

Applying Hill's Criteria to the Study of Autism Spectrum Disorders and Exposure to Mercury

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Abstract

Purpose. *Autism Spectrum Disorders (ASDs) are neuropsychiatric disorders that include Autistic Disorder, Asperger syndrome and Pervasive Developmental Disorders – not otherwise specified (PDD-NOS). Prevalence rates of ASDs are reported to have increased in the last decade, raising concern amongst researchers, service providers, policymakers, and families. While current aetiological research is exploring the interface between genetic and environmental factors, exposure to heavy metals, in particular mercury has retained public interest.*

Methods. *A systematic review of publications from 1980 to 2010 inclusive was used to examine the hypothesized link between ASD and mercury exposure. Hill's criteria for causation were applied to critically appraise the reviewed studies.*

Results. *Reviewed studies failed to demonstrate strength and consistency of association as well as establish a temporal link between the onset of ASD symptoms and mercury exposure.*

Conclusions. *The risk of developing and being diagnosed with an ASD as a result of mercury exposure remains unclear because of methodological flaws in studies conducted to date. Rigorous research is needed to provide adequate information to families, clinicians and decision-makers. They must be provided with up-to-date, critically appraised information to help them make informed decisions.*

Autism Spectrum Disorders (ASDs) are neuropsychiatric disorders that include Autistic Disorder, Asperger syndrome and Pervasive Developmental Disorders – Not Otherwise Specified (PDD-NOS) (DSM-IV-TR) (American Psychiatric Association (APA), 2000). Reported prevalence rates of ASD are inconsistent across the literature and may range from 10.1/10,000 to 64.9/10,000 (Bryson, Clark, & Smith, 1988; Fombonne, Zakarian, Bennett, Meng, & McLean-Heywood, 2006; Ouellette-Kuntz et al., 2006). Prevalence rates are reported to have increased in the last decade raising concern amongst researchers, service providers, policymakers, and family members (Ouellette-Kuntz et al., 2007; Rice et al.,

2007). A review of 43 epidemiological studies revealed that only a small fraction (0% to 16.7%; mean = 5.9%) of the cases of Autistic Disorder could be related to any known aetiologically significant medical condition (Fombonne, 2005). While current aetiological research is exploring the interface between genetic and environmental factors, exposure to heavy metals, in particular mercury, has retained public interest (Hertz-Picciotto et al., 2006). This paper aims to improve critical thinking towards studies investigating the causal link between ASD and exposure to mercury by applying the nine criteria for causality as defined by Hill to the literature related to these associations (Hill, 1965). Hill's criteria include: (1) the strength of the association, (2) the consistency of the observed association, (3) the specificity of the association, (4) the temporal relationship of the association, (5) a biological gradient or dose-response curve, (6) the biological plausibility, (7) the coherence with the current knowledge, (8) experimental or semi-experimental evidence, (9) and the analogy with similar evidence. The strength and the consistency of the association are two criteria that must invariably be considered to prove causation (Hill, 1965). Other criteria might be difficult to establish or irrelevant to the nature of the observed association, but should nevertheless be systematically examined. If evidence exists for the remaining seven criteria, conclusions may be drawn without hesitation, although the lack of evidence does not nullify a causal association.

In neuropsychiatry, four of Hill's nine criteria are considered critical to assess causality: the strength of the association (criterion 1), the consistency of the observed association (criterion 2), the biologic rationale (criterion 6), and the temporal relationship of the association (criterion 4) (van Reekum, Streiner, & Conn, 2001). Arguments of a biologic gradient (i.e., severity of symptoms increases with exposure; criterion 5) and specificity of the relation between the causal agent and the outcome (criterion 3) may not be feasible to prove, since symptom severity is difficult to determine and multiple causal factors are generally identified for one neuropsychiatric disorder. Coherence with known facts (criterion 7) and analogy with similar evidence (criterion 9) might add to our understanding of the association; however, aetiological understanding in neuropsychiatry is still limited and

a lack of evidence to prove these three criteria is not sufficient to rule out the causal relationship. Experimental studies (criterion 8) provide greater evidence of a causal link but may be unethical to perform in neuropsychiatry since it is unconceivable to induce some form of brain dysfunctions experimentally in humans.

