Development of Reporting Guidelines for Systematic Review for Environmental Epidemiology Studies

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Abstract

Systematic review is a type of review that identifies, assesses, and combines all the published empirical evidence on a specific topic by using explicit, systematic methods. This type of reviews often includes a meta-analysis, a statistical tool used to combine the collected data into a quantitative summary estimate. Guidance documents such as the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) were developed to improve the reporting of systematic reviews of randomized controlled trials. Systematic reviews are commonly used in the field of healthcare research and are increasingly being employed in the field of environmental epidemiology. Environmental epidemiology studies examine exposures in populations and their associations with health outcomes. This field contains several unique considerations that require a careful and critical assessment to ensure the validity of results and to make the data or information more useful for the readers. However, to our knowledge, there is currently no guidance document for conducting systematic reviews that comprehensively addresses the specific issues in this field. Therefore, the objectives of this proposal are twofold: (1) to conduct a systematic review of the currently published epidemiology systematic reviews on a specific topic (mercury exposure and children autism spectrum disorder) to identify analytical issues encountered, and (2) use the experience and other potential solution identified in the literature to develop a guidance or recommendation document for conducting systematic reviews of environmental epidemiology studies. Akin to reporting guidelines for randomized controlled trials, a reporting guideline for environmental epidemiology is anticipated to increase the clarity and transparency of publications and enhance the usefulness of systematic reviews for knowledge synthesis.

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List of Abbreviations

ADD	Attention Deficit Disorder
ADHD	Attention Deficit Hyperactivity Disorder
AMSTAR-2	A MeaSurement Tool to Assess systematic Reviews ver.2
ASD	Autism Spectrum Disorder
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th Edition
Hg	Mercury
IQR	Interquartile range
MeHg	Methyl-mercury
MD	Mean difference
MOOSE	Meta-analyses Of Observational Studies in Epidemiology
NDD	Neurodevelopmental disorder
OR	Odds ratio
PDD	Pervasive Developmental Disorder
PICOS	Patient/Population/Problem, Intervention/exposure, Comparison/Control, Outcome, Study type
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RoB	Risk of Bias
SMD	Standardized Mean Difference
TCV	Thimerosal-containing vaccine
TD	Tic Disorder

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Chapter 1: Introduction

1.1 General Introduction

The main purpose of a systematic review is to provide a high level of evidence for clinical decision-makers or policymakers (Aromataris et al., 2015; Sofaer & Strech, 2012). With the overwhelming amount of published research evidence, systematic reviews were developed to give access to the decision-makers a summary of evidence that is maximally informed and minimally biased and ready to be applied to their decision-making.

Systematic reviews often include a statistical analysis of the primary data, called a meta-analysis. Meta-analysis is the pooling of quantitative results from individual studies to arrive at summary estimates. It is commonly used in healthcare research to pool the results of randomized controlled trials. Methods have also been developed to pool other types of study designs, such as observational studies and indirect comparisons (Higgins & Green, 2011; Mills et al., 2013). Meta-analysis is increasingly being employed in reviews of occupational and environmental contaminant exposures and health outcomes (Chen et al., 2016; Yoshimasu et al., 2014; Zani et al., 2017; Zhang et al., 2015). Blair et al. developed a set of guidelines for meta-analysis in environmental epidemiology. They provided recommendations for study identification and selection, data extraction, data analysis (addressing heterogeneity, exposure characterization, study quality, and aggregation of study outcomes), and presentation/interpretation of results (Blair et al., 1995). Sheehan et al. evaluated methods and reporting of environmental epidemiology meta-analyses according to the guidance of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), MOOSE (Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies), and the recommendations of Blair et al. (Sheehan & Lam, 2015). They

found that although most studies adhered to the guidelines, weaknesses were present in specific areas such as study selection, data extraction, and exposure heterogeneity. The authors recommended that guidelines specific to environmental health/epidemiology be developed (Sheehan & Lam, 2015). Environmental epidemiology examines exposures in populations and their associations with health outcomes. The field contains several unique considerations that require a careful and critical assessment to judge the validity of results. For example, the data are often non-normally distributed, co-exposures are usually present, and different matrices and/or biomarkers may be used to measure exposure. Many studies also fail to report key pieces of information and data. Considerable heterogeneity also exists in methodologies, even among studies in the same topic area. Studies may present exposures in different ways (i.e., continuous, dichotomous, tertiles, quantiles), adjust for different covariates, use untransformed or transformed data, and contain reference groups with non-comparable exposure levels. Such inconsistencies present challenges in assessing the validity of results and combining studies in meta-analyses.

A reporting guideline for environmental epidemiology is anticipated to increase the clarity and transparency of publications and facilitate the conduct of systematic reviews and meta-analyses. However, to our knowledge, there is currently no guidance document for conducting meta-analysis that comprehensively addresses these issues in this field. Although Blair et al. provide some direction for conducting environmental health meta-analysis, recommendations specific to the analytical issues commonly encountered, such as the best methods to handle detection limits and non-linear dose-response effects, are absent.

1.2 ASD (Autism Spectrum Disorder) and Hg exposure

Methylmercury (MeHg) was first identified as a developmental neurotoxicant during the Minamata Bay disaster in 1956 where methylmercury from industrial wastewater was released into Minamata Bay, Japan; children who were born from mothers who consumed fish from the contaminated waters demonstrated mental retardation and other symptoms such as blindness, muscle weakness and paralysis (Harada, 1995). Its neurotoxicity is most prominent in the developing nervous system (Andersen et al., 2000; Snoj Tratnik et al., 2017) because, in comparison to adults, children have heavier exposures to environmental toxicants in relation to their body weight (Ruggieri et al., 2017; Selevan, Kimmel, & Medola, 2004). In addition, infants are at a higher risk of neurological disorders because of their physiological immaturity in homeostasis and detoxification mechanisms while having a very efficient gastrointestinal absorption (Ruggieri et al., 2017). A study of blood and hair mercury levels in pregnant women with maternal fish consumption during pregnancy showed that the maternal blood and hair mercury levels decreased during the second and third trimesters of pregnancy, but the cord blood mercury level was significantly higher than maternal blood at birth (Morrissette et al., 2004; Garrecht and Austin, 2011). These study results may suggest that increased maternal fish consumption during pregnancy can lead to higher fetal mercury accumulation; decrease in maternal blood and hair mercury levels in accordance to increase in cord blood mercury levels may suggest that there is an active placental transfer of mercury to the fetus (Morrissette et al., 2004). Moreover, this study shows that the critical window of vulnerability to mercury exposure may be during the last three months of pregnancy (last two trimesters), which is when there was a significant increase in placental transfer of mercury. Studies have proven that mercury exposure in high- and lose-doses targets the central nervous system (CNS) and cause significant and long-

term neurological damage (Johansson et al., 2007; Zahir et al., 2005). The mechanisms underlying mercury neurotoxicity are still unknown. However, studies have shown that mercury is able to cross the blood brain and placental barriers and induce reactive oxygen species (ROS) production and oxidative stress (Shenker et al., 2002).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that includes impairments in language, communication skills, and social interactions combined with restricted and repetitive behaviours, interests or activities (National Institute of Mental Health, 2018; Ofner et al., 2018). ASD consists of a variety of mental disorders that differ in type and severity of symptoms; these conditions include autistic disorder, Asperger's syndrome, and pervasive developmental disorder (PDD) (National Institute of Mental Health, 2018). Based on numerous twin studies, ASD is known to be a disorder with strong genetic components; concordance rates for autism are 90% in monozygotic twins and 10% in dizygotic twins (Folstein & Rosen-Sheidley, 2001; Ritvo et al., 1985; Rutter, 2000). However, there are 1-2% of the ASD cases not related to any genetic mutations, which suggests other factors such as environmental factors can play a role (Abrahams & Geschwind, 2008). The mechanism behind ASD are still unknown but studies on oxidative stress in autism has demonstrated that some of autistic children show elevated oxidative stress (Garrecht & Austin, 2011). Studies on GST (glutathione-S-transferase) genes have demonstrated that polymorphisms in these genes is associated with an increased sensitivity to mercury and may be linked to autism (James et al., 2006; T. A. Williams et al., 2007). GST enzymes are responsible for the detoxification of the toxic by-products of oxidative stress and mercury and mercury damage; polymorphism of the GST genes will impact the GST enzyme functions (Garrecht & Austin, 2011; Hayes et al., 2005).

Even though there are many systematic reviews published on the relationship between mercury exposure and autism, the evidence is still inconsistent. Therefore, we have decided to conduct a systematic review of systematic reviews to identify the analytical issues of the published systematic reviews.

1.3 Research Objectives

The objective of this project is to develop a guidance document for conducting meta-analyses of environmental epidemiology studies. The guidance document will make use of meta-analysis case examples to illustrate analytical issues and potential solutions. The objective will be addressed through the following research questions, each question corresponding to a thesis chapter:

1) What are the common analytical issues and challenges faced in conducting meta-analysis in the field of environmental epidemiology?

2) How should systematic reviews be conducted in the field of environmental epidemiology in order to facilitate the process and address the common challenges faced in this field?

Each research questions will be addressed through the following specific objectives:

1) Conduct a systematic review of the currently published systematic reviews on mercury exposure and the association with ASD (autism spectrum disorder) to identify the types of challenges encountered during the meta-analysis process.

2) Develop a guidance document of recommendations for improving the quality of systematic reviews of environmental epidemiology studies.

The research conducted for each objective is presented in a manuscript format in Chapters 2, and 3. The Final Chapter presents the summary findings and the conclusion of the thesis.

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Chapter 2: Environmental mercury exposure and autism spectrum disorder: a systematic review of systematic reviews.

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Contribution Statement

KJL was responsible for the conception and design of the study, data acquisition, analysis and interpretation, and drafting and revising the manuscript. XFH and HMC were responsible for funding requisition, supervision of data acquisition and analysis, result interpretation, and revising the manuscript.

Abstract

Background: The link between Autism Spectrum Disorder (ASD) and mercury exposure is controversial.

Objectives: 1) To review the published systematic reviews on mercury exposure and ASD in children; 2) To present a summary of the meta-analysis of the findings; 3) To conduct a meta-analysis and meta-regression on the primary data reported in the review papers.

