of AUT, AD, and FDD-NOS into the single dimension of ASD. Previous studies have typically compared participants according to the DSM-IV diagnostic labels or grouped children with an AUT and PDD-NOS diagnosis. It is possible that by grouping together the individual diagnostic categories in the present study, nuances between porphyrin levels may have been lost. For example, the Nataf et al. (2006) study identified a possible dose-response relationship across diagnostic groups with porphyrin levels showing a positive association with ASD severity.

The impact of several limitations of the present study should be taken into account. The first is that participants were recruited from across Australia and, as such, it was not economically feasible to conduct independent ASD assessments. The second limitation is in regard to the study age range (2-6 years). As already noted in the paper, the age range was specifically chosen to reflect the naturalistic ASD diagnostic period. It is possible that following completion of the study, some of the participating children who were in one of the two control groups were subsequently diagnosed with an ASD. It is also possible, although less likely, that a child may have been misdiagnosed with an ASD (false positive). A final limitation relates to the second study aim which was to examine whether porphyrin levels could predict ASD severity. Only 29 parents were able to provide a CAIS score and, therefore, the sample size available for analysis was modest, and this will likely have had a bearing on the results. However, it should be noted that the sample size is directly comparable to the study by Geier et al. (2009).

There are a number of clear strengths to the study. The present study was the first to incorporate two differing healthy control groups: a sibling control and an external control (comprising of neurotypical children with no known blood relatives with a diagnosis of ASD). The study employed a very strict inclusion criteria designed as best as possible to eliminate possible confounds. For example, participants were not taking any prescription medications as they are known to affect porphyrin levels. Although noted as a limitation, the tight age range is also a strength of the study. If urinary porphyrins are to be considered as a genuine diagnostic tool, then not only do they need to reliably distinguish between groups, they also need to be applicable during the typical diagnostic period. In summary, however, the findings of this study failed to support the use of urinary porphyrins as a biomarker of ASD.

Acknowledgments

We gratefully acknowledge donations made to the Swindon Autism Bio-Research Initiative (SABI). Without these generous donations it would have been impossible to conduct the study. We would also like to acknowledge the support and encouragement we have received from many parents (and grandparents) of children diagnosed with ASD to continue conducting biological studies. All authors declare no conflict of interest.

References


INSAR

Standley et al. Are porphyrins a valid biomarker of ASD?
An investigation of the potential association between mercury and ASD


Chapter 6: Ancestry of Pink Disease (infantile acrodynia) identified as a risk factor for Autism Spectrum Disorders.

Contrary to expectation, the second empirical study presented in this thesis (Shandley, Austin & Bhowmik, 2014) failed to find a difference between urinary porphyrins across three groups of children (ASD, sibling control, external control). Furthermore, neither group membership nor ASD severity could be predicted based upon urinary porphyrins. Therefore, the final study will take a different approach to examine the potential association between Hg and ASD. The concept for this study is based on a component of the Hg-autism hypothesis which, as discussed earlier, postulates that ASD develops due to an interaction between a genetic susceptibility and environmental trigger. This study will investigate whether an individual susceptibility to Hg is heritable (and therefore genetic). To address this question, a cohort of people with a known sensitivity to Hg, (survivors of pink disease) were surveyed to determine the prevalence of ASD among their descendants.

Pink disease, also known as infantile acrodynia, was an especially prevalent condition in Australia, North America, and Central Europe in the first half of the 20th century (Rocaz, 1933). Two studies by Warkany and Hubbard (1948, 1951) led to the discovery that a form of inorganic Hg, calomel (mercurous chloride), widely used in teething powders, was responsible for the disease. Following the removal of this ingredient from most teething powders in the mid-1950s, pink disease essentially disappeared (Curtis, Ferguson, Kell & Samuel, 1987). Critically, it was determined that merely being exposed to Hg was not sufficient for the disease to develop but, rather, an individual sensitivity to Hg must also be present (Bivings, 1949; Bivings & Lewis, 1948; Warkany & Hubbard, 1948).
The following paper reports on the final study presented in this thesis. Pink disease survivors were surveyed (N=522) to obtain details of the clinical status of their children (n=1086) and grandchildren (n=1366). The prevalence rates of a range of clinical conditions (ASD, attention deficit/hyperactivity disorder, epilepsy, Fragile X syndrome and Down syndrome) was determined and compared to their respective general population prevalence rates (matched by year of birth).
Paper 4


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<th>Email or phone</th>
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<td>David Austin</td>
<td>School of Psychology</td>
<td><a href="mailto:david.austin@deakin.edu.au">david.austin@deakin.edu.au</a></td>
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<tr>
<td>Kerrie Shandley</td>
<td>School of Psychology</td>
<td>An investigation of the potential association between mercury and Autism Spectrum Disorder; An Interdisciplinary approach</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

Conceptual development of study, design and administration of questionnaire, liaison with public health and education group facilitator, ethics application, recruitment and liaison with participants, data management and analysis, drafting of manuscript and revision, principal author.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: 30/4/14

Kerrie Shandley

4. Description of all author contributions

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
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<tr>
<td>Kerrie Shandley</td>
<td>Conceptual development of study, design and administration of questionnaire, ethics application, recruitment and liaison with participants, data management and analysis, drafting of manuscript and revision, principal author.</td>
</tr>
<tr>
<td>David Austin</td>
<td>Feedback on questionnaire, review and feedback of manuscript.</td>
</tr>
</tbody>
</table>
5. Author Declarations
I agree to be named as one of the authors of this work, and confirm:
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<td><em>signature</em></td>
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</tr>
<tr>
<td>David Austin</td>
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6. Other contributor declarations
I agree to be named as a non-author contributor to this work.