Criteria for Causation Between Mercury and ASD

Mercury is just one of a handful of heavy metals that have been suggested as environmental contributors to the development of ASD. The heightened public interest regarding the until-recent presence of thimerosal (a mercury-based preservative) in widely distributed vaccinations amongst other sources of exposure raises concern over the insufficient evidence tying the heavy metal to the manifestation of ASD. In this next section, a brief comment is made on issues relevant to each of Hill's nine criteria regarding the association between ASD and mercury.

Criterion 1: Strength of the Association

The strength of an association is the statistical or clinical significance of the association, is observed in a well-defined population but does not occur to the same extent in an appropriate control group. Case-definition, sample size and statistical power are thus crucial to determine the strength of an association. Differences in participant selection and identification procedures across studies are key methodological issues that must be considered. ASD was first introduced in the third edition of the Diagnostic and Statistical Manual (DSM-III) in 1980 (American Psychiatric Association (APA), 1980) and subtypes and diagnostic criteria have been revised several times since (DSM-III-R; DSM-IV; DSM-IV-TR) (APA, 1987; APA, 1994; APA, 2000). Two- to threefold variation in the prevalence rates of ASD can result from applying different diagnostic criteria to the same survey data (Kielinen, Linna, & Moilanen, 2000). Social and demographic characteristics of the cases with ASD may also influence study results. Attention should be given to the participants' gender as the male: female ratio

is approximately 4:1, with a more pronounced difference among individuals with intellectual disabilities (Fombonne, 2005). To appraise the multiple factors that could impact the strength of the observed association, a systematic review of the literature on ASD and exposure to mercury was conducted. Systematic reviews are designed to assess the strength of the observed associations as they help in collecting and critiquing relevant methodological information in all selected studies.

Criterion 2: Consistency of the Observed Association

An assessment of consistency requires determining whether the association has been repeatedly observed by different persons, in different places, circumstances and times (Hill, 1965). Methodological issues may explain discrepancies, but if no reason justifies discrepancies, evidence seriously undermines the argument of causation (van Reekum et al., 2001). Systematic reviews are particularly useful in assessing consistency across studies while taking into account methodological issues.

Criterion 3: Specificity of the Association

Although evidence remains limited, ASD is known to be partially determined by genetic factors (Leonard et al., 2010). Specificity of the association is not confirmed, but it does not constitute a necessary criterion for causation in neuropsychiatry (van Reekum et al., 2001). Consequently, this third criterion is not relevant to the study of the association between ASD and exposure to heavy metals. Other criteria must be considered.

Criterion 4: Temporality

The time of exposure to the potential agent must occur prior to the onset of the symptoms. The method used to estimate cases exposure to mercury is crucial in making assumptions about temporal sequence. Multiple sources of exposure to mercury may exist, and there is difficulty in assessing the risk of ASD with prenatal or early childhood exposure. Relying on biomarkers to estimate mercury exposure offers the advantage of measuring exposure

from all possible sources, but not all of them can provide information on past exposures (World Health Organization (WHO), 2006). Mercury accumulates in the bones and teeth, and can be excreted through sweat, nails, urine and feces. Measurement of heavy metals from body tissues is not only affected by tissue-specific half-lives, but also may largely be a result of differential accumulation and excretion capabilities and mechanisms amongst different populations (Axel Weiner & Nylander, 1993; Grandjean, PAL, & White, 1995; Holmes, Blaxill, & Haley, 2003; Kern, Geier, Adams, & Geier, 2010). The reported half-lives of mercuric compounds in blood and body tissues are variable. Data from five publications found the half-life of methyl mercury in blood or hair to range from 45 to 70 days (Al-Shahristani & Shihab, 1974; Kershaw, Dhahir, & Clarkson, 1980; Miettinen, Rahola, Hattula, Rissanen, & Tillander, 1971; Sherlock, Lindsay, Hislop, Evans, & Collier, 1982; Smith et al., 1994). Therefore, prenatal and early childhood exposures are not likely to be identified through blood or hair mercury levels measured later in life. Mercury levels in bones and teeth provide more information on past exposure compared to blood or hair levels. Modelling may also be used to estimate exposure (WHO, 2006). This relies on available information on the concentration of mercury in air, water or soil along with information on when, where and how individuals might have been exposed to the metal. Where data are available, this indirect method can provide a measure of exposure prior to the onset of symptoms of ASD thereby respecting the temporality criterion in studies of risk factors. However, information on exposure and cases of ASD typically comes from different sources and this may lead to imprecise or unreliable data.