Methods: Two databases, PubMed and Scopus, were searched for eligible reviews according to the inclusion criteria developed *a priori*. AMSTAR-2 score was used to assess the quality of each systematic review. Data were extracted from case-control studies included in the reviews, and mercury exposure levels were converted to hair mercury concentrations. Standardized mean difference (SMD) in hair mercury concentration was calculated between ASD patients and

neurotypical children. Subgroup analyses were performed based on the continents where the study was conducted, the age of the patients, and the type of ASD.

Results: A total of 10 review papers were included (7 systematic review papers and three non-systematic review papers). The association between mercury exposure and risk of ASD in children was not consistent across different biomarkers. A total of 39 original studies (case-control studies) with 4759 participants were extracted to be included in the meta-analysis. The calculated SMD was 0.30 (-0.05, 0.65), suggesting that hair mercury concentration was not significantly different between the ASD patients and the control group. Moreover, the subgroup analysis showed that there was a significant difference between hair mercury concentrations between ASD patients and neurotypical controls in Asia and Africa but not in America and Europe. There was no significant difference between younger (age 0-6 years) and older (age 7 or older) age groups. Similarly, subgroup analysis on different labelling of ASD types (ASD vs. autism) showed no significant difference. Meta-regression shows that there were no significant differences observed between SMD values of different studies despite the different levels of hair mercury levels in controls.

Conclusions*:* There was no consistent relationship between mercury exposure and ASD from case-control studies. More researchneeded to investigate the potential regional difference.

2.1. Introduction/Background:

Mercury (Hg) is a toxic metal found in air, water and soil, and it is distributed throughout the environment by both natural and anthropogenic processes (UNEP & WHO, 2008). There are three principal forms of mercury that affect human health: elemental mercury, inorganic mercury, and organic mercury (methylmercury). Industrial workers at gold mines and mercury mines and

other occupations such as dentists are exposed to inorganic mercury in their workplace (Park & Zheng, 2012; UNEP & WHO, 2008). Dental fillings made with mercury amalgam can also be a source of inorganic mercury to the general population (UNEP, 2018; UNEP & WHO, 2008). Seafood consumption is the main source of MeHg (methylmercury) exposure in the populations worldwide (NRC, 2000; UNEP, 2018). MeHg bioaccumulates through the food chain as predator organisms such as plants and animals (especially aquatic animals such as fish and crustaceans) absorb the contaminants that their food sources (small organisms) absorbed from the environment. Its neurotoxicity is most prominent in the developing nervous system (Andersen et al., 2000; Snoj Tratnik et al., 2017) because, in comparison to adults, children are more vulnerable to environmental hazards for they have excessively heavier exposures to environmental toxicants (Ruggieri et al., 2017; Selevan et al., 2004).

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that includes impairments in language, communication skills, and social interactions combined with restricted and repetitive behaviours, interests or activities (National Institute of Mental Health, 2018; Ofner et al., 2018). It is often recognized in the first two years of age and, therefore, is labelled as a developmental disorder. ASD consists of a variety of mental disorders that differ in type and severity of symptoms; these conditions include autistic disorder, Asperger's syndrome, and pervasive developmental disorder (PDD) (National Institute of Mental Health, 2018). Fifty-two million cases of ASDs were estimated in 2010, which is equivalent to the prevalence rate of 7.6 per 1000 or one in 132 persons. A recent global burden of disease report for ASD reported that autistic disorders accounted for more than 58 DALYs per 100 000 population globally, while other ASDs accounted for 53 DALYs per 100 000 (Baxter et al., 2015). ASD prevalence estimate in the United States for the year 2000 was 6.7 per 1000 children, and it has more than doubled to 14.7

per 1000 children in 2010 (Boat & Wu, 2015). National Autism Spectrum Disorder Surveillance System (NASS) of Canada has reported a combined prevalence of 15.2 per 1000 children and youth aged 5-17 for ASD diagnosis in 2015 across the seven provinces and territories (PEI, Yukon, Newfoundland and Labrador, Nova Scotia, New Brunswick, Quebec, British Columbia) (Ofner et al., 2018). One review paper reported the prevalence of ASD worldwide estimates to a median of 62 per 10 000 children (Elsabbagh et al., 2012). Research suggests that the cause of ASD can be a combination of genetic and environmental factors; however, genetic inheritance may only have 30-40% attribution to ASD (Landrigan et al., 2012). There is an increasing level of evidence on the association between neurodevelopmental disorders such as ASDs environmental factors such as toxic chemicals. Toxic chemicals can directly injure the developing brain or interact with genes that confer susceptibility and indirectly affect the brain function (Karimi et al., 2017; Landrigan et al., 2012). Studies have shown that MeHg exposure can interfere with the important brain development processes such as the function of cytoskeleton elements that transports the neuronal cells from the germination site to the site of function, the formation of dendrites and axons and the induction of apoptosis (Antunes dos Santos et al., 2016; Kunimoto & Suzuki, 1997; Miura et al., 2000; K. Nagashima et al., 1995; Kazuo Nagashima, 1997). A review of epidemiology studies identified industrial chemicals, including MeHg as developmental neurotoxicants (Grandjean & Landrigan, 2006). Furthermore, mercury has been suspected of causing autism in the past due to a suspected link with vaccinations containing thimerosal (an organic compound that contains ethylmercury) as a preservative in a number of biological and drug products including many vaccines used on children since the 1930s (Baker, 2008). However, experts alike have disproven the link between thimerosal-containing-vaccines (TCVs) and increasing risk for ASD through

numerous epidemiological studies and have proven that thimerosal for use in vaccines is safe (Price et al., 2010; Schultz, 2010).

Numerous epidemiology studies analyzed the Hg concentrations within biomarkers of ASD patients and compared them with neurotypical controls. However, these primary studies present inconsistent findings. These discrepancies may be due to the heterogeneous nature of ASD etiology but also from the differences in methodology between the studies. The inconsistent findings makes it difficult for the readers to derive clear conclusions from the literature. To overcome these inconsistencies and summarize the findings, systematic reviews have been conducted by scientists. However, due to differences in scope and methodological quality of the systematic reviews, readers are now faced with a multitude of systematic reviews with conflicting findings. It is now necessary to conduct a systematic review of the systematic reviews to summarize and analyze the known evidence reported by the systematic reviews to address the inconsistencies in their findings and identify the limitations of the previous reviews. Systematic review on systematic reviews, also known as umbrella reviews, compare and compile the findings of separate reviews of different levels (i.e. different route of exposure and population) to provide an overall picture of the findings and assess the quality of each systematic review (study eligibility, risk of bias of included studies, etc.) so that the readers can access the best evidence on a specific topic (Aromataris et al., 2015; Smith et al., 2011).

The objectives of this systematic review of systematic reviews are: 1) To review the systematic reviews on mercury exposure and ASD in children; 2) To summarize the meta-analysis findings and the trends seen in the review papers, 3) To conduct a meta-analysis and meta-regression on the primary data reported in the included review papers.

2.2 Methods:

This systematic review was done following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Moher et al., 2009) as a guide, in combination with the guide to conducting systematic reviews of systematic reviews (Smith et al., 2011). As defined in the Diagnostic and Statistical Manual of Mental Disorders (4th Edition) (Bell, 1994), ASDs include autistic disorder (299.00); Asperger's disorder (299.80); pervasive developmental disorders not otherwise specified, including atypical autism (PDD-NOS) (299.80); Rett's disorder (299.80); and childhood disintegrative disorder (299.10). In our systematic review, we defined "ASD" as a disorder that is synonymous with "autism," "Asperger's syndrome" and "pervasive developmental disorder (PDD)", and we labelled all three as "ASD" in our review.

Therefore, the included systematic reviews and literature reviews in our study presented data on any of these four disorder "names".

2.2.1 Literature Search and Study Selection

A literature search was done in April 2019 on two databases, PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and Scopus (https://www.scopus.com/search/form.uri?display=basic), in order to find all published systematic reviews on the relationship between mercury exposure and ASD. The search protocol used for PubMed was:

(("mercury" OR "methylmercury" OR "mercury exposure")) AND ("neurodevelopment" OR "neurotoxicity" OR "neuropsychological" OR "neurobehavioral" OR "neurobehavioural" OR "cognitive development" OR "autism" OR "ASD" OR "autism spectrum disorder")

This search was then limited to study type: reviews.

The search protocol used for Scopus was:

(TITLE-ABS-KEY ("mercury" OR "methylmercury" OR "mercury exposure") AND TITLE-ABS-KEY ("neurodevelopment" OR "neurotoxicity" OR "neuropsychological" OR "neurobehavioral" OR "neurobehavioural" OR "cognitive development" OR "autism" OR "ASD" OR "autism spectrum disorder")) AND DOCTYPE (re)

We adopted a broad searching strategy for health outcomes to increase sensitivity because autism or ASD can be reported in studies with other neurodevelopmental or neurotoxic health conditions. The search period was limited to the period of January 2000 through April 2019. The reference lists of selected papers were also searched for additional eligible papers. Four papers were included in this process (De Palma et al., 2012; Karagas et al., 2012; Lam et al., 2016; Taylor et al., 2014) and two of these review papers (De Palma et al., 2012; Taylor et al., 2014) was included in our systematic review. **Figure A1** of the **Appendix** shows the flow diagram of the literature search.

An eligibility criteria list was made beforehand. Only systematic reviews that used epidemiological studies and evidence to conduct qualitative and quantitative analyses were included (both with and without meta-analysis); narrative reviews that did not show specific data were not included. Studies that study a mixture of metal compounds were excluded. Moreover, systematic reviews that focused solely on the metabolic processes of mercury were also excluded from this study. The main exposure type included was biomarker measurements of Hg. I included review papers that look at infants, children or adolescents only (<20 years).

For the meta-analysis, a second literature search was performed on two databases, PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) and Scopus (https://www.scopus.com/search/form.uri?display=basic), to find all published original studies (case-control studies) on the relationship between mercury exposure and ASD. The search protocol used for PubMed was:

((("mercury" [Mesh]) OR "mercury compounds" [Mesh]) OR "Mercury isotopes" [Mesh]) AND ("Autism" OR "Autistic Disorder"[Mesh] OR "Autism Spectrum Disorder"[Mesh])

The search period was limited to the period of January 2016 through 2020 since the most recent original studies from the meta-analysis papers was published in 2017. The search strategy was adopted from Jafari et al., 2017.