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An investigation of the potential association between mercury and ASD

ANCESTRY OF PINK DISEASE (INFANTILE ACRODYNIA) IDENTIFIED AS A RISK FACTOR FOR AUTISM SPECTRUM DISORDERS

Kerrie Shandley, David W. Austin
Swinburne Autism Bio-Research Initiative (SABRI), Brain and Psychological Sciences Research Centre, Swinburne University of Technology, Hawthorn, Victoria, Australia

Pink disease (infantile acrodynia) was especially prevalent in the first half of the 20th century. Primarily attributed to exposure to mercury (Hg) commonly found in teething powders, the condition was developed by approximately 1 in 500 exposed children. The differential risk factor was identified as an idiosyncratic sensitivity to Hg. Autism spectrum disorders (ASD) have also been postulated to be produced by Hg. Analogous to the pink disease experience, Hg exposure is widespread yet only a fraction of exposed children develop an ASD, suggesting sensitivity to Hg may also be present in children with an ASD. The objective of this study was to test the hypothesis that individuals with a known hypersensitivity to Hg (pink disease survivors) may be more likely to have descendants with an ASD. Five hundred and twenty-two participants who had previously been diagnosed with pink disease completed a survey on the health outcomes of their descendants. The prevalence rates of ASD and a variety of other clinical conditions diagnosed in childhood (attention deficit hyperactivity disorder, epilepsy, fragile X syndrome, and Down syndrome) were compared to well-established general population prevalence rates. The results showed the prevalence rate of ASD among the grandchildren of pink disease survivors (1 in 25) to be significantly higher than the comparable general population prevalence rate (1 in 160). The results support the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for ASD.

Pink disease, or infantile acrodynia as it was also known (primarily in Europe and America), was an especially prevalent condition in Australia, North America, and Central Europe in the first half of the 20th century (Rocca 1933). The first description of pink disease in the literature dates back to 1903 by Selter, a German physician, although cases in Australia predate this time by at least two decades (Selter 1903; Wood and Wood 1935). Pink disease remained in relative obscurity in the greater medical community until 1914, when it was again described, this time by Swift, an Australian-born physician, at an Australasian medical congress in New Zealand (Swift 1914).

Case studies provided a comprehensive clinical picture of pink disease long before its etiology was established. The most commonly reported symptoms included: irritability, nervousness, photophobia (light sensitivity), hyperhidrosis (excessive sweating), hypotonia (low muscle tone), ataxia (lack of coordination), digestive problems (including loss of weight, loss of appetite, vomiting, and constipation), anemia, excessive salivation, respiratory problems, lethargy, extreme misery, slurring/loss

Received 29 January 2011; accepted 5 May 2011

This study was funded by donations generously made to the Swinburne Autism BioResearch Initiative (www.sabri.org.au). We thank Dr. Farnsworth, the facilitator of the Pink Disease Support Group (www.pinkdisease.org), for her support of the study and role in the recruitment of Pink Disease survivors. We also thank Tom Critchley for her assistance in entering the survey data and Dr. Denny Meyer and Dr. Jorah Rhoenik for their statistical assistance.

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of speech, loosening/loss of teeth, swollen extremities, and perhaps most famously (and from which the name "pink disease" was derived), marked reddening of the extremities, particularly the hands and feet (Rocaz 1933; Wood and Wood 1935: Leys 1950). Fatality was reasonably high, with death resulting in 10–33% of cases (Rocaz 1933). For the survivors, recovery was considered to be complete, although research conducted in recent decades revealed medical sequelae to be high in this group, including Young's syndrome (infertility in men) and bronchiectasis (Hendry et al. 1993; Williams and O'Reilly 1959).

In 1945, a critical study became the catalyst that led to the discovery of the etiology of pink disease. Upon noticing a similarity of pink disease symptomatology to arsenic and thallium poisoning, American physicians Warkany and Hubbard (1948) undertook a quantitative metal determination of 14 children diagnosed with pink disease and found that 12 of the 14 children had elevated levels of urinary mercury (Hg). A replication study by Warkany and Hubbard (1951) consolidated the finding with Hg levels elevated in 92% of 41 children diagnosed with pink disease in comparison to 15% of controls. Although Hg was used in a wide range of medicinal products at the time, the primary culprit was determined to be teething powders containing calomel (mercurous chloride). Following the removal of calomel from most teething powders in 1954, pink disease essentially disappeared (Curtis et al. 1987). Interestingly, however, while millions of teething powders were sold (some 7 million annually in North England alone of one of the most famous brands, Steedman’s Teething Powder), only 1 in 300 exposed children developed pink disease (Emmley 2005). The differential risk factor was identified as an idiosyncratic sensitivity to Hg (Warkany and Hubbard 1948; Bivings 1949; Bivings and Lewis 1948).

It was postulated that autism spectrum disorders (ASD), which are primarily comprised of autistic disorder and Asperger’s disorder, also have a pathogenesis stemming from Hg exposure (Austin 2000; Bernard et al. 2001; Mutter et al. 2003). The mounting evidence is compelling. Behavioral changes, hyperactivity, and alterations in spontaneous and learned behaviors have been observed in animals exposed to Hg during the prenatal or early postnatal period (Agency for Toxic Substances and Disease Registry 1999). Studies also showed that there were significant elevations of Hg in the urine of children with autism in comparison to controls, as well as in urinary markers of Hg damage (Austin and Shandley 2000; Bratina et al. 2000; Gerber and Gerber 2007; Nataf et al. 2006). Other investigations found the severity of autism to positively correlate with the child’s body burden of toxic metals (Adams et al. 2009; Geier et al. 2009; Holness et al. 2003). In addition, some epidemiological studies demonstrated an association between Hg exposure and ASD prevalence (Gallagher and Goodman 2010; Gerber and Gerber 2006a; Palmer et al. 2006; 2009; Windham et al. 2006), but not all studies confirmed this correlation (Hvid et al. 2003; Verstraeten et al. 2003).