Criterion 7: Coherence with Known Facts

Mercury is primarily released in the environment as a result of human industrial activities and, consequently, human exposure has increased since industrialization. Mercury exists as a range of organic and inorganic matters that vary in toxicity and persistence in living organisms. Methyl mercury is an organic compound much more toxic than pure metal

mercury itself, and has the ability to migrate across cell membranes and accumulate in living organisms (Environment Canada, 2010a). Children are disproportionately more vulnerable to chemical toxicants as their metabolic pathways are comparatively immature (Landrigan & Garg, 2002). An additional vulnerability in children is their rapid pace of neurodevelopment and the incompletely formed blood brain barrier which then makes the developing brain much more susceptible to any toxic insults (WHO, 2006). Ingestion of methyl mercury in foods is the most prevalent pathway of exposure to mercury in human populations (U.S. Department of Health and Human Services, 2006). Thimerosal, a mercury-containing anti-microbial preservative, is used in vaccines and immunoglobulins around the world, but efforts have been made to decrease its use. In the U.S., “thimerosal has been removed from or reduced to trace amounts in all vaccines routinely recommended for children 6 years of age and younger, with the exception of inactivated influenza vaccine” (FDA, 2010). In Canada, most vaccines do not contain thimerosal; the only thimerosal-containing vaccine in routine use in the infant immunization schedules of some Canadian jurisdictions is the hepatitis B vaccine (Health Canada, 2007). See also the Public Health Agency of Canada (2007) for a list of other vaccines, including the multi-val influenza vaccine, that contain thimerosal.

Criteria 6 and 9: Biological Plausibility and Analogy with Similar Evidence

Chronic exposure to mercury can cause damage to the brain, spinal cord, kidneys and liver, even at a very low level (Environment Canada, 2010b). Exposure to mercury while in the womb can lead to neurodevelopmental problems in children. Damage to the developing brain following mercury exposure is widespread. Excess exposure may inhibit brain transport mechanisms and critical enzymes of normal neuronal development and appears to involve oxidative stress mechanisms and impairment of microtubule assembly (Aschner & Aschner, 1990; D. A. Geier, King, Sykes, & Geier, 2008). Teratogenic effects on the foetus vary depending on the developmental stage

at the time of exposure, although no exposure level is completely safe (Sadler, 2004).

Criteria 5 and 8: Biological Gradient and Experimental or Quasi-experimental Evidence

Mercury poisoning is characterized by symptoms similar to autistic traits such as an indifference to others, irritability, anxiety, and obsessive-compulsive traits (WHO, 2007). Furthermore, a decrease in the severity of ASD-like symptoms in children suffering from mercury poisoning has been observed following the substitution of metabolites implicated in crucial mercury detoxification pathways (chelation therapy) (James et al., 2004).

Given that the putative association between ASD and exposure to mercury is plausible, evidence is required to assess the strength and consistency of this association to establish causality. Temporality between the exposure and the onset of symptoms is also a critical criterion that requires attention. Studies on the potential link between ASD and exposure to mercury are presented and discussed with these three essential criteria in mind.

Materials and Methods

A systematic review was used to examine the hypothesized link between ASD and mercury exposure. The guidelines from the Centre for Reviews and Dissemination (CRD, 2008) were followed to identify, analyze and compare relevant studies.