The literature searches were carried out by one reviewer (K.L.). Duplicates were removed before the screening of the studies can begin. The parallel screening of the extracted literature was done by two reviewers (K.L. and X.F.H.). Primary screening of the literature involved screening of the title and abstracts from the literature search against the eligibility criteria. Then secondary screening was carried out by obtaining the full text of all titles included in the first screening. Full text reports were screened to decide whether they meet the inclusion criteria. Through discussion and consensus, final list of included studies was decided. Although we were looking for only systematic reviews, due to small number of systematic reviews published in this field, we also included some non-systematic review papers that reported relevant primary data that can be included in the meta-analysis (Geier et al., 2015; Kern et al., 2016; Sharma et al., 2019).

2.2.2 Data Extraction and Quality Assessment

Two authors (K.L. and X.F.H.) collected the following data from each review paper: objective of the review, health outcomes of interest (the type of ASD outcomes), number of included studies, type of included studies (i.e. case-control), number of samples, demographics of the population (location, age, sex distribution, etc.), mercury exposure biomarker, exposure levels, study results (meta-analysis findings) and other potential confounders that can be accounted for in the quantitative analysis. The methodological quality of each systematic review was assessed using the AMSTAR (A MeaSurement Tool to Assess systematic Reviews)-2 score (Shea et al., 2017),

which consists of 16 items. The final grades of each systematic review were based on the overall score out of 16 (lowest score one study can get is 0 and the highest score would be 16); a study was ranked either "high" for score of 12 or higher, "medium" for score of 6-11 or "low" for score of 5 or lower. Each reviewer independently assessed the quality of each systematic review and via discussion and consensus, final scores of the review papers were finalized.

2.2.3 Statistical Analysis

Out of the systematic reviews that we included, we selected the ones with meta-analysis and extracted the meta-analysis results such as standard mean difference (SMD) and odds ratios (OR) for summary purposes. For systematic review papers that do not have meta-analysis and non-systematic reviews, the reported data that is relevant was summarized in **Table 2** to see the trend in the conclusions of each review paper. We then extracted the original studies (case-control studies, in specific) from each of the included reviews. To ensure all the primary data are up to date, we also conducted a literature search on the recent primary studies that also looked at the relationship between mercury exposure and ASD. We then combined all the extracted data to perform our own meta-analysis at a larger scale.

For studies that reported effect sizes on multiple biomarkers, we used the data for the most appropriate biomarker; the order of most preferred to least preferred biomarkers of MeHg was hair > blood > urine. This order was chosen because most studies measured mercury in hair or blood, and hair mercury most accurately reflects long-term exposure (Ye et al., 2016). In the end, we converted all the exposure measurements into equivalent levels of mercury that would be found in hair samples for the data to be comparable. For conversion of blood mercury levels (μg/L) into hair mercury μg/g), we used the conversion ratio of 250 (Clarkson and Magos, 2006).

$$Hair\ Hg\ \left(\frac{\mu g}{g}\right) = Blood\ Hg\ \left(\frac{\mu g}{L}\right) \times \frac{250}{1000}$$

As for conversion of urine mercury (µg/L) into hair mercury µg/g), we used the regression model developed by Ohno et al., 2007.

$$Hair\ Hg\ \left(\frac{\mu g}{g}\right) = \frac{urine\ Hg\ \left(\frac{\mu g}{g}\ creatinine\right) + 0.009}{0.57}$$

We estimated the SMD (standard mean difference) and 95% confidence interval (CI) of mean hair mercury levels between cases (ASD patients) and controls (neurotypical individuals). SMDs were then presented in a forest plot. All statistical analyses were performed using Stata software, version 16.0 (StataCorp, College Station, TX, USA).

2.3. Results

2.3.1 Study Characteristics

We included ten reviews for our review; seven of these papers were systematic reviews (De Palma et al., 2012; Jafari et al., 2017; Parker et al., 2004; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017; Taylor et al., 2014; Yoshimasu et al., 2014) and 3 of these papers were non-systematic reviews (Geier et al., 2015; Kern et al., 2016; Sharma et al., 2019) that reported relevant data. All reviews were published between 2004 and 2019. Five of the systematic review papers included meta-analysis (De Palma et al., 2012; Jafari et al., 2017; Saghazadeh & Rezaei, 2017; Taylor et al., 2014; Yoshimasu et al., 2014). Our included review papers covered data from 136 original studies in total, excluding the overlapping studies (**Table 1**). According to the AMSTAR-2 score, 4/7 systematic review papers (Jafari et al., 2017; Saghazadeh & Rezaei, 2017; Taylor et al., 2014; Yoshimasu et al., 2014) were classified as high-quality studies (score ≥ 12) and 2/7 studies (De Palma et al., 2012; Parker et al., 2004) were classified as medium-quality studies

(score 6-11) and 1/7 study (Rossignol et al., 2014)was ranked as low-quality study (score ≤ 5). The detailed scores can be seen in the **AMSTAR-2 (Shea et al., 2017) Score** of the **Appendix**.

Table 1. Summary of included review papers

Type of study	Number of papers	Number of primary literature (total)	Number of primary literature (net)
Systematic review without meta-analysis	2[1]	41	41
Systematic review with meta-analysis	5[2]	119	75
Non-systematic review	3[3]	140	108
NET	**10**	**300**	**136**

[1] Rossignol et al., 2014; Parker et al., 2004

[2] Yoshimasu et al., 2014; Taylor et al., 2014; Saghazadeh and Rezaei, 2017; Jafari et al., 2017; De Palma et al., 2014

[3] Sharma et al., 2019; Kern et al., 2016; Geier et al., 2015

All systematic reviews conducted their searches on PubMed, three reviews used Web of Science databases (Jafari et al., 2017; Rossignolet al., 2014; Yoshimasu et al., 2014). Three systematic reviews and two literature reviews searched Google Scholar (Jafari et al., 2017; Kern et al., 2016; Rossignol et al., 2014; Sharma et al., 2019; Taylor et al., 2014). Other databases used included PsychoINFO (Rossignol et al., 2014; Yoshimasu et al., 2014), EMBASE (Rossignol et al., 2014; Taylor et al., 2014), Medline (Parker et al., 2004; Taylor et al., 2014), the Cochrane library (Jafari et al., 2017; Parker et al., 2004), OvidSP (Yoshimasu et al., 2014), MagIran (Jafari et al., 2017), CINAHL, ERIC, AMED (Rossignol et al., 2014), Web of Knowledge, ScienceDirect and ResearchGate (Sharma et al., 2019).

Age ranges of the participants in the included reviews varied slightly. Four systematic reviews and three literature reviews included children aged 0-17 years old diagnosed with ASD by a health clinician (clinical diagnosis) (De Palma et al., 2012; Geier et al., 2015; Kern et al., 2016; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017; Sharma et al., 2019; Taylor et al., 2014), while one systematic review included children aged 1.4-23.5 years diagnosed with ASD by a health clinician (clinical diagnosis) (Yoshimasu et al., 2014). All reviews provided outcome data relating to the comparison between ASD patients vs. neurotypical individuals. Some reviews included data for other neurodevelopmental disorders (NDDs) such as motor and behavioural problems and ADHD (Geier et al., 2015; Parker et al., 2004; Sharma et al., 2019). All included systematic reviews provided data on outcomes diagnosed clinically. The types of diagnostic tests used by the clinicians varied among the primary studies, and they are listed in **Table A2 of the Appendix**. The most common method of diagnosis used was DSM IV (Diagnostic and Statistical manual of Mental Disorders 4th Edition).

Five review papers examined inorganic mercury exposure via TCV (thimerosal containing vaccines)(Geier et al., 2015; Kern et al., 2016; Parker et al., 2004; Taylor et al., 2014; Yoshimasu et al., 2014). There were 25 original studies on the relationship between TCV exposure and ASD diagnosis; the type of TCVs included DTaP vaccines and Hepatitis B vaccines vaccinated during the first month of life. Except for one study (Geier et al., 2015), the review papers concluded that there was no relationship between ASD and thimerosal (ethylmercury) exposure on children. Two meta-analysis papers calculated odds ratio of the relationship between inorganic mercury exposure via TCVs and ASD in exposed children; Yoshimasu et al., 2014 reported OR = 0.99 (0.90, 1.08, p = 0.*180*) and Taylor et al., 2014 reported OR = 1.00 (0.93, 1.07, p = 0.92) (**Table 2**).

Two review papers looked at inorganic mercury exposure via air pollution (Kern et al., 2016; Yoshimasu et al., 2014). There were nine original studies included in these two reviews. Both review papers reported a positive relationship between mercury exposure via air pollution and ASD diagnosis. None of the review papers included a meta-analysis.

The rest of the review papers observed the relationship between mercury levels in biomarkers and ASD; the type of biomarkers measured included mercury in hair, urine, blood, erythrocytes, teeth, serum and brain tissues of the ASD patients or maternal biomarkers such as maternal blood, maternal hair, cord blood, and breast milk. Some reviews included other chemicals such as lead and cadmium (De Palma et al., 2012; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017). However, for the purpose of our review, we only reviewed primary data on the relationship between mercury and ASD. Some of the these review papers included original studies on other types of neurodevelopmental disorders (ADHD, ADD, Tic disorder (TD), and developmental/speech/language delay) (Geier et al., 2015; Parker et al., 2004; Sharma et al., 2019; Yoshimasu et al., 2014). De Palma et al.(2012) included a meta-analysis along with a cross-sectional case-control study; this meta-analysis combined the data from other primary studies with the data of their own.

Three review papers investigated mercury level in the red blood cell (RBC; erythrocyte) of ASD patients (Jafari et al., 2017; Kern et al., 2016; Saghazadeh & Rezaei, 2017). They included results from five original studies. All three review papers reported a positive relationship between ASD and RBC mercury levels. Two of these review papers included meta-analyses (Jafari et al., 2017; Saghazadeh & Rezaei, 2017) and compared the RBC mercury levels between ASD patients and neurotypical controls. Saghazadeh and Rezaei reported standardized mean difference (SMD = 1.562 (0.420-2.704)) and Jafari et al. reported a mean difference (MD = 0.43 µg/L (0.12, 0.74));

both demonstrated that the RBC mercury level in ASD patients is significantly higher than the RBC mercury level in neurotypical individuals.