Mercury contained in vaccines (as a preservative under the tradename Thiomersal, but more commonly known as thiomersal/thimerosal), dental amalgams (silver fillings), seafood, and the atmosphere is argued to be the primary set of sources of Hg exposure for infants both in utero and in their early years (Austin 2008). However, not all children exposed to such sources of Hg develop an ASD, suggesting, as was the case with pink disease, that a hypersensitivity to the adverse effects of Hg needs to be present in addition to the Hg exposure for the condition to manifest. Therefore, the Hg-autism hypothesis is, in reality, a two-part hypothesis that states that Hg exposure combined with a genetic/physiological sensitivity to Hg or a predisposition to impaired Hg excretion capacity leads to a chronic elevation of Hg in the brain and body (Bernard et al. 2001).

The purpose of the present study was to test the Hg-autism hypothesis. If the hypothesis is indeed correct, and a sensitivity to Hg is heritable (genetic), the prevalence of ASD among the descendants of a cohort confirmed as having a hypersensitivity to Hg (pink disease survivors) should be higher than a comparable general population prevalence.
MATERIALS AND METHODS

Participants and Study Design

Ethical approval to conduct the present study was received from the Swinburne University Human Research Ethics Committee. Individuals who had previously been diagnosed with pink disease were invited to participate in this study by completing a survey online, by mail, or via telephone interview. In cases where the pink disease survivor was incapacitated or deceased, family members were able to complete the survey as a proxy. The Australian Pink Disease Support Group (PDSC) estimated that approximately 5000 survivors were still living at the time of study commencement in July 2009 (D Farnsworth personal communication 19 November 2009).

Participants were recruited via the PDSC, an Australian not-for-profit group dedicated to providing support and information to pink disease survivors and their families—the only such group in the world. The PDSC maintains a membership database and sent out the survey to all of its past and present members, in addition to advertising the study on its website (www.pinkdisease.org). In order to minimize response bias, the true purpose of the study was not included on recruitment materials sent out to potential participants; instead, recruitment materials indicated that the purpose of the study was to investigate the general health outcomes of the descendants of pink disease survivors. In total, 2600 surveys were sent, 2000 by mail and 600 by e-mail, with an anticipated overlap of approximately 300 members receiving the survey by both mail and e-mail. A chance to win one of 10 shopping vouchers to the value of $75 was offered as an incentive to participate.

The survey included sociodemographic questions regarding the pink disease survivor (gender, date of birth, current place of residence), the relationship of the respondent to the survivor (if the survey was completed by a proxy), and information pertaining to the pink disease survivor’s descendants (children and grandchildren). With respect to the descendants, survey respondents were asked to provide details regarding the number of children and grandchildren, the gender and age of each, and whether they had been diagnosed with any of the following conditions prior to the age of 16 years: autism, Asperger’s disorder, attention deficit hyperactivity disorder (ADHD), epilepsy, Fragile X syndrome, mental retardation, and/or Down syndrome.

The survey was commenced or returned by 531 people (a response rate of 23.1%); however, 9 surveys were removed from analysis, as 6 were repeated entries, and 3 surveys were incomplete. This left a total of 522 surveys that were included in the analysis. The characteristics of the pink disease survivor cohort are provided in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
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<td>Age</td>
<td>Mean</td>
<td>64.07</td>
<td>63.78</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>6.63</td>
<td>6.06</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>44-86</td>
<td>44-86</td>
</tr>
<tr>
<td>Survey completion type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>30</td>
<td>62</td>
</tr>
<tr>
<td>Postal</td>
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<td>75</td>
<td>341</td>
</tr>
<tr>
<td>Telephone</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Survey respondent status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD survivor</td>
<td>509</td>
<td>100</td>
<td>409</td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Current place of residence</td>
<td></td>
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<tr>
<td>New South Wales</td>
<td>218</td>
<td>42</td>
<td>173</td>
</tr>
<tr>
<td>Queensland</td>
<td>86</td>
<td>15</td>
<td>70</td>
</tr>
<tr>
<td>Victoria</td>
<td>68</td>
<td>11</td>
<td>56</td>
</tr>
<tr>
<td>South Australia</td>
<td>33</td>
<td>6</td>
<td>27</td>
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<td>Western Australia</td>
<td>31</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Tasmania</td>
<td>13</td>
<td>2</td>
<td>11</td>
</tr>
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<td>Australian Capital Territory</td>
<td>7</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Northern Territory</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Overseas</td>
<td>61</td>
<td>20</td>
<td>41</td>
</tr>
</tbody>
</table>

Note: PD = pink disease.

*Gender was not provided by eight survey respondents; therefore, the total is not the sum of male and female in all cases.

**Where the survey was completed by proxy the most common person was the mother (n = 5), followed by the daughter (n = 4), wife/widow (n = 3), and granddaughter (n = 1).

*Place of residence was not provided by one survey respondent.

*Where the survivor was not located in Australia, the next most common location was the United Kingdom (n = 38), followed by New Zealand (n = 14) and Canada (n = 3).
Descendants of Pink Disease Survivors

Children  Pink disease survivors had a cumulative total of 1103 children. Only live births, biological children, and children surviving to at least 5 yr were included in the analysis; therefore, 17 children were not included for the following reasons: 7 children were adopted, 4 stillborn, and 6 died at an early age (cot death: $n = 2$, prematurity: $n = 1$, congenital heart disease: $n = 1$, immature lungs: $n = 1$, unspecified: $n = 1$). Additionally, three of the survey respondents stated that they had children but failed to provide details and were therefore not included in the analysis. This left a total of 1086 children that were included. Of the 522 survey respondents, 79.5% ($n = 415$) stated that they had at least one child, with the average being 2.2 children (range: 1–8). The mean age of the children was 37.1 yr (SD = 8.81), ranging from 3 to 61 yr.