Study Identification and Selection

Studies were identified through several sources. Databases (OVID Medline, OVID Medline In-progress, OVID Healthstar, PsycINFO, CINAHL, EMBASE, Global Health, AMED, and EBM Reviews - Cochrane DSR, ACP Journal Club, DARE, and CCTR) were searched for publications from 1980 to 2010 inclusive, with combinations of keywords such as autism, pervasive developmental disorders, and mercury. The starting date of 1980 was chosen as it correlates with the first introduction of ASD in the DSM (American Psychiatric Association, 1980).

Citations from chosen articles and relevant reviews were hand-searched. Grey literature not published commercially or indexed by major databases was searched to include unpublished articles, reports, and conference abstracts valuable to the systematic review, so as to minimize publication bias (CRD, 2008). Grey literature was identified through a Google and Google Scholar search and by visiting autism-related research society websites (Autism Canada Foundation, the Autism Research Centre, the Autism Research Institute, the Autism Society Canada, the Autism Society of America, and the Autism Spectrum Disorders – Canadian-American Research Consortium).

Titles and abstracts were screened to select relevant papers based on the list of criteria presented below (all inclusion and exclusion criteria were applied to identified papers). Papers were included if they examined:

- the relationship between exposure to mercury and ASD,
- a hypothesized source of exposure, prenatal or otherwise,
- a sample population of individuals with ASD.

Exclusion criteria were set in order to provide the best available evidence and avoid flaws in the appraisal of causal relationships between mercury exposure and ASD, particularly regarding the strength and consistency of the association, as well as its temporality. Papers were excluded if they:

- reported measurement of mercury from body tissues without reporting upon sources and/or exposure status of participants,
- recruited cases amongst persons diagnosed with mercury poisoning,
- failed to report the use of appropriate statistical analyses testing a stated hypothesis.

We also excluded review papers and articles reporting findings from ecological studies. Systematic reviews summarize, compare and analyze findings from original papers. We excluded ecological studies from this review to help with analyses and comparisons. Their

population-based design requires a different approach to extract data, appraise their quality and analyze their findings.

Data Extraction and Quality Assessment

A data extraction form was tailored to suit the review questions. Information regarding the purpose, methods and results of each study was collected. Samples and population characteristics including age, gender and diagnoses, as well as location, sample size and selection methods were recorded into common categories to facilitate cross-study comparisons.

Quality assessment served to determine if results were unduly influenced by methodological bias and provided an indication of the strength of the presented evidence (CRD, 2008). Factors used to assess the quality of the included studies were: sample selection, case identification, measurement of exposure to mercury, including the ability to determine temporality between onset of the symptoms and exposure. High quality studies confirmed case status following all or part of the Canadian best practice guidelines for the diagnosis of ASD (Nachshen et al., 2008). These guidelines recommend the use of the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the International Classification of Diseases (ICD) diagnostic criteria *and* the combination of clinical judgement *and* standardized assessment tools. The Autism Diagnostic Observation Scale (ADOS) (Lord, Rutter, & Couteur, 1994; Lord, Rutter, DiLavore, & Risi, 2002), the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994) and the Childhood Autism Rating Scale (CARS) (Schopler, Reichler, & Renner, 1988) are the recommended instruments. Clinical judgement requires significant training and experience. An interdisciplinary approach is also recommended. Studies that assessed exposure using objective criteria consistent across both cases and controls were deemed to be of high quality. Furthermore, as the aim of the review was to determine whether mercury exposure could be a risk factor for ASD, high quality studies had to be able to demonstrate that exposure occurred prior to the first symptoms of ASD, that is during pregnancy or early childhood.