Five review papers studied the hair mercury level of patients versus the neurtypical control groups (De Palma et al., 2012; Jafari et al., 2017; Kern et al., 2016; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017). They included results from 24 original studies. 2/5 review papers did not include a meta-analysis (Kern et al., 2016; Rossignol et al., 2014b) and they acknowledged that the conclusions found in the original studies on the relationship between hair mercury and ASD were divergent and therefore the relationship is inconsistent. 3/5 review papers included meta-analysis (De Palma et al., 2012; Jafari et al., 2017; Saghazadeh & Rezaei, 2017) and compared the RBC mercury levels between ASD patients and neurotypical controls and each concluded on a different type of relationships. Saghazadeh and Rezaei reported SMD = 0.082 (-0.201, 0.364) and concluded that the relationship is inconsistent. Meanwhile, De Palma et al. reported SMD = 0.086 (-0.318, 0.49) and concluded that there is no relationship between ASD and hair mercury levels. Jafari et al. reported MD = -0.14 mg/g (-0.28, -0.01) and concluded that the relationship is negative.

Five review papers studied blood mercury levels (Jafari et al., 2017; Kern et al., 2016; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017; Sharma et al., 2019). There were 17 original studies included. 3/5 review papers did not include a meta-analysis component (Rossignol et al., 2014; Sharma et al., 2019; Kern et al., 2016) and stated that the relationship is inconsistent or there is no relationship. Saghazadeh and Rezaei calculated the SMD between blood mercury levels in ASD cases and neurotypical controls (SMD = 0.193 (-0.278, 0.664)) and concluded that there is no relationship between ASD and blood mercury. Jafari et al. calculated the MD between blood

mercury levels in cases and controls (MD = 0.43 µg/L (0.12, 0.74)) and concluded that higher blood mercury levels have a moderately positive effect on the risk of ASD diagnosis.

Four review papers looked at mercury levels in urine (Jafari et al., 2017; Kern et al., 2016; Rossignol et al., 2014b; Saghazadeh & Rezaei, 2017). There were nine original studies included. 2/4 review papers did not have a meta-analysis component (Kern et al., 2016; Rossignol et al., 2014) and concluded that the findings of the primary literature are inconsistent. Saghazadeh and Rezaei calculated the SMD between blood mercury levels in ASD cases, and neurotypical controls (SMD = 0.125 (-0.114, 0.364)) and Jafari et al. calculated the MD between blood mercury levels in cases and controls (MD = 0.51 mg/g creatinine (-0.14, 1.16)). Both meta-analysis papers reported that there is no relationship between urine mercury levels and ASD diagnosis.

Table 2. Characteristics of the included reviews

Review Paper	Type of Review	Outcome (method or process of diagnosis)	Age	Number of primary literature	Dose/Exposure	Relationship
Yoshimasu et al., 2014	Meta-analysis	ASD*/autism (Clinical diagnosis)	1.4-23.5 yrs	20	Ethylmercury (thimerosal vaccines)	NR* OR* = 0.99 (0.90, 1.08, p = 0.180)
		ASD (Clinical diagnosis)			Inorganic environmental mercury (Air pollution)	Positive OR = 1.66, (1.14-2.17, p = 0.27)
Taylor et al., 2014	Meta-analysis	Autism, ASD (Clinical diagnosis)	0-11 yrs	10	Ethylmercury (thimerosal vaccines)	NR OR = 1.00 (0.93, 1.07, p = 0.92)
Saghazadeh and Rezaei, 2017	Meta-analysis	ASD/autism (Clinical diagnosis)	0-14 yrs	38	Erythrocyte Hg	Positive SMD* = 1.562 (0.420-2.704) (p = 0.007)
					Hair Hg	Inconsistent SMD = 0.082 (-0.201, 0.364) (p = 0.570)
					Blood Hg	NR SMD = 0.193 (-0.278, 0.664) (p = 0.422)
					Urine Hg	NR SMD = 0.125 (-0.114, 0.364) (p = 0.306)
Jafari et al., 2017	Meta-analysis	ASD/autism (Clinical diagnosis)	1.38-9.6 yrs	44	Hair Hg	Negative MD* = -0.14 mg/g (-0.28, -0.01) (p = 0.039)
					Urine Hg	NR

						MD = 0.51 mg/g creatinine (-0.14, 1.16) (p = 0.121)
					Whole Blood Hg	Positive MD = 0.43 µg/L (0.12, 0.74 (p = 0.007)
					Erythrocyte Hg	Positive MD = 1.61 µg/L (0.83, 2.38) (p <0.001)
					Brain Hg	Positive MD = 0.61 ng/g (0.02, 1.19) (p = 0.043)
De Palma et al., 2014	Meta-analysis	ASD/autism (Clinical diagnosis)	2-17 yrs	7	Hair Hg	NR SMD = 0.086 (-0.318, 0.49) (p = 0.831)
Rossignol et al., 2014	Systematic review	ASD/autism (Clinical diagnosis)	0-14 yrs	29	Hair Hg, Urine Hg, Blood Hg, Brain Hg, Teeth Hg	Inconsistent
Parker et al., 2004	Systematic review	ASD, NDD*s (Clinical diagnosis)	1-7 months	12	Ethylmercury (thimerosal vaccines)	NR
Sharma et al., 2019	Literature Review	Autism, motor and behavioural problems, ADHD* (Clinical diagnosis)	0-14 yrs	50	Whole blood, cord blood, breast milk	NR
Kern et al., 2016	Literature Review	ASD/autism severity (Clinical diagnosis)	0-16 yrs	70	Blood mercury, Hair Hg, Serum Hg, Urine Hg, erythrocyte Hg, ethylmercury (thimerosal vaccines), Air pollution	NR (Thimerosal) Inconsistent (tissue Hg levels)

Geier et al., 2015	Literature Review	ASD/autism, ADHD/ADD*, TD*, Developmental/speech/language delay (Clinical diagnosis)	0-14 yrs	20	Ethylmercury (thimerosal vaccines)	Positive

* ADD = Attention Deficit Disorder; ADHD = Attention Deficit Hyperactivity Disorder; ASD = Autism Spectrum Disorder; MD = Mean Difference; NDD = NeuroDevelopmental Disorder; NR = No relationship; OR = Odds Ratio; SMD = Standardized Mean Difference; TD = Tic Disorder

2.3.2 Meta-Analysis for ASD and Hair Mercury

For our meta-analysis, we extracted primary studies that look at hair mercury levels in children with ASD and neurotypical children (control) from all included review papers. The inclusion criteria for the original studies were case-control studies that provide mean mercury levels in hair, urine, or blood in ASD patients and controls. First, we extracted 33 original studies from meta-analysis papers that meet the inclusion criteria (Adams et al., 2013; Adams et al., 2006; Adams et al., 2008; Al-Ayadhi, 2005; Albizzati et al., 2012; Blaurock-Busch et al., 2011; Bradstreet et al., 2003; De Palma et al., 2012; Domingues et al., 2016; El-baz et al., 2010; Elsheshtawy et al., 2011; Hertz-Picciotto et al., 2010; Holmes et al., 2003; Ip et al., 2004; Jung et al., 2008; Kern et al., 2007; Lakshmi Priya & Geetha, 2011; Li et al., 2017; Macedoni-Lukšič et al., 2015; Majewska et al., 2010; McKean et al., 2015; Mohamed et al., 2015; Mostafa et al., 2016; Mostafa & Refai, 2007; Obrenovich et al., 2011; Skalny et al., 2017a; Skalny et al., 2017b; Stamova et al., 2011; Wecker et al., 1985; Williams et al., 2008; Woods et al., 2010; Wright et al., 2012; Yau et al., 2014). We excluded one primary study (Rahbar et al., 2013) because the study did not provide us with SD values for the mean mercury exposure measurements. We then went through the 14 primary studies from the systematic reviews (that do not overlap with the studies in meta-analysis papers) and found four studies (Fido & Al-Saad, 2005; Hodgson et al., 2014; Metwally et al., 2015; Yassa, 2014) with relevant data for our meta-analysis. Moreover, we went through the original studies included in the non-systematic reviews and did not find any non-overlapping studies. Lastly, to update our search of original studies, we conducted a literature search and found two additional studies (Gil-Hernández et al., 2020; Qin et al., 2018). The flowchart of study selection for the primary studies published 2017-2020 can be seen in **Figure**

A2-2 of the Appendix. In total, we conducted our meta-analysis on 39 individual original studies (**Figure A2-1 in the Appendix**) with 4759 participants (**Table A1 in the Appendix**).

When extracting the reported data from each study, we chose to only include one type of biomarker measurements when exposure data was reported for more than one biomarker for the same group of participants within the study. The order of preference was hair mercury > blood mercury > urine mercury. A summary of the characteristics of each included original study is presented in **Table A2 of the Appendix**.

2.3.2.1 Standardized Mean Difference

Meta-analysis on the 39 studies (45 datasets) with 4759 participants (2540 cases and 2219 controls) (see **Table A1** of the **Appendix**) showed that the hair mercury concentration in autistic children is not significantly different compared to neurotypical children (see **Figure A3** of the **Appendix**: SMD = 2.74, 95% CI: -0.22. 5.70, *p*-value for heterogeneity <0.0001; I^2 = 99.95%). According to I^2 (99.95%) and the Cochrane Q test (P<0.0001), there was a high level of heterogeneity between the studies. Egger's test showed no small-study effects. Begg's funnel plot shows that three studies (Fido & Al-Saad, 2005; Hodgson et al., 2014; Yassa, 2014) report very high effect sizes that cause asymmetry in the funnel plot and therefore indicating publication bias. The lower bound of 95% CI interval for these three studies (24.3; 64.7; 19.7) was higher than the higher bound of the pooled effect CI (5.70), indicating that these studies present extremely large effect sizes that strongly impacts the pooled effect value. A funnel plot without these three studies (see **Figure A9** of the **Appendix**) show 7 studies (8 datasets) that are on the right (positive) side of the outside of the funnel (Al-Ayadhi, 2005; El-baz et al., 2010; Ip et al., 2004; Lakshmi Priya & Geetha, 2011; Metwally et al., 2015; Mohamed et al., 2015; Qin et al., 2018); these studies were conducted in Asian and African continents. 7 studies (7 datasets) conducted in Europe, America

and Asia (1 study) were found on the left side (negative) of the outside of the funnel (Domingues et al., 2016; Gil-Hernández et al., 2020; Holmes et al., 2003; Li et al., 2017; Majewska et al., 2010; Obrenovich et al., 2011; Skalny, et al., 2017a; Skalny et al., 2017b).