Grandchildren  Pink disease survivors had a cumulative total of 1380 grandchildren. As was the case for children, only live births, biological grandchildren, and grandchildren surviving to at least 5 yr were included in the analysis. Therefore, 14 grandchildren were not included, as 8 grandchildren were stillborn and 6 died at an early age (Potter’s syndrome: $n = 1$, Werdnig Hoffmann’s disease: $n = 1$, stroke in the womb: $n = 1$, congenital heart defect: $n = 1$, unspecified: $n = 1$). This left a total of 1366 grandchildren that were included in the analysis. Of the 522 survey respondents, 60.7% ($n = 317$) stated that the pink disease survivor had at least one biological grandchild, with an average of 4.3 grandchildren (range: 1–15). The mean age of the grandchildren was 11.3 yr (SD = 7.65), ranging from <1 to 38 yr.

RESULTS

The numbers of children and grandchildren diagnosed with autism, Asperger’s disorder, ADHD, epilepsy, fragile X syndrome, mental retardation or Down syndrome are presented in Table 2.

ASD Prevalence

The general population ASD prevalence rates used as the comparison group for this study were taken from a report commissioned by the Australian Advisory Board on Autism Spectrum Disorders (MacDermott et al. 2007). ASD prevalence is well understood to be difficult to measure, due, in part, to changing diagnostic criteria and databases of variable quality (Charman et al. 2009). The Australian Advisory Board data, however, were largely immune from these confounding variables as they were gathered over a period of time when diagnostic criteria for autism and Asperger’s disorder did not change. Data were collected from multiple sources across health, disability, and education sectors in addition to Australian state and territory autism associations, allowing for the vast

<table>
<thead>
<tr>
<th>Condition</th>
<th>Children</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Grandchildren</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>($n = 1086$)</td>
<td>($n = 545$)</td>
<td>($n = 541$)</td>
<td>($n = 1366$)</td>
<td>($n = 630$)</td>
<td>($n = 736$)</td>
<td>($n = 1366$)</td>
<td>($n = 630$)</td>
<td>($n = 736$)</td>
</tr>
<tr>
<td>Autism</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>12</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Asperger's disorder</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>23</td>
<td>17</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>ADHD</td>
<td>28</td>
<td>20</td>
<td>8</td>
<td>29</td>
<td>19</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>18</td>
<td>10</td>
<td>7</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*Note: ADHD = attention deficit hyperactivity disorder.

**Gender not provided for all children and grandchildren, therefore the total is not the sum of male and female in all cases.
majority of Australian ASD cases to be captured. Furthermore, the reported rates were highly consistent with other ASD prevalence studies conducted internationally around that same time (Centers for Disease Control and Prevention 2007).

This study presents the comparative rates for two age groups used in the Australian Advisory Board report (6–12 and 13–16 yr) for the most recent year for which data is available, 2005. The Australian Advisory Board calculated its ASD prevalence rates from Centrelink data. Centrelink is an Australian federal government social service agency and is considered the most comprehensive single source of Australian ASD data. Figures 1 and 2 provide a graphical comparison of the prevalence rates of autism and Asperger’s disorder, respectively, among the general population reported by the Australian Advisory Board and the age-matched grandchildren of pink disease survivors. Only data for grandchildren are reported here, as the children of the pink disease cohort were mainly born in the 30-year period from 1950 to 1980, a period for which no reliable Australian ASD population prevalence rates are available. The most recent Australian ASD prevalence rate for children 6–12 years is 1 in 160, 13–16 years 1 in 272, and for 6–16 years, 1 in 789 (MacDermott et al. 2007). This figure is a combination of the autism and Asperger’s disorder rates in 2005. The comparative ASD prevalence rate for the grandchildren of pink disease survivors aged 6–12 years is 1 in 25 (n = 398), 13–16 years 1 in 35 (n = 141), and for 6–16 years, 1 in 27 (n = 539).

To determine whether the difference in ASD prevalence rate between the pink disease grandchildren and the general population is significant and, furthermore, whether there is an elevated risk for disease generally among pink disease descendants (for the clinical conditions captured in our survey: ADHD, epilepsy, Fragile X syndrome, and Down Syndrome), one-sided Poison probabilities were calculated, the results of which are presented in Table 3. Ninety-five percent confidence intervals were calculated using Bay’s approximation. Well-established population prevalence rates were used as a comparison for fragile X syndrome (Fragile X Association of Australia 2009) and Down syndrome (Down’s Syndrome Association of Victoria 2009). For ADHD, there is no definitive agreement regarding an accepted prevalence rate, with figures ranging from 1.7 to 6%; consequently, the midpoint (3.85%) was used as the comparison rate (Buckmeister 2004). In Australia, there have been no apparent studies undertaken to determine the prevalence of epilepsy in childhood. Consequently, for this study,

![Graph showing ASD prevalence rates](image_url)

**FIGURE 1.** Autism prevalence rates for children aged 6–12 and 13–16 years among the Australian population and the grandchildren of pink disease survivors.
An investigation of the potential association between mercury and ASD

FIGURE 2. Asperger's disorder prevalence rates for children aged 6-12 and 13-16 years among the Australian population and the grandchildren of pink disease survivors.

TABLE 3. Comparison of Observed and Expected Cases of Clinical Conditions Among the Grandchildren of Pink Disease Survivors Aged 6-16 Years at 2005 (n = 539)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Observed cases</th>
<th>Expected cases</th>
<th>SIR</th>
<th>95% confidence interval</th>
<th>1-Sided Poison Probability (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD</td>
<td>20</td>
<td>2.85</td>
<td>7.02</td>
<td>4.20-10.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ADHD</td>
<td>13</td>
<td>20.75</td>
<td>0.63</td>
<td>0.33-3.07</td>
<td>0.97</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>3</td>
<td>3.67</td>
<td>0.82</td>
<td>0.10-2.39</td>
<td>0.71</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>0</td>
<td>0.14</td>
<td>0.00</td>
<td>+26.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Females</td>
<td>1</td>
<td>0.13</td>
<td>7.69</td>
<td>0.10-42.80</td>
<td>0.12</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>0</td>
<td>0.67</td>
<td>0.00</td>
<td>+5.47</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note: SIR = Standardized incidence ratio; ASD = autism spectrum disorder; ADHD = attention deficit hyperactivity disorder; Asterisk indicates lower limit for fragile X males and Down syndrome not calculable. Data for males and females were pooled unless otherwise stated.

the Australian adult epilepsy prevalence rate was used for comparison (0.68%) (Australian Bureau of Statistics 1998). This figure is broadly equivalent to international studies reporting both childhood and adult prevalence rates for epilepsy (Sridharan 2002; Oka et al. 2006). Mental retardation was not included in this analysis as an accepted prevalence rate is not available due to complications in measuring intellectual disability among school-aged children (Wen 1997). As is shown in Table 3, the elevated risk for an ASD among pink disease grandchildren was significant. There were no significant elevations in prevalence among the grandchildren on any of the non-ASD conditions.