Table 1. Details of the Reviewed Studies Exploring the Link Between Autism Spectrum Disorders and Mercury Exposure

Author (Year), Location	Sample Selection	n	Age ¹	Gender Ratio ²	Exposure	Results ¹
Adams et al. (2007), Phoenix, Arizona, USA	Cases: Autism, PDD-NOS or Asperger syndrome. Diagnostic Validity: No Controls: Friends and neighbours of the cases. Without developmental delay, illness, or other medical conditions.	16 11	6.1±2.2 7.0±1.7	4.26:1 0.82:1	Prenatal and postnatal exposure to thimerosal, maternal amalgams, seafood and paint consumption Temporality established: No	Significant Lower Exposure <u>Anti-RhoD immunoglobulin:</u> Cases: 0% Controls: 36% P<0.05 Significant Higher Exposure <u>Antibiotic Usage 0-12 mo.</u> Cases: 3.6±2.4 rounds Controls: 1.5±1.5 rounds P<0.01 <u>Antibiotic Usage 0-36 mo.</u> Cases: 10.8±9.5 rounds Controls: 5.1±4.3 rounds P<0.05
Adams et al. (2008), USA	Cases: Autism. Diagnostic Validity: No Controls: Friends with no immediate relation to the cases. Normal development. Normal vaccinations scheduled up to the 1 st haircut.	78 31	16.6±4.4 mo. 16.5±5.0 mo. Age-matched	6.09:1 1.91:1	Prenatal and postnatal exposure to thimerosal, seafood and dental amalgams Temporality established: No	Significant <u>Antibiotic usage 0-18 mo.</u> Cases: 3.8±3.7 rounds Controls: 1.5±2.1 rounds p<0.01

¹ The format used to present information in the column designated by (1) is range (mean± SD) when data are available. Unless otherwise noted, units of age are in years.

² The format used to present information on gender ratio is males:females, when data are available.

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Table 1. Details of the Reviewed Studies Exploring the Link Between Autism Spectrum Disorders and Mercury Exposure (continued)

Author (Year), Location	Sample Selection	n	Age ¹	Gender Ratio ²	Exposure	Results ¹
Andrews et al. (2004), United Kingdom	Cases: Autism <i>Diagnostic Validity: No</i> Controls: Randomly selected, case-matched individuals with no diagnosis of an ASD.	104 410	Median age: 4.4 Age-matched	8.43:1 4.5:1	Postnatal thimerosal exposure <i>Temporality established: No</i>	Non Significant No greater hazard with increasing thimerosal exposure for autism. HR per dose by 3 months: 0.89 HR per dose by 4 months: 0.94
Croen et al. (2008), Northern California	Cases: Children with at least one diagnosis of an ASD. <i>Diagnostic Validity: No</i> Controls: No diagnosis of an ASD.	400 410	4-7 years 4-7 years	4.7:1 4.5:1 Sex-matched	Prenatal thimerosal exposure <i>Temporality established: Yes</i>	Non Significant Association between prenatal exposure to anti-D immunoglobulin and ASD (Odds Ratios) <i>Autistic Disorder</i> 1.03 (0.60-1.76) <i>Asperger/PDD-NOS</i> 1.20 (0.65-2.22)
Geier & Geier (2005), USA	Vaccine Safety Datalink database and CDC-VSD database. Children received at least 2 polio vaccines within the HMO by age 1 year. ICD-9 codes indicative of congenital disorders, severe perinatal disorders, recipients of hepatitis B immunoglobulins, and premature infants were excluded. <i>Diagnosis Validity: No</i>	127	At first diagnosis: 42 mos.	4.88:1	Postnatal thimerosal exposure <i>Temporality established: No</i>	Non Significant P values not reported.