The analysis was repeated without the three identified outliers (36 studies; 42 datasets) and the results showed that the overall standardized mean difference between the hair mercury concentrations of the ASD patients and the neurotypical controls was very small, and the relationship was non-significant (see **Figure A4** of the **Appendix**: 0.30, 95% CI: -0.05, 0.64, p-value for heterogeneity <0.0001; I^2 = 96.60%). The heterogeneity among studies stayed significantly high (I^2 = 96.60%; Cochrane Q test P<0.0001). In **Figure A4** of the **Appendix,** the studies were listed in ascending order of the mean mercury levels in the ASD patients.

2.3.2.2 Subgroup Analysis

The heterogeneity in the relationship between hair mercury and ASD diagnosis was explored by subgroup analyses based on different types of ASD, continents and age of diagnosis for the ASD cases. The analyses were conducted, excluding the three outlying studies (Fido & Al-Saad, 2005; Hodgson et al., 2014; Yassa, 2014).

A total of 17 studies (18 datasets) reported data for patients diagnosed with autism (subjects described as “autistic” or patients of “autism” or “autistic disorder”) (Al-Ayadhi, 2005; De Palma et al., 2012; El-baz et al., 2010; Elsheshtawy et al., 2011; Hertz-Picciotto et al., 2010; Holmes et al., 2003; Ip et al., 2004; Lakshmi Priya & Geetha, 2011; Majewska et al., 2010; McKean et al., 2015; Mohamed et al., 2015; Mostafa & Refai, 2007; Park & Zheng, 2012; Stamova et al., 2011; Wecker et al., 1985; Williams et al., 2008; Woods et al., 2010) and 19 studies (23 datasets) that reported data for patients diagnosed with ASD (Adams et al., 2013, 2006, 2008; Albizzati et al., 2012; Blaurock-Busch et al., 2012; Bradstreet et al., 2003; Domingues et al., 2016; Gil-Hernández

et al., 2020; Kern et al., 2007; Li et al., 2017; Macedoni-Lukšič et al., 2015; Metwally et al., 2015; Mostafa et al., 2016; Obrenovich et al., 2011; Qin et al., 2018; Skalny et al., 2017a, 2017b; Wright et al., 2012; Yau et al., 2014). There was only one dataset that reported data on patients with Asperger's syndrome (Al-Ayadhi, 2005), so we excluded it from our subgroup analysis. The pooled SMD between ASD patients and neuotypical controls was 0.05 (95% CI: -0.33, 0.42), while pooled SMD between "autistic" patients and controls was 0.15 (95% CI: -0.12, 0.89) (see **Figure A5** of the **Appendix**). The pooled SMDs between cases and controls for both the ASD patients and autistic patients were very small and non-significant for a relationship. There was not a significant difference between the two SMD values and in comparison to the overall SMD calculated (SMD = 0.30, 95% CI: -0.05, 0.65). The heterogeneity among studies for ASD patients (I^2 = 93.97%; Cochrane Q test P<0.0001) and autistic patients (I^2 = 96.63%; Cochrane Q test P<0.0001) were both significantly high.

There were 6 studies (6 datasets) from Africa (El-baz et al., 2010; Elsheshtawy et al., 2011; Metwally et al., 2015; Mohamed et al., 2015; Mostafa et al., 2016; Mostafa & Refai, 2007), 13 studies (16 datasets) from America (Adams et al., 2013, 2006, 2008; Hertz-Picciotto et al., 2010; Holmes et al., 2003; Kern et al., 2007; McKean et al., 2015; Obrenovich et al., 2011; Stamova et al., 2011; Wecker et al., 1985; Williams et al., 2008; Woods et al., 2010; Yau et al., 2014), 8 studies (9 datasets) from Asia (Al-Ayadhi, 2005; Blaurock-Busch et al., 2011; Bradstreet et al., 2003; Ip et al., 2004; Jung et al., 2008; Lakshmi Priya & Geetha, 2011; Li et al., 2017; Qin et al., 2018), and 9 studies (11 datasets) from Europe (Albizzati et al., 2012; De Palma et al., 2012; Domingues et al., 2016; Gil-Hernández et al., 2020; Macedoni-Lukšič et al., 2015; Majewska et al., 2010; Skalny et al., 2017a, 2017b; Wright et al., 2012). The pooled SMD between ASD patients and neurotypical controls in Africa, America, Asia and Europe was 0.67 (95% CI: -0.03, 1.38), -0.16

(95% CI: -0.38, 0.07), 1.60 (95% CI: 0.26, 2.93), -0.23 (95% CI: -0.52, 0.06) respectively (see **Figure A6** of the **Appendix**). The pooled SMDs between ASD patients and neurotypical controls were highest in Asia and Africa and displayed positive relationships. However, the heterogeneity among studies for Africa (I^2 = 94.66%; Cochrane Q test P<0.0001) and Asia (I^2 = 98.71%; Cochrane Q test P<0.0001) were very high. The pooled SMD in America and Europe were negative and small in magnitude. The heterogeneity among studies for America (I^2 = 77.53%; Cochrane Q test P<0.0001) and Europe (I^2 = 76.72%; Cochrane Q test P<0.0001) were also significantly high but lower compared to the other two continents.

The subgroup analysis on the age of ASD patients was conducted after excluding the four outlying studies as well as one study (Obrenovich et al., 2011) that did not provide the age of the participants. The age of the ASD patients were divided into two groups: age 0-6 years and age 7 years or older. For studies that only provided the age range, we looked at the highest age of the range and categorized the study accordingly. For studies that only provided the age mean, we assumed the mean represented the whole group and categorized the study accordingly. After categorization, there were 16 studies (19 datasets) with patients of age 0-6 years (Adams et al., 2008; Gil-Hernández et al., 2020; Majewska et al., 2010; McKean et al., 2015; Metwally et al., 2015; Qin et al., 2018; Skalny et al., 2017a, 2017b; Stamova et al., 2011; Williams et al., 2008; Woods et al., 2010; Yau et al., 2014), 21 studies (22 datasets) with patients of age 7 years or older (Adams et al., 2013, 2006; Al-Ayadhi, 2005; Albizzati et al., 2012; Blaurock-Busch et al., 2011; Bradstreet et al., 2003; De Palma et al., 2012; Domingues et al., 2016; El-baz et al., 2010; Ip et al., 2004; Jung et al., 2008; Lakshmi Priya & Geetha, 2011; Li et al., 2017; Majewska et al., 2010; Mohamed et al., 2015; Mostafa et al., 2016; Mostafa & Refai, 2007; Skalny et al., 2017a, 2017b; Wecker et al., 1985; Wright et al., 2012). The pooled SMD between ASD patients sampled at age

0 to 6 years, and age-matched neurotypical controls was 0.09 (95% CI: -0.32, 0.51) while pooled SMD between ASD patients sampled at age 7 or older and controls was 0.32 (95% CI: -0.05, 1.10) (see **Figure A7** of the **Appendix**). The pooled standardized mean differences in all ages were very small and not significant enough for a relationship. The heterogeneity among studies for both age groups is high; age 0-6 years (I^2 = 94.46%; Cochrane Q test P<0.0001) and age 7 years or older (I^2 = 96.63%; Cochrane Q test P<0.0001).

Table 3. Summary of meta-analysis results

Comparison	**Number of Subjects (studies; datasets)**	**SMD (95% CI)**	**I^2**
ASD patients vs. neurotypical controls	4535 (36; 42)	0.30 (-0.05, 0.64)	96.60%
Autistic patients vs. neurotypical controls	2286 (17;18)	0.15 (-0.12, 0.89)	96.63%
ASD patients vs. neurotypical controls (Africa)	679 (6;6)	0.67 (-0.03, 1.38)	94.66%
ASD patients vs. neurotypical controls (America)	1719 (13;16)	-0.16 (-0.38, 0.07)	77.53%
ASD patients vs. neurotypical controls (Asia)	1234 (8;9)	1.60 (0.26, 2.93)	98.71%
ASD patients vs. neurotypical controls (Europe)	913 (9;11)	-0.23 (-0.52, 0.06)	76.72%
ASD patients vs. neurotypical controls (Age 0-6 yrs)	2046 (16;19)	0.09 (-0.32, 0.51)	94.46%
ASD patients vs. neurotypical controls (Age ≥7 yrs)	2494 (21;22)	0.32 (-0.05, 1.10)	96.63%

2.4. Discussion

This systematic review of systematic review provides a definitive, comprehensive analysis of the known evidence on the relationship between hair Hg levels and ASD diagnosis, given the

mixed findings to date, and identify the limitations of the previous systematic reviews. Consequently, this review critically evaluated the methodological quality of the published systematic reviews to discover the causes of inconsistent findings in the literature. Through this review, the outcome data from included systematic reviews were re-extracted, and re-analyzed; this summary of evidence enabled for the comparison of findings among the systematic reviews. In the current review, we have conducted a meta-analysis based on 39 studies (45 datasets) with 4759 participants from 15 countries. Our meta-analysis calculated the SMD between the hair mercury levels of ASD patients and neurotypical controls (SMD = 0.30 (-0.05, 0.64) and demonstrated that the mercury concentrations in hair of ASD patients compared to neurotypical individuals are not significantly different. Calculated SMD value was not significant enough to suggest a possible relationship between hair mercury and ASD diagnosis; SMD value of less than 0.5 corresponds to significance level or error rate less than 0.05 or 5% and therefore is considered to have small significance (Cohen, 1988). The scientific data we have compiled in the current meta-analysis include various mercury species measurements in different levels and different biomarkers in various study populations. This explains the heterogeneity and inconsistencies in the data of the published studies. This is the first systematic review of systematic reviews to combine the data for mercury levels in multiple biomarkers into one unit of measurement (hair mercury) to assess the relationship between mercury biomarker levels and ASD.

The topic of the relationship between mercury level in biomarkers such as hair and ASD has been controversial. Studies that reported higher mercury levels in the hair of ASD patients compared to neurotypical individuals (Al-Ayadhi, 2005; El-baz et al., 2010; Fido & Al-Saad, 2005; Hodgson et al., 2014; Lakshmi Priya & Geetha, 2011; Yassa, 2014) defended their findings by stating that ASD patients have a greater ability to accumulate mercury in their body compared to

the neurotypical children. However, there are numerous studies that reported lower mercury levels in the hair of ASD patients compared to neurotypical individuals (Domingues et al., 2016; Gil-Hernández et al., 2020; Holmes et al., 2003); they supported their results by stating that ASD patients have impaired detoxification and excretory pathways and therefore cannot excrete toxic agents such as mercury to the hair follicles as efficiently as neurotypical individuals. The included systematic reviews on this topic reported divergent conclusions on the relationship between ASD diagnosis and level of mercury, depending on the type of tissue. Our systematic review of these review papers summarized their findings and discovered that the data reported on hair, blood and urine mercury in relation to ASD diagnosis is often inconsistent or indicates that there is no relationship (**Table A4**). Accordingly, our meta-analysis combined these primary data and found that the data reported in the original studies show inconsistent results, and it is highly likely that there is no significant relationship between body burden of mercury as shown by the biomarker concentrations and ASD.