DISCUSSION

The prevalence of ASD was found to be significantly higher among the grandchildren of pink disease survivors in comparison to the general population, providing support for the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for ASD. Furthermore, an examination of the prevalence rates of a group of non-ASD clinical conditions (ADHD, epilepsy, Fragile X syndrome, and Down syndrome) among the pink disease descendants and the general population indicates there is not a general elevated risk for disease among this cohort, but rather a specific risk for ASD. An alternative explanation for the
findings may relate to the higher body burden of Hg in a parent being passed to their offspring. It is difficult to conceptualize how this may occur in terms of paternal transfer, but the phenomenon of Hg preferentially distributing to the developing fetus via the umbilical cord in Hg-exposed mothers is well documented (Sakamoto et al. 2010).

As identified earlier, numerous studies demonstrated a relationship between ASD and Hg (Agency for Toxic Substances and Disease Registry 1999; Austin and Shandley 2008; Bradstreet et al. 2003; Geier and Geier 2006a; 2007; Nataf et al. 2007; Adams et al. 2009; Geier et al. 2009; Holmes et al. 2003; Gallagher and Goodman 2010; Palmer et al. 2006; 2009; Windham et al. 2006), and our results add further compelling evidence in support of this relationship. The unique contribution of this study is that, to our knowledge, it is the first to examine the “individual susceptibility” variable inherent in the Hg-autism hypothesis. Our results suggest that this variable may have a heritable component and therefore, of course, a genetic basis. What our results do not do, however, is enable an understanding of the degree to which the susceptibility is inheritable and the mechanism by which this may occur. This is clearly an important focus for future research.

A possible mechanism for Hg-induced autism was proposed by James et al. (2004) and Geier and Geier (2006b), who demonstrated that oxidative stress is high and glutathione (GSH) levels are low in children with autism. This is entirely consistent with an etiology of autism based on a gene-Hg interaction, as low GSH predisposes an individual to damage from Hg exposure by limiting the body’s capacity to both minimize oxidative damage produced by the metal and excrete the toxin.

Of additional note is the gender ratio in which the descendants of the pink disease cohort were diagnosed with ASD. ASD is reported to occur more commonly in males; typically the quoted ratio is four males to one female (MacDermott et al. 2007). Interestingly, the present study replicates this pattern, with male descendants approximately fourfold more likely to be reported as diagnosed with an ASD in comparison to female descendants. Unfortunately, data are not available to confirm (or disconfirm) whether males were more likely to be stricken with pink disease, as the condition was never mandated as a reportable medical condition.

The Hg-autism hypothesis engenders passionate debate on both sides; however, the cumulative science in this field is now of such depth and breadth that it is difficult, if not impossible, to be completely dismissive of the Hg-autism link. Furthermore, our findings clearly suggest that individuals with a family history of pink disease are at significantly greater risk of having a grandchild with an ASD than the general population. Irrespective of the Hg-autism hypothesis, this has implications for public and environmental health, fields of genetic counseling and family planning, and autism research generally.

The present study design had several notable strengths. In order to minimize response biases and maximize the validity of the data, the true purpose of the study was not included on recruitment materials sent out to potential participants (i.e., information statement); instead, recruitment materials merely stated that the researchers were investigating the health outcomes of their descendants. Recruitment was maximized by obtaining the assistance of the Australian Pink Disease Support Group, which sent out survey packs to its members, both past and present, and publicized the study online. In addition, pink disease survivors were provided with a variety of response format options (online, telephone, mail). This is especially important as we were largely dealing with an elderly cohort. A further strength was that our comparison groups (grandchildren and general population) were matched for age and birth year.

A challenge encountered with this study was finding an appropriate comparative Australian population prevalence rate for ADHD. As mentioned earlier, no agreement has been reached regarding a definitive prevalence rate for ADHD with estimates ranging
from 1.7 to 6%. Therefore, the difference between the observed and expected cases displayed in Table 3 for ADHD needs to be viewed with caution. Weaknesses of this study include the fact that independent assessments of the pink disease survivors' descendants were not conducted in order to validate the accuracy of the self-reported diagnoses. Information on other variables hypothesized to play a role in the development of ASD, such as birth weight, breastfeeding history, or maternal and paternal age, was not collected. Nevertheless, the results shed significant light on the gene–environment interaction hypothesized to underpin the etiology of ASD. The role that accessory factors such as birth weight and breastfeeding history may play in the etiology warrants further research.

CONCLUSIONS

Given that Hg is well established as a potent neurotoxin (especially to developing fetuses and young children), and that the findings from this study show that individuals with a confirmed hypersensitivity to Hg are at significantly greater risk of having a descendant with an ASD, it would appear a matter of fundamental ethics for health professionals and health policy makers to minimize Hg exposure (particularly for pregnant and nursing mothers and their children). Furthermore, there is an urgent need to fund research programs designed to build upon our understanding of the gene–environment pathogenesis (and possibly pathogeneses) of ASD. This research effort needs to be multidisciplinary in nature so as to facilitate research into multivariate models of ASD etiology, with the primary focus on environmental triggers (i.e., Hg) and heritable (genetic) risk factors.