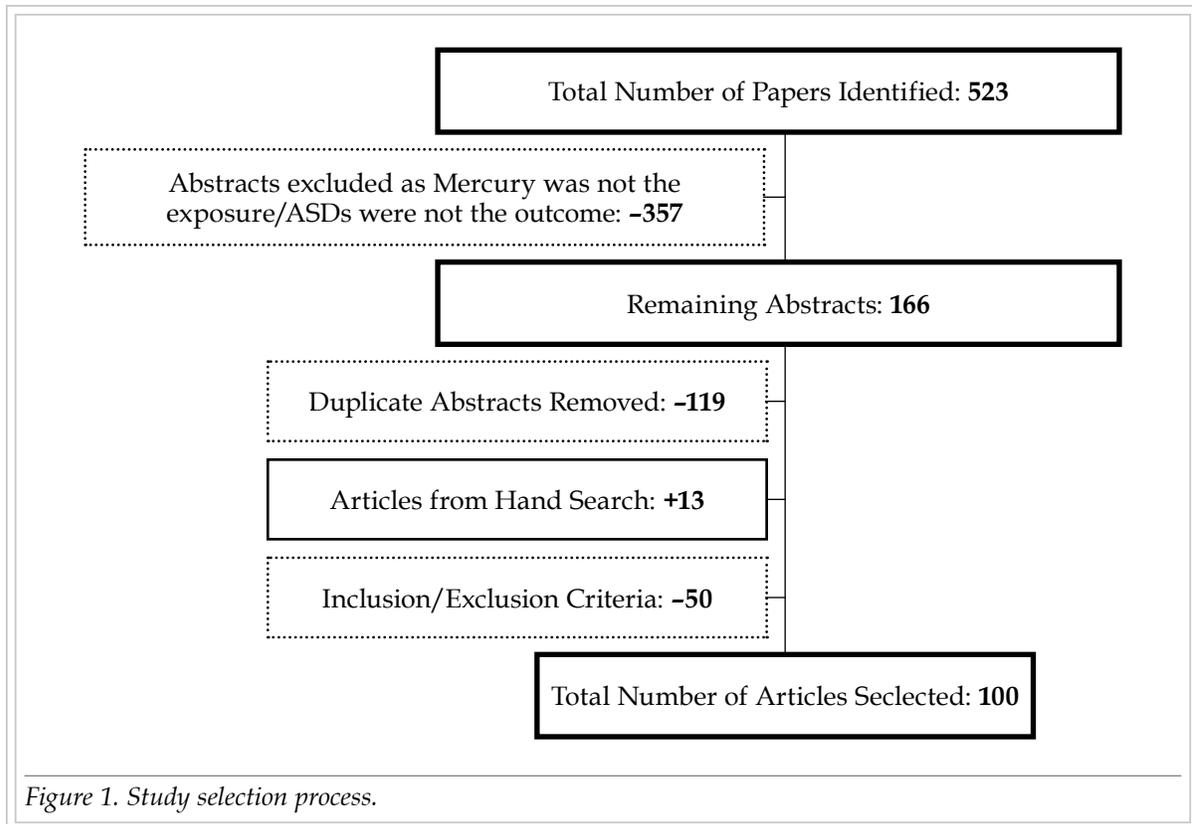
Table 1. Details of the Reviewed Studies Exploring the Link Between Autism Spectrum Disorders and Mercury Exposure (continued)

Author (Year), Location	Sample Selection	n	Age ¹	Gender Ratio ²	Exposure	Results ¹
Holloway et al. (2003), Arizona, USA	Cases: Individuals with an ASD <i>Diagnostic Validity: No</i> Controls: Individuals with no ASDs	53	7.1±3.0	3.42:1	Prenatal and postnatal exposures [Maternal and fetal seafood consumption, dental amalgams, vaccinations, paint consumption] <i>Temporality established: No</i>	Non Significant No significant increases when comparing investigated risk factors (P>0.05)
Holmes et al. (2003), 94%USA, 6%International, [Canada, England, Mexico]	Cases: Autism. <i>Diagnostic Validity: No</i> Controls: Individuals with no developmental disabilities or chronic illnesses.	94	Median months: 17.7 Range: 11-24	3.5:1	Prenatal mercury exposure [Rho D immunoglobulin injections, dental amalgams] <i>Temporality established: Yes</i>	Significant <i>Prenatal Thimerosal:</i> Cases: 0.53±0.67 Controls: 0.09±0.29 P<0.0000004 <i># Maternal Amalgams:</i> Cases: 8.35±3.43 Controls: 6.60±3.55 P<0.01
Hviid et al. (2003), Denmark	Cases: Autistic Disorder, PDD-NOS. Vaccinated at least once with a thimerosal containing vaccine. <i>Diagnostic Validity: No</i> Controls: Autistic Disorder, PDD-NOS. Vaccinated with thimerosal-free formulations of the same vaccines as controls.	425	At diagnosis AD: 4.7±1.7 PDD-NOS: 6.0±1.9 Born before 1992	Not specified	Postnatal thimerosal exposure <i>Temporality established: No</i>	Non Significant No evidence of a dose-response association between the dose of ethyl mercury received and ASDs