In this study, we analyzed the variance in the association of mercury and ASD in different populations and different ages of participants by performing subgroup analyses. The subgroup analysis based on continents demonstrated that there is a higher hair mercury concentrations found in ASD patients compared to the neurotypical children in Asia and Africa. In comparison, there is no significant difference in hair mercury levels among the participants in America and Europe. There were a considerably higher number of studies conducted in America and Europe compared to Asia and Africa; lack of data may have introduced a level of heterogeneity in results. Some possible confounding variables that may have affected these results include the diet and lifestyle of the participants, as well as their family history of ASD. One meta-analysis study reported that the developmental status of countries has a significant influence on the overall effect size of the

mean difference in hair mercury between ASD patients and neurotypical controls; ASD patients in developing countries demonstrated higher hair mercury levels in ASD patients while this trend was not seen in developed countries (Saghazadeh & Rezaei, 2017). More studies with larger sample sizes should be conducted outside America are needed to compare with the American data. Moreover, we performed our second subgroup analysis based on the age at which the hair samples were taken from the participants. Our results suggested that there is no difference in hair mercury levels between ASD patients and neurotypical children in both age groups (ages 0-6 and 7 or older). However, a recent study compared the hair mercury levels of two age groups (age 3-4 and age 7-9) and found that the difference between hair mercury concentrations in ASD patients and neurotypical children was higher in the older age group compared to the younger age group (Majewska et al., 2010). Older ASD patients (age 7-9) demonstrated higher mercury hair concentrations compared to age-matched neurotypical children. In comparison, younger ASD patients (age 3-4) demonstrated lower mercury hair concentrations compared to age-matched neurotypical children. They suggested that this may be due to the impaired ability to excrete mercury in younger ASD patients. This discrepancy in the results suggests that the age categories defined in this study were too broad to show a significant effect age has on the mercury excretion to hair follicles.

Regarding the association between ASD and mercury levels in hair, the current meta-analysis did not provide any significant associations. We conducted a meta-regression of SMD between hair mercury levels in ASD patients and neurotypical controls in relation to the mean mercury hair levels in the neurotypical controls to test whether the level of mercury exposure in controls of each study impact the SMD values (**Appendix Figure A8**). Results of the meta-regression demonstrated that there is no correlation (correlation coefficient = 0.10; **Appendix**

Table A5). This result suggests that there were no significant differences observed between SMD values of different studies despite the different levels of hair mercury levels in controls.

There are some limitations regarding the current review paper. First, we only included case-control studies in the meta-analysis. The problem with case-control studies is that the time of exposure and outcome cannot be captured by these studies. Also, we only included review papers written in English, which could have introduced a level of publication bias. Moreover, the study design and method used in each meta-analysis papers were different and difficult to compare them in summarizing their findings into one systematic review. Regarding the meta-analysis portion of the current paper, we had to extract the primary data from each review paper. However, the review papers often did not present their primary data clearly, and it was necessary for us to go back to each primary study to extract the data. For example, the mercury exposure levels measured for cases and controls were not recorded clearly in the review papers (missing unit of measurement, missing SD values, etc.). The method of diagnosis and confounding variables that were considered in each primary study was almost always missing in the review papers. Lastly, a systematic review of systematic reviews is useful in summarizing and assessing the quality of the data presented by other review papers, but it is limited in a way that we can miss possible important primary papers that are not included in these reviews.

2.5. Conclusion

The current systematic review of published systematic reviews demonstrated that there are inconsistent data reported regarding the association between mercury levels in biomarkers and the risk of ASD in children. The current meta-analysis summarized the primary data collected from the published systematic review papers. It demonstrated that mercury concentrations in hair are not significantly associated with an increase in the risk of ASD. Moreover, the first subgroup

analysis demonstrated that the continents in which each primary study was conducted might influence the hair mercury levels in ASD patients. It is suggested that future studies worldwide (especially in Asia and Africa) should be conducted to examine the concentration of mercury in mercury biomarkers to create a more robust dataset. Finally, the second subgroup analysis demonstrated that there is no difference in hair mercury levels in ASD patients of different age groups. However, to test the hypothesis of previous studies on the relationship between age of diagnosis or sampling and hair mercury levels in ASD patients, better knowledge on patients' age of diagnosis would be required.

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Chapter 3: Assessment on the reporting and methodological quality of systematic reviews in environmental epidemiology

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Contribution Statement

KJL was responsible for the conception and design of the study, data acquisition, analysis and interpretation, and drafting and revising the manuscript. XFH and HMC were responsible for funding requisition, supervision of data acquisition and analysis, result interpretation, and revising the manuscript.

Abstract

Background: Systematic reviews are now increasingly being employed in reviews of environmental epidemiology studies. A reporting guideline for environmental epidemiology can increase the clarity and transparency of publications and facilitate the conduct of systematic reviews and meta-analyses.

Objectives: 1) To investigate the adherence of published systematic reviews of environmental epidemiology studies to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses); 2) To provide suggestions and recommendations for developing a guidance document designed specially for environmental epidemiology systematic reviews.

Methods: Systematic reviews collected from a previously conducted systematic review of systematic reviews on the relationship between Hg exposure and neurodevelopmental disorders were assessed using PRISMA (guidance document) and AMSTAR-2 (quality assessment tool). The overall adherence rates in percentages (median and IQR) to each item of the PRISMA and

AMSTAR-2 were calculated. Subgroup analyses were performed without the items specifically targeted towards meta-analysis.

Results: A total of 14 systematic reviews were included. The median overall adherence to the 27 items of the PRISMA statement was 64.3% (IQR: 35.7-85.7%). The subgroup analysis without the eight items specific to meta-analysis revealed an adherence rate of 78.6% (IQR: 60.7-92.9%). The median overall adherence of the systematic reviews to the 16 items of the AMSTAR-2 checklist was 46.45% (IQR: 41.1-74.98%). The subgroup analysis without the two items specific to meta-analysis revealed an adherence rate of 46.45% (IQR: 37.5-82.13%). Items 5 and 10 of the PRISMA guideline and items 2, 5, 6, 7, 9, 13 and 14 of the AMSTAR-2 checklist obtained low adherence ratings (<50%) among the included reviews.

Conclusions: The most common reporting items that were lacking in the included systematic reviews were : 1) properly developed protocol a priori, 2) availability of a full literature search 3) reporting of the process in study selection, data extraction/collection, 4) assessment of RoB and discussion in relation to the study results, 5) for meta-analysis, the detailed reporting of primary data, 6) assessment of heterogeneity in the review findings. These limitations were most prominent in systematic reviews without meta-analysis, while meta-analysis papers presented higher adherence to the items of PRISMA and AMSTAR-2.

3.1. Introduction/Background:

Systematic reviews and meta-analyses are essential tools in the field of evidence-based healthcare to provide the highest level of evidence to inform decision-making (Aromataris et al., 2015; Sofaer & Strech, 2012). This type of review was first used to combine randomized clinical

trials, but they are now being used to combine different types of literature, such as observational studies and indirect comparisons (Higgins & Green, 2011; Mills et al., 2013). There is an increasing number of systematic reviews being published on occupational and environmental contaminant exposures and health outcomes (Chen et al., 2016; Hu et al., 2018; Yoshimasu et al., 2014; Zani et al., 2017; Zhang et al., 2015).

Systematic reviews often involve the use of a guidance document such as PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009) and MOOSE (Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies) (Stroup et al., 2000). These documents guide the reviewers through the structure of how to report a proper systematic review; some of the special requirements for the reporting of systematic reviews include a detailed and comprehensive plan and search strategy derived *a priori* and the development of a very focused well-defined research question. In the case of environmental epidemiology studies, there is no customized guidance document developed for conducting a systematic review in this field; the majority of reviews in this field uses either PRISMA or MOOSE as their guidance document. Blair et al. proposed a guideline for meta-analysis in the field of environmental epidemiology (Blair et al., 1995). Sheehan and Lam assessed the methodology and reporting of environmental epidemiology systematic reviews by using the guidance of PRISMA and MOOSE and the recommendations of Blair et al. (Sheehan & Lam, 2015). They conducted a systematic review on the systematic reviews and meta-analyses on the associations between chronic low-dose chemical exposures and related health effects and compared the reported methodologies with the checklist they have created based on available published guidelines. They found that although most studies adhered to the guidelines, weaknesses were present in certain areas such as study selection, data extraction, and exposure heterogeneity.

The authors recommended that guidelines specific to environmental health/epidemiology be developed (Sheehan & Lam, 2015).

The objective of this project is to investigate the adherence of published systematic reviews of environmental epidemiology studies to the PRISMA statement (Moher et al., 2009) and AMSTAR (A MeaSurement Tool to Assess systematic Reviews)-2 checklist (Shea et al., 2017) and test for the methodological rigour and reporting quality of the included systematic reviews on environmental epidemiology. This project aims to update the study by Sheehan and Lam by assessing more recent systematic reviews of environmental epidemiology studies. Moreover, unlike the review by Sheehan and Lam, this study assesses the quality of reporting and methodology of the systematic reviews separately and proposes solutions for each reporting and methodology item that the reviews are missing. Through this project,we aim to provide suggestions and recommendations for developing a guidance document designed specially for systematic reviews of environmental epidemiology studies.

3.2. Methodology

In Chapter 2 of this thesis, we conducted a systematic review of the published systematic reviews on the relationship between Hg exposure and ASD diagnosis. The seven systematic reviews from Chapter 2 were used in this chapter.

To include more systematic reviews, we expanded our inclusion criteria. We searched through the list of excluded reviews from Chapter 2 to find additional reviews to be included. Seven additional systematic reviews that were excluded because 1) they were on different types of neurodevelopmental conditions (e.g. ADHD, neurobehavioural abnormalities), 2) focused on the etiology of mercury exposure and ASD or 3) were descriptive and reported no numerical data, were also included.