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Chapter 7: Discussion

ASD is a complex neurodevelopmental disorder and, although the pathogenesis of ASD is yet to be established, contemporary research indicates that an environmental trigger may play a critical role. Research investigating potential environmental triggers has accelerated in recent decades identifying a range of possibilities, including pesticides, pollution, chemicals, parental age, and maternal or neonatal infection. One proposed environmental trigger that has been the subject of considerable interest (and controversy) is that of Hg.

The mounting evidence that Hg is associated with ASD is compelling, but not definitive. Consequently, the question as to whether Hg is involved in the pathogenesis of ASD remains unanswered. More research addressing this potential association is critical. It was therefore the intention of this thesis to take a biological and hereditary approach to the question of whether a relationship exists between Hg and ASD. Three published studies were conducted. The first study (Chapter 4; Austin & Shandley, 2008) sought to establish whether the porphyrin profiles of a convenience sample of Australian children diagnosed with ASD reflected a pattern consistent with exposure to, and the toxic effects of, Hg (e.g., an elevation in urinary levels of 5CP, PrCP and CP). The second study (Chapter 5; Shandley, Austin & Bhowmik, 2014) sought to address the limitations of the first study (small sample size, lack of appropriate control group) and extend upon previous porphyrin studies by determining whether group membership and ASD severity could be predicted using porphyrin profiles. The final study (Chapter 6; Shandley & Austin, 2011) examined individual sensitivity to Hg by exploring ASD prevalence rates among the descendants of a cohort of the general population with a known sensitivity to Hg (pink disease survivors).
Urinary Porphyrins

Porphyrin profiles have been identified as a reliable tool for measuring Hg damage in humans, wildlife and rodents in both developed and developing countries (Henshel et al., 2007). Furthermore, the process of collecting urine for porphyrin analysis is non-invasive and reasonably low-stress and therefore suitable for vulnerable populations, such as children with ASD. Two published studies in this thesis (Austin & Shandley, 2008; Shandley, Austin & Bhowmik, 2014) used urinary porphyrin profiles as the primary outcome measure.

The first porphyrin study (Austin & Shandley, 2008) utilised a convenience sample of Australian children with ASD presenting to a psychology clinic that had formerly undergone porphyrin testing. The mean urinary porphyrin levels for this ASD group were compared against the mean urinary porphyrin levels reported by Nataf et al. (2006) and Geier and Geier (2007) for their ASD and control groups. In addition, the porphyrin levels from all studies were compared to an external control group of normally developing children (Minder & Schneider-Yin, 1996). The results of the study supported the hypothesis that the triad of porphyrins associated with Hg toxicity (5CP, PrCP and CP) among the Australian ASD group would be elevated in comparison to the control groups reported by the other three studies. Nevertheless, there were substantial limitations to this study including a small sample size, wide age-range and lack of control group matched for age and geographic location (country). The results, however, did indicate that a larger, controlled urinary porphyrin study was warranted.

The second larger porphyrin study presented in this thesis (Shandley et al. 2014) compared a group of Australian children 2-6 years of age diagnosed with ASD
against two healthy control groups; a sibling control (children with a sibling diagnosed with ASD) and an external control (children with no known blood relatives diagnosed with ASD). In contrast to the first study, and indeed all known urinary porphyrin and ASD studies previously published (Austin & Shandley, 2008; Nataf et al., 2006; Geier & Geier, 2006d, 2007; Heyer et al., 2012; Kern et al., 2011; Woods et al., 2010; Youn et al., 2010), the hypothesis that there would be a significant difference in the urinary porphyrin levels between the ASD group and control groups was not supported. Furthermore, neither group membership, nor ASD severity could be predicted based upon urinary porphyrins. Essentially, the results do not support that there is an association between Hg and ASD. Nevertheless, there are several other possibilities that may explain this finding.

The issue of age has previously been raised as a key variable in respect to urinary porphyrin measurement (Woods et al., 2009, 2010). Porphyrin levels are typically higher and more unstable at younger ages, decreasing throughout childhood and becoming more stable as the individual approaches adulthood. Therefore, studies using older children or adults as controls could be misleading due to natural age-related variation in urinary porphyrin concentrations. However, the issue of an age-appropriate control group was addressed in the Shandley et al. (2014) porphyrin study by recruiting two age-matched control groups. Furthermore, any unanticipated age variation was addressed by controlling for age in the analysis. Interestingly though, in contrast to our study, the results of an earlier, smaller study by Kern et al. (2011) found a significant difference between children with ASD and an age- and sex-matched control group.

A second issue in regards to age also needs to be considered. The Shandley et al. (2014) porphyrin study recruited children within the typical ASD diagnostic age-
range (2-6 years). This age-range is both considerably younger and more restricted than those used in other porphyrin studies. The majority of prior porphyrin studies have recruited children from 2-12 years of age, effectively doubling the upper limit of the Shandley et al. (2014) study. As mentioned above, porphyrins tend to be higher, and less stable among younger age groups. Consequently, it is possible that urinary porphyrins are an ill-suited measure of Hg damage among younger cohorts. Further studies examining the validity of urinary porphyrins as a marker of Hg damage among infants and pre-school aged children would be a valuable addition to the knowledge base on porphyrins.

The Shandley et al. (2014) study is the first in the series of studies exploring ASD and urinary porphyrins to align with the DSM 5 nosology, merging children with a diagnosis of Autistic Disorder, Asperger’s Disorder and PDD-NOS into the single dimension of ASD. ASD is undeniably a broad spectrum given that it includes severely disabled children at one end of the spectrum who may require intensive, specialised lifelong care, while the other end of the spectrum includes children who are often able to attend mainstream schooling and will possibly be able to go on to live an independent adult life. There is some suggestion that elevated porphyrin levels may be more pronounced among more severely disabled groups of children.