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Table 1. Details of the Reviewed Studies Exploring the Link Between Autism Spectrum Disorders and Mercury Exposure (continued)

Author (Year), Location	Sample Selection	n	Age ¹	Gender Ratio ²	Exposure	Results ¹
Miles et al., (2007), Missouri, USA	Cases: AD, Asperger Syndrome <i>Diagnostic Validity: No</i>	208	Pregnancy prior to 2002 1.4-23.5; 7.2±4.3	Not Specified	Prenatal thimerosal exposure <i>Temporality established: Yes</i>	Non Significant Cases: 13.9% exposed to ante partum Rhlg (29/208) P=0.22 Controls: 14.8% exposed to ante partum Rhlg. (4/29)
Verstraeten et al. (2003), USA	Controls: Children with Down syndrome and other de novo chromosome disorders. Participants: Autism <i>Diagnostic Validity: No</i>	27 202	Pregnancy prior to 2002 Median age at 1 st diagnosis (months) 44	Not Specified 8:1	Not Specified Postnatal thimerosal exposure Temporality established: No	Non Significant No significant associations were found with cumulative exposure at any age and risk for autism in either continuous or categorical exposure analyses. <i>Risk Ratios:</i> <u>Cumulative: (95% CI)</u> 1 mo: 1.16 (0.78-1.71) 3 mo: 1.06 (0.88-1.28) 7 mo: 1.00 (0.90-1.09) P>0.05 <u>Categorical: (95%CI)</u> 0-75 ug: 1.00 87-162.5 ug: 0.95 (0.62-1.46) ≥175 ug: 0.65(0.27-1.52) P = 0.58



Data extraction and quality assessment were conducted independently by two reviewers.

Results

From 523 papers identified through database searches, only 146 articles investigated the link between mercury exposure and ASD. Ten studies were retained for final analysis (Figure 1). These studies are summarized in Table 1.

Three of the ten papers found significant correlations between increased mercury exposure and ASD diagnosis (Adams, Romdalvik, Ramanujam, & Legator, 2007; Adams, Romdalvik, Levine, & Hu, 2008; Holmes et al., 2003), one of which (Adams et al., 2007) also found a significant correlation between ASD and a lower exposure to mercury. However, methodological issues could explain the lack of both consistency and strength of the association.

Case definition differed between studies. Some included participants with any diagnosis of ASD, while others limited their study population to individuals with "autism." None of the

studies met the criteria for diagnostic validity. They did not report using standardized diagnostic methods to confirm ASD case status. However, little information is available in the published papers to appraise accurately the diagnostic validity. Diagnostic validity is therefore questionable in all of the reviewed studies. The diagnostic criteria applied in studies may impact the results by changing the study population (Kielinen et al., 2000).

Selection of comparison groups is also crucial when exploring differences between populations. Two papers (Adams et al., 2008; Holmes et al., 2003) used age-matched controls, while only Holmes et al. employed gender matching. Gender ratios in cases deviated from the 4:1 male: female ratio expected to exist in the base population with ASD, and all studies reported using imbalanced case: control ratios. None of the studies controlled for pica, although persons presenting with this condition have been shown to be at higher risk of exposure to heavy metals (Rutter & Taylor, 2002). A common methodological flaw in vaccine studies was observed: so-called "controls" are not controls in the scientific sense. In terms of postnatal

vaccines, “controls” were most commonly those who were not receiving vaccinations known to contain thimerosal, but still received some level of vaccination (Hviid et al., 2003). Studies looking at vaccination during pregnancy selected controls that did not receive any vaccination.

Sample sizes were generally small with cases varying between 16 and 425 participants, and controls between 11 and 733 participants. Thus, some of the studies may not have had sufficient statistical power to detect differences between cases and controls, and this may explain observed discrepancies.