The quality of reporting and methodological rigour of the included systematic reviews was assessed using the PRISMA statement (Moher et al., 2009) and AMSTAR-2 checklist (Shea et al., 2017). We reviewed the adherence to the items of PRISMA and AMSTAR-2 of each review and examined which items had the lowest adherence levels. PRISMA statement consists of 27 reporting items that are used as guidance in reporting systematic reviews. Assessment of reporting quality reflects on the reviewer's ability to present their findings in a comprehensible manner, rather than the process of how the review was conducted (Shea et al., 2017). On the other hand, AMSTAR-2 is a 16-item assessment tool developed to evaluate the quality of the methodology used for systematic reviews, i.e. how the reviews were planned and conducted.

We tracked which parts of the PRISMA and AMSTAR-2 the systematic reviews were lacking and identified the common conducting and reporting limitations of the published systematic reviews. Each item of the PRISMA and AMSTAR-2 was given a score of 1 if the item was followed correctly and 0 if the item was missing. For items that were not applicable, we recorded N/A. Frequencies, percentages, median and IQR were tabulated.

3.3. Results

A total of 14 systematic reviews were included. In our systematic review of systematic reviews (Chapter 2), a total of 7 systematic reviews (De Palma et al., 2012; Jafari et al., 2017; Parker et al., 2004; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017; Taylor et al., 2014; Yoshimasu et al., 2014) were included. For this chapter, seven additional systematic reviews were included from the list of excluded reviews (Julvez & Grandjean, 2009; Kern et al., 2017; Koning et al., 2017; Pajewska-Szmyt et al., 2019; Schoeman et al., 2009; Williams & Ross, 2007; Wołowiec et al., 2013).

7/14 systematic reviews presented relevant data for the relationship between Hg exposure and ASD/autism (De Palma et al., 2012; Jafari et al., 2017; Parker et al., 2004; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017; Taylor et al., 2014; Yoshimasu et al., 2014). 3/14 systematic reviews (Kern et al., 2017; Williams & Ross, 2007; Wołowiec et al., 2013) were on neurodevelopment and chemical exposure; these reviews included findings for the relationship between ASD and Hg exposure but did not provide relevant data for Chapter 2. 4/14 systematic reviews (Julvez et al., 2013; Koning et al., 2017; Pajewska-Szmyt et al., 2019; Schoeman et al., 2009) were on neurodevelopmental disorders other than ASD. Only 5 of the included systematic reviews included a meta-analysis (De Palma et al., 2012; Jafari et al., 2017; Saghazadeh & Rezaei, 2017; Taylor et al., 2014; Yoshimasu et al., 2014).

4-14 systematic reviews (28.5%) (Jafari et al., 2017; Rossignol et al., 2014; Saghazadeh & Rezaei, 2017; Taylor et al., 2014) reported having followed PRISMA as their reporting guideline. 9/14 systematic reviews (64.3%) (Julvez et al., 2013; Kern et al., 2017; Koning et al., 2017; Pajewska-Szmyt et al., 2019; Parker et al., 2004; Schoeman et al., 2009; Williams & Ross, 2007; Wołowiec et al., 2013; Yoshimasu et al., 2014) did not report to have followed any type of reporting guidance documents. De Palma et al., 2012 reported that they followed the guidelines of MOOSE (Stroup et al., 2000).

The median overall adherence of the systematic reviews to the 27 items of the PRISMA statement was 64.3% (IQR: 35.7-85.7%) (Table 4). The median adherence to the PRISMA was 80% (IQR: 64.3-100%) after withdrawing those “not applicable” items. Items 13-16 and 20-23 are reporting items specific to the process of conducting statistical analysis or meta-analysis. Since not all reviews conducted a meta-analysis, we performed a subgroup analysis without these eight

reporting items. The median adherence of the systematic reviews to the 19 items of the PRISMA not related to meta-analysis was 78.6% (IQR: 60.7-92.9%).

The median overall adherence of the systematic reviews to the 16 items of the AMSTAR-2 checklist was 46.45% (IQR: 41.1-74.98%) (Table 5). The median adherence was 50% (IQR: 41.1-92.9%) after withdrawing those "not applicable" items. Items 11 and 12 are reporting items specific to the process of conducting statistical analysis or meta-analysis. We also conducted a subgroup analysis without these two items. The median adherence of the systematic reviews to the 14 items of the AMSTAR-2 not related to meta-analysis was 46.45% (IQR: 37.5-82.13%).

Table 4. Summary table for PRISMA comparison

Section/Topic	#	Checklist item	Yes	No	N/A
Title					
Title	1	Identify the report as a systematic review, meta-analysis, or both	9 (64.3%)	5 (35.7%)	0 (0%)
Abstract					
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	11 (78.6%)	3 (21.4%)	0 (0%)
Introduction					
Rationale	3	Describe the rationale for the review in the context of what is already known.	14 (100%)	0 (0%)	0 (0%)
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	13 (92.9%)	1 (7.1%)	0 (0%)
Methods					
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3 (21.4%)	11 (78.6%)	0 (0%)

Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	13 (92.9%)	1 (7.1%)	0 (0%)
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	14 (100%)	0 (0%)	0 (0%)
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7 (50%)	7 (50%)	0 (0%)
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	9 (64.3%)	5 (35.7%)	0 (0%)
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6 (42.9%)	8 (57.1%)	0 (0%)
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8 (57.1%)	6 (42.9%)	0 (0%)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7 (50%)	7 (50%)	0 (0%)
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	5 (35.7%)	0 (0%)	9 (64.3%)
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	5 (35.7%)	0 (0%)	9 (64.3%)
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	4 (28.6%)	1 (7.1%)	9 (64.3%)
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	1 (7.1%)	2 (14.2%)	11 (78.6%)
Results					
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	12 (85.7%)	2 (14.2%)	0 (0%)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	13 (92.9%)	1 (7.1%)	0 (0%)

Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see Item 12).	7 (50%)	7 (50%)	0 (0%)
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot.	5 (35.7%)	0 (0%)	9 (64.3%)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	5 (35.7%)	0 (0%)	9 (64.3%)
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	4 (28.6%)	1 (7.1%)	9 (64.3%)
Additional analyses	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	3 (21.4%)	0 (0%)	11 (78.6%)
Discussion					
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).	12 (85.7%)	2 (28.6%)	0 (0%)
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias).	10 (71.4%)	4 (28.6%)	0 (0%)
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	14 (100%)	0 (0%)	0 (0%)
Funding					
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	9 (64.3%)	5 (35.7%)	0 (0%)
Overall Median (IQR)			**64.3% (35.7-85.7%)**		
Overall w/o N/A items Median (IQR)			**80% (64.3-100%)**		

Table 5. Summary table for AMSTAR-2 comparison

#	Checklist item	Yes	No	N/A
1	Did the research questions and inclusion criteria for the review include the components of PICOS?	13 (92.9%)	1 (7.1%)	0 (0%)
2	Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	3 (21.4%)	11 (78.6%)	0 (0%)
3	Did the review authors explain their selection of the study designs for inclusion in the review?	13 (92.9%)	1 (7.1%)	0 (0%)
4	Did the review authors use a comprehensive literature search strategy?	14 (100%)	0 (0%)	0 (0%)
5	Did the review authors perform study selection in duplicate (in pairs)?	6 (42.9%)	8 (57.1%)	0 (0%)
6	Did the review authors perform data extraction in duplicate?	4 (28.6%)	10 (71.4%)	0 (0%)
7	Did the review authors provide a list of excluded studies and justify the exclusions?	4 (28.6%)	10 (71.4%)	0 (0%)
8	Did the review authors describe the included studies in adequate detail?	12 (85.7%)	2 (14.3%)	0 (0%)
9	Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	6 (42.9%)	8 (57.1%)	0 (0%)
10	Did the review authors report on the sources of funding for the studies included in the review?	7 (50%)	7 (50%)	0 (0%)
11	If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	5 (71.4%)	0 (0%)	9 (64.3%)
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	3 (42.9%)	2 (28.6%)	9 (64.3%)
13	Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	5 (35.7%)	9 (64.3%)	0 (0%)
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	6 (42.9%)	8 (57.1%)	0 (0%)
15	If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	5 (71.4%)	0 (0%)	9 (64.3%)
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	7 (50%)	7 (50%)	0 (0%)
Overall Median (IQR)		**46.45% (41.1-74.98%)**		
Overall w/o N/A items Median (IQR)		**50% (41.1-92.9%)**		

Items 5 and 10 of the PRISMA guideline and items 2, 5, 6, 7, 9, 13 and 14 of the AMSTAR-2 checklist obtained low adherence ratings among the included reviews (more than 50% of the reviews were missing these items in their reporting).

Item 5 of PRISMA and item 2 of AMSTAR-2 are on developing a protocol of the systematic review a priori. Only 21.4% (3/14 studies) have reported having developed a proper protocol before conducting the systematic reviews.

Item 10 of PRISMA and items 5-6 of the AMSTAR-2 are on the study selection and data extraction process. Only 42.9% (6/14 studies) have conducted the study selection and data collection in pairs (item 5-6 of AMSTAR-2) and reported the steps of this process clearly in their methodology section (item 10 of PRISMA).

Item 7 of AMSTAR-2 is on providing a list of excluded studies from the literature search. Only 42.9% (6/14 studies) have provided a list of excluded studies.

Item 9 and 13 of AMSTAR-2 is on assessing for the risk of bias (RoB) in the included literature in the reviews. 42.9% (6/14 studies) used a satisfactory technique for assessing the RoB for each included study in their methodology section (Item 9 of AMSTAR-2), but only 35.7% (5/14 studies) accounted for the risk of bias when discussing their findings in the results section (Item 13 of AMSTAR-2).

Item 14 of AMSTAR-2 is on assessing for any heterogeneity that is observed in the results of the review. Only 42.9% (6/14 studies) assessed the heterogeneity among the included literature and discussed them in the results section.

3.4. Discussion

3.4.1 Adherence to PRISMA and AMSTAR-2

In this study, we assessed the reporting and methodological quality of systematic reviews in environmental epidemiology using the PRISMA statement (reporting quality) and AMSTAR-2 checklist (methodological quality). Only 28.6% (4/14 studies) of the included systematic reviews stated to have followed the PRISMA statement. This percentage is similar to that previously reported for nursing journals (28%, 30/107) (Tam et al., 2017) and internal and specialty medical journals (27%, 40/146) (Tao et al., 2011). The level of adherence generally conforms to the level of endorsement of other reporting guidelines. For example, Sheehan and Lam reported that among the systematic reviews in the field of environmental epidemiology, one quarter referred to the use of a guidance document such as PRISMA and MOOSE (Sheehan & Lam, 2015). We observed that the reviews that stated to have followed the PRISMA statement demonstrated higher adherence levels to the AMSTAR-2 checklist; the methodological quality of the systematic reviews that followed PRISMA was found to be higher. In this study, only systematic reviews on the relationship between neurodevelopment and mercury exposure are covered. Nevertheless, it is hoped that the present study will provide a benchmark against which the reporting of further environmental epidemiology systematic reviews can be compared.