Consider, for example, the study by Nataf et al. (2006), where the urinary porphyrin markers associated with Hg (5CP, PrCP, and CP) were significantly elevated among children with AUT in comparison to a control group, however, they were not significantly elevated in children with Asperger’s Disorder or PDD-NOS. If the children diagnosed with Autistic Disorder, Asperger’s and PDD-NOS were grouped according to the DSM 5, one wonders if there would be a significant difference between the newly formed ASD group and the control group. Clearly the
classification issue driven by DSM-5 whereby previously distinct diagnostic groups are collapsed into a single ASD entity needs to be accounted for by any future research in this area.

**Pink Disease**

Pink disease survivors are a diminishing population of individuals with an idiosyncratic sensitivity to Hg and therefore represent a unique opportunity to better understand the clinical ramifications and mechanisms of Hg on human health. Few cases of pink disease have been diagnosed since the 1960s following the removal of calomel from medicinal products used with newborns and infants, primarily teething powders. Consequently, pink disease survivors are an ageing cohort of the population with the youngest afflicted individuals typically aged in their 50-60s.

Shandley and Austin (2011) found ASD to be significantly higher (6-7 fold) among the grandchildren of pink disease survivors in comparison to the general population matched by birth year. Furthermore, the results indicate that there was not an elevated risk for disease generally among the offspring of pink disease survivors, but rather a specific risk for ASD. The results are therefore consistent with the hypothesis that a hypersensitivity to Hg may be a heritable risk factor for the development of ASD. However, the results of this study do not provide information as to the mechanism by which a sensitivity to Hg might be inherited. Some possible mechanisms by which this may occur have been identified by others. For example, elevated oxidative stress and decreased glutathione have been identified as a possible mechanism by which Hg may induce ASD (James et al. 2004; Geier & Geier, 2006a; Youn et al. 2010). An individual low in glutathione would have both a diminished capacity to minimise oxidative damage produced by Hg and excrete the toxicant. A
recent case-control study by Stamova et al. (2011) investigated gene expression and blood Hg levels in boys diagnosed with ASD and an age-matched control group of typically developing boys. The authors identified the expression of 189 genes to correlate with Hg levels in the ASD group alone. The primary biological function of the genes was reported to be cell morphology, amino acid metabolism, and antigen presentation. This suggests that the underlying mechanism may relate to the inability of children with ASD to effectively metabolise toxic substances.

An issue for consideration in the Shandley and Austin (2011) study is the use of the second generation of descendants (grandchildren) of pink disease survivors. Obtaining comparable ASD prevalence rates was a critical component of the study, however, in Australia, only one ASD prevalence study has been conducted (MacDermott et al., 2008). This had a substantial impact on the study as the mean age of the children of pink disease survivors was 37 years and Australian general population ASD prevalence rates are not available for this cohort born in the 1960s and 1970s. Consequently the next best option was to compare the prevalence rates of grandchildren who could be matched for birth year. Whether this issue mediates or obscures the results in some manner is unclear.

A further issue for consideration is the lack of additional information collected in respect to other variables hypothesised to play a role in the development of ASD, such as parental age, maternal or neonatal infection, and birth weight. Pink disease survivors report a wide range of medical issues experienced during adulthood. It is possible that ASD may only be indirectly associated with pink disease and therefore Hg. That is, pink disease survivors may be more likely to experience biomedical issues due to Hg which are responsible for the higher prevalence of ASD among the descendants as opposed to the descendants inheriting a sensitivity to Hg. For
example, fertility issues have been reported among pink disease survivors, such as Young’s syndrome which is a condition relating to male infertility (Hendry et al., 1993). Fertility issues may lead to a greater frequency of babies in the low birth weight range, a variable that has been associated with ASD. There is a clear need for more research in this area, and given pink disease survivors are a rare ageing cohort of the population, the need is urgent.

**Strengths**

The body of work presented in this thesis has made a significant and unique contribution to the body of research exploring the potential association between Hg and ASD. Firstly, the two empirical studies utilising urinary porphyrins as a measure of Hg damage (Austin & Shandley, 2008; Shandley et al., 2014) remain the only published studies of their type conducted in Australia. The Shandley et al. (2014) paper offers a particularly unique perspective to the collection of porphyrin studies as this was a well powered study with well-defined groups and, in contrast to all other comparable published studies, found no significant differences between an ASD group and two healthy control groups. Consequently, the results from the paper do not support the assumption that there is a relationship between Hg and ASD. Despite this, there remains a question over the reliability of urinary porphyrins as a measure of Hg toxicity among very young (infants/pre-school aged) children, and this requires further investigation.

The Shandley and Austin (2011) paper was the first to examine the notion of individual susceptibility to Hg among a cohort of pink disease survivors. A substantial recruitment campaign was carried out in conjunction with the Australian Pink Disease Support Group (the only group in the world dedicated to pink disease
survivors) allowing current and past members from their membership list to be contacted. Given pink disease survivors are largely an elderly cohort, a variety of response format options were provided allowing pink disease survivors to select the option with which they were most comfortable. Working closely with the Pink Disease Support Group and providing a variety of response format options created the best chance for recruiting as many remaining pink disease survivors into the study as possible. In order to minimise response bias, the purpose of the study was withheld from participants with recruitment materials merely stating that the researchers were interested in the health outcomes of their descendants. The study was furthered strengthened by matching comparison groups for age and birth year. The study found that descendants of pink disease survivors were significantly more likely to have a diagnosis of ASD; critically highlighting that heritability of Hg sensitivity may be a key factor in understanding the aetiology of ASD.