The review identified only three studies that could establish mercury exposure prior to the clinical onset of symptoms typical of ASD (Croen, Matevia, Yoshida, & Grether, 2008; Holmes et al., 2003; Miles & Takahashi, 2007) by investigating potential sources of exposure limited to the prenatal period.

Discussion

It remains clear that evidence is lacking in the case of mercury-induced ASD pathologies. More stringent methods of study conduction need to be established and strictly adhered to before claims regarding any potential role of the heavy metal in the clinical onset of ASD may be made. The strength and the consistency of the observed association must be considered when examining causality (Hill, 1965). The reviewed studies failed to establish both of these criteria. Consequently, it is impossible to confirm or nullify the hypothesis of an aetiological link between ASD and exposure to mercury without addressing these methodological flaws.

Future research should assess mercury exposure using methods that allow for the investigation of the timing between exposure and the onset of symptoms. In addition, given the high variability in the levels of the exposure and the expected diversity in genetic susceptibility to ASD, larger sample sizes are needed to detect such associations with adequate power. Alternatively, statistical modelling using data from existing databases on chemical and heavy metal concentrations in the air, water or soil during participants’ *in utero* development and early childhood offers an inexpensive

approach for the identification of populations which appear to be at high risk for ASD due to mercury exposure. Ideally, such databases would capture mercury exposure from multiple sources (Adgate et al., 2004; Sax, Bennett, Chillrud, Kinney, & Spengler, 2004). Well-characterized cases and matched controls could then be sampled from these populations so as to measure mercury in calcified tissues.

In addition to temporality and power issues, consideration needs to be given to case definition. An independent diagnostic assessment is advised to better describe the sample and exclude children who do not meet diagnostic criteria when assessed using best practice guidelines. However, confirming diagnoses using a standardized approach represents many challenges for researchers, and might not be feasible. A confirmed diagnosis of DSM IV autism by a paediatric neurologist or developmental paediatrician is the most feasible procedure, unless one is part of an extensive clinical team that also does research. Similarly, sibling and relative controls should be used with caution, as genetic vulnerabilities are already known to be involved in the aetiology of ASD. Studies on siblings may still be highly relevant, but comparison groups should either be exclusively composed of siblings or of unrelated children.

Complex interactions between genetic and environmental factors are now considered to be involved in the aetiology of ASD. As a consequence, future research investigating these underlying interactions is strongly recommended. None of the reviewed articles considered genetic factors in their analyses. The Childhood Autism Risks from Genetics and the Environment study (CHARGE) (Hertz-Picciotto et al., 2006) is an ongoing population-based study collecting information on 1,000 to 2,000 cases and controls. Information is collected about the children’s social, intellectual and behavioural development, their exposure to chemicals in the environment at home and elsewhere, their medical history, their diet and other aspects of their lives, before and after birth. Findings from this investigation are promising to shed light on the aetiology of ASD.

In conclusion, the risk of developing and being diagnosed with an ASD as a result of mercury exposure remains unclear because of methodo-

logical flaws in the studies conducted to date. Rigorous research is needed to provide adequate information to families, clinicians and decision-makers. They must be provided with up-to-date, critically appraised information to help them make informed decisions. One additional issue that one might consider addressing is whether the public should really wait until hazardous effects of a potentially toxic substance are proven beyond a doubt before one should make the effort to reduce mercury intake or exposure, especially for babies and young children, and pregnant or nursing mothers.

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Key Messages From This Article

People with disabilities: We don't know yet what causes autism spectrum disorders (ASDs). Exposure to pollutants, in particular mercury, could be one of the causes, but the research to-date is too weak to demonstrate a link.

Professionals and policymakers: Being well-informed on the current evidence on the aetiology of autism spectrum disorder (ASD) is crucial to adequately answer questions from persons with ASD and their parents, and to develop policies to prevent the exposure to causal agents. This paper provides information to improve critical thinking towards published research of the link between ASD and exposure to mercury.

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