**Limitations**

The published studies presented in this thesis need to be viewed in light of a number of limitations. Firstly, a limitation common to all three empirical studies (Austin & Shandley, 2008; Shandley & Austin, 2011; Shandley et al., 2014) was the lack of independent clinical assessments. Due to cost of conducting assessments, the sample sizes and location of participants (across rural and metropolitan regions in most states and territories in Australia) it was not economically feasible to conduct independent clinical assessments to validate the accuracy of all self-reported diagnoses. It is reasonable, therefore, to assume that a small number of diagnostic errors may be present; however, where possible, any doubts that arose over diagnosis were followed up with the individual participant/family. Furthermore, a cautious
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approach was adopted whereby strict inclusion criteria was applied to each study, only accepting a child as an ASD case if an assessment had been carried out by a qualified health professional. Children who were in the process of undergoing an ASD assessment or who exhibited symptoms seemingly consistent with an ASD diagnosis were not accepted as an ASD case. Consequently, if any errors in diagnosis did occur, it is unlikely that they did so in large enough numbers to influence the results of any of the studies.

As previously discussed, the presence of elevated levels of 5CP, PrCP and CP are associated with heavy metal toxicity, with PrCP specifically associated with Hg toxicity. However, PrCP is an unstandardised porphyrin (largely unvalidated biochemical entity). For this reason, not all laboratories that conduct porphyrin testing include PrCP in their urinary porphyrin profiles. Until such time as PrCP is standardised (if this is to occur), studies placing emphasis on the importance of this porphyrin in respect to Hg toxicity need to be viewed cautiously.

A further limitation not raised in the Shandley et al. (2014) paper is that the analyses did not consider exposure to contaminants. As discussed in Chapter 3, Hg is ubiquitous in the environment and humans are particularly exposed through dental amalgam, diet, vaccinations, and geographic location. It is possible that the study groups may have been differentially exposed to Hg, and whether this would have had an impact on the results is unknown. However, it is recommended that future studies take into account as many contaminant variables as possible.

**Future research**

The number of studies investigating urinary porphyrins and ASD are steadily increasing. The studies presented in this thesis suggest that there is need to consider
the reliability of urinary porphyrins as a measure of Hg damage, particularly in regards to younger cohorts. Longitudinal studies investigating changes in porphyrin levels across age between children with ASD and normally developing children would substantially improve the knowledge base on urinary porphyrins and what value they have in furthering our understanding of any possible association between Hg and ASD. There is also need for a replication study of the Shandley et al. (2014) study. The results of this study were markedly different from previous porphyrin studies and it is important to determine whether the results can be replicated.

Based upon the results presented in this thesis, further genetic and biological studies, particularly with pink disease survivors, may further our understanding of the mechanisms by which Hg may induce ASD. For example, studies seeking to identify genetic variables that may differentiate pink disease survivors from non-pink disease survivors are encouraged. Genetic markers unique to pink disease survivors may play a critical role in one’s susceptibility to Hg. It would then be beneficial to explore any identified genetic markers among individuals with ASD. Moreover, enquiry into potential mechanisms by which Hg could disrupt epigenetic regulation is important, in addition to determining whether there is a transgenerational effect (i.e., whether epigenetic changes are passed from parent to offspring).

Further research examining the association between pink disease and other hypothesised risk factors in the pathogenesis of ASD (i.e., birth weight, parental age, maternal/neonatal infection and birth weight) would also be highly beneficial. To further establish whether an individual sensitivity to Hg is heritable it is necessary to determine whether other ASD risk factors are common among pink disease survivors. If this is the case, then the apparent relationship between Hg and ASD demonstrated in the Shandley and Austin (2011) paper may in fact be an indirect
relationship, mediated by biomedical issues experienced by pink disease survivors as a consequence of Hg.

**Conclusion**

Research investigating possible environmental triggers in the pathogenesis of ASD has increased dramatically in recent years. In particular, Hg as a possible environmental trigger has been the topic of much debate since the publication of a paper by Bernard et al. (2001) which proposed that ASD is a form of Hg poisoning. Although some of the evidence to date is compelling, scientifically, it is not definitive. The present thesis took an interdisciplinary approach to investigate the potential association between Hg and ASD. Three empirical studies were conducted, two biological studies utilising urinary porphyrins as the main outcome measure, and a heritability study examining whether an individual sensitivity to Hg may be genetic.

The two published studies (Austin & Shandley, 2008; Shandley et al., 2014) undertaken to investigate Hg toxicity among Australian children via the use of urinary porphyrin profiles ultimately failed to support the association between Hg and ASD. The first study conducted in the present thesis (Austin & Shandley, 2008), a pilot trial, found a significant difference between the porphyrin levels of a small convenience sample of Australian children with ASD in comparison to the control groups of three other studies (Geier & Geier, 2007; Minder & Schneider-Yin, 1996; Nataf et al., 2006), indicating that a further study was warranted. However, the larger case-control study (Shandley et al., 2014), designed to address the limitations of the pilot trial (small sample size, lack of appropriate control group) and extend upon previous porphyrin studies, failed to find a significant difference in urinary
porphyrins levels between three groups: (1) children diagnosed with ASD; (2) children with a sibling diagnosed with ASD (sibling control group); and (3) children with no known blood relatives diagnosed with an ASD (external control group).

Furthermore, neither group membership, nor ASD severity could be predicted based upon urinary porphyrins. However, this result must be considered in light of the potential unreliability of urinary porphyrins as a measure of Hg damage for young children.

The Shandley & Austin (2011) study provides support for a possible heritable and/or genetic basis for a sensitivity to Hg and warrants further investigation into mechanisms by which this may occur. In addition, it is necessary to determine the nature of the relationship between pink disease and other hypothesised risk factors for ASD in order to establish whether there is a direct or indirect link between pink disease, ASD and Hg.

Based upon the studies undertaken in the present thesis it is not possible to definitively conclude whether there is an association between Hg and ASD. The apparent association between Hg and ASD is clearly complex and likely mediated by several factors. Mediating factors may vary across individuals based on physiological characteristics (i.e., genetics, age, weight and gender) and/or Hg characteristics (i.e., age of exposure, form, and dose). Several challenging, multifactorial studies will be required to understand the nature of the association between Hg and ASD, if one indeed exists.
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