A systematic review must consist of these essential items: a detailed research question, a reproducible protocol, a detailed and systematic literature search, careful assessment of the selected studies, and a transparent and comprehensible presentation of the findings (Brooks & McNeely, 2013).

The median overall adherence of the systematic reviews to the 27 items of the PRISMA statement was 64.3%. Without the 8 items on meta-analysis, the median adherence was 80%. Our

findings were very similar to the values reported for gastroenterology and hepatology journals (83.1–91.1%) (Panic et al., 2013), and radiology journals (83.7%) (Tunis et al., 2013). A relatively high adherence rate was achieved when the items specifically for meta-analysis reporting were removed because the majority of the systematic reviews included in this study did not include a meta-analysis.

The median overall adherence of the systematic reviews to the 16 items of the AMSTAR-2 checklist was 46.45%. Without the two items on meta-analysis, the median adherence was 50%. The adherence rates for AMSTAR-2 were lower than the ones for PRISMA. One reason for this may be due to the fact AMSTAR-2 has a smaller number of items compared to PRISMA. Another reason for this was that four items (i.e. items 7, 9, 13, 14) in AMSTAR-2 obtained a relatively low adherence rating among the systematic reviews; the PRISMA statement does not have equivalent items for these specific AMSTAR-2 items. These items solely focus on the detailed methodological quality (assessment of RoB, etc.).

In this study, only 21.4% (3/14 studies) of the systematic reviews have conducted a protocol *a priori* (item 2 of AMSTAR-2) and reported the existence of the protocol and where it can be accessed (item 5 of PRISMA). Although international organizations such as Cochrane Database for Systematic Reviews and the Agency for Healthcare Research and Quality (AHRQ) require protocols for systematic reviews, majority of systematic reviews of other traditional journals (89%) did not report working from a protocol (Moher et al., 2007; Shamseer et al., 2015; Shea et al., 2017; Tam et al., 2017). Other reviews on PRISMA endorsement in systematic reviews and meta-analyses have reported that only 4.4% of systematic reviews in gastroenterology and hepatology journals (Panic et al., 2013), 15% of systematic review in radiology journals (Tunis et al., 2013), 27% of reviews in orthodontic journals (Fleming et al., 2013), 2.7% of systematic

reviews in nursing journals (Tam et al., 2017) had developed a proper protocol. Similarly, a systematic review of the published epidemiological literature has discovered that only 8% of the systematic reviews reported having a proper protocol or plan (Sheehan & Lam, 2015). It can be concluded that the development of a protocol before the review process is not commonly done by the majority of the study fields. A review protocol not only prespecifies the objectives and methods of a systematic review, but it also enhances transparency and objectivity and reduces bias (Shamseer et al., 2015; Whiting, 2009). The credibility and reliability of the results of a systematic review depend largely on the *a priori* planning and documentation of a protocol.

Only 42.9% (6/14 studies) of the systematic reviews had followed item 10 of PRISMA and items 5-6 of AMSTAR-2 and reported to have conducted the study selection and data collection in pairs and reported the steps of this process clearly in their methodology section. Similarly, only 37.8% of the systematic reviews in nursing journals (Tam et al., 2017) had reported having followed item 10. On the other hand, more than 80% of systematic reviews in gastroenterology and hepatology journals (Panic et al., 2013), radiology journals (Tunis et al., 2013) and orthodontic journals (Fleming et al., 2013) reported having adequately followed item 10. The goal of a systematic review is to present their review process transparently and clearly so that the systematic methodology can be replicated and analyzed for credibility and risk of bias. To minimize the bias, an explicit methodology of the review must be reported. According to the Cochrane Handbook, more than one reviewer is recommended to carry out data extraction and study selection to minimize errors and reduce the potential biases that can be introduced (selection bias) (Higgins & Green, 2011).

Among the 14 systematic reviews, only 6 (42.9%) have provided a list of excluded studies (item 7 of AMSTAR-2). In a review assessing the adherence level of gastroenterology and

hepatology journals, this item (item 5 of the original AMSTAR checklist) was excluded in the assessment because the PRISMA guideline does not recommend the authors to report a list of excluded studies (Panic et al., 2013). In addition, a review of systematic reviews in nursing journals reported an adherence level of 0% for this item. According to these results, it can be deduced that the majority of scientific reviews do not report a list of excluded studies. However, this can decrease the level of transparency in reporting of systematic reviews as the excluded studies remain invisible, and the impact of their exclusion from the reviews is unknown.

A total of 42.9% (6/14 studies) of the systematic reviews reported to have followed item 9 of AMSTAR-2 and satisfactorily assessed the RoB for each included study in their methodology section, but only 35.7% (5/14 studies) of the systematic reviews followed item 13 of AMSTAR-2 and accounted for the RoB in presenting their findings in the results section. Items 9 and 13 are newly added items in AMSTAR-2 (Shea et al., 2017); reviews that assessed the reporting quality of systematic reviews used the original AMSTAR score and therefore did not assess the RoB of the included studies of each review. There is great diversity in the study designs of primary studies included in systematic reviews. Therefore, RoB assessment is essential in identifying possible biases that can impact the results of the review (Shea et al., 2017). In the current study, among the systematic reviews that assessed the RoB of the included literature, many did not even discuss the RoB whendescribing their results. The reviewers did not consider the RoB of each original study in combining their data; for studies with higher often overestimate effect sizes, combining data from the original studies with low RoB and data from the original studies with high RoB can result in unreliable recommendations for practice or policy (Higgins & Green, 2011; Katikireddi et al., 2015). It is, therefore, essential for reviewers to categorize their included studies according

to their level of RoBs to identify which studies provide the most reliable data and should be emphasized in the review results.

Only 42.9% (6/14 studies) of the systematic reviews followed item 14 of AMSTAR-2 and discussed the heterogeneity they have found in the results section. Items 14 is also a newly added item in AMSTAR-2 (Shea et al., 2017); reviews that assessed the reporting quality of systematic reviews used the original AMSTAR score and therefore did not assess the heterogeneity in their results. The reviewers must discuss the potential causes of heterogeneity in their results; the causes can come from the differences in the PICOS (population, intervention, control group, outcome, study design) framework or study design and methodological considerations (Shea et al., 2017).

An important observation from this study was that the majority of the systematic reviews that had high adherence levels for the PRISMA statement items and AMSTAR-2 score items were the ones that conducted a meta-analysis. Review papers that addressed themselves as systematic reviews but did not conduct meta-analysis often lacked the specific characteristics of a proper systematic review (i.e. proper protocol registered, assessment of RoB, discussion of heterogeneity, etc.); they were similar to an ordinary literature review, rather than a systematic review. These reviews almost always never followed a specific guideline such as PRISMA, which explains the lack of proper structure for a systematic review. This study has shown that the majority of the systematic reviews in environmental epidemiology do not follow a guidance document such as PRISMA, and therefore the reviewers lack the knowledge on how to properly report a good systematic review.

3.4.2 Other Methodological and Reporting Limitations

In Chapter 2 of this thesis, while we conducted a systematic review of systematic reviews, we identified the common methodological reporting limitations of the published systematic reviews.

We have discovered that some of the systematic reviews did not follow the common structure of a scientific study (i.e. introduction, methodology, results, discussion, conclusion) and made it difficult for the readers to extract the important results and data reported by the review. In addition to this, there were some systematic reviews that were not clear on the methodology of conducting the review; the process of development of search strategy, study selection, and data extraction was often not described in detail. This made it unclear on what type of primary data they dealt with, and whether there was any source of selection bias. This was most evident in cases where the systematic review was used as a part of a study (e.g. De Palma et al., 2012).

In some of the systematic reviews, the reported search strategy was often very vague; some keywords were given, but the details of a full electronic search strategy was not presented (e.g. Schoeman et al., 2015). Flowcharts were also often missing, especially for systematic reviews without meta-analysis.

In particular, when trying to extract primary data from the systematic reviews, we discovered that the elements such as the exposure levels and their SD values, conversion ratios (if the values were converted to a different unit of exposure), the unit of exposure (such as μg/g of hair Hg), and other extra details on the participants such as age, gender and method of diagnosis were often missing. This led us to go back to the primary literature and seek for details. This limitation goes against the purpose of a systematic review; systematic reviews aim to enable increased and efficient access to the evidence given by the overwhelming number of original

studies. Systematic reviews should be able to provide a comprehensive summary of primary data and not require the readers to go back to the originalstudies for clarification.

These observations were focused on whether the findings or data results of the reviews were clearly presented or not. These limitations we have found made the process of conducting a systematic review of systematic reviews challenging (Chapter 2).

3.4.3 Recommendations for Future Systematic Reviews

1) Developing a protocol ahead of conducting the review

To increase the methodological and reporting quality of the future systematic reviews in environmental epidemiology, future reviewers should first develop a proper protocol before conducting the review. This will allow the reviewers to plan the review process carefully to avoid potential problems. The most recently published guidance document for conducting protocols for systematic reviews is Shamseer et al., 2015.

2) Writing the review using the scientific writing format

To facilitate the reading of the review, reviewers should all follow the classic format of a scientific review (introduction, methodology, results, discussion, conclusion).

3) Presenting a full electronic search

Reviewers should present a full electronic search in the review. Reviewers should provide the full list of keywords and restrictions (i.e. time of publication, language, type of document) for at least one of the databases they have used. This will allow the readers to be able to repeat the search and have access to the literature used in the review.

4) Reporting the process of study selection and data extraction

It is important that reviewers report the process of the study selection and data extraction/collection very clearly. The number of reviewers involved should be mentioned (review must be done by more than one reviewer), and the process of how the consensus between the reviewers was made should also be reported. A visual representation (flowchart) of study selection should always be presented in the review.

5) Reporting assessment of RoBs of individual studies

Reviewers should report the methodology of how RoBs of individual studies were assessed and how these assessment results are applied to the data synthesis. Assessed RoBs must be discussed in the results section as well as the discussion section to present the limitations at study and outcome level.

6) Reporting of primary data

If meta-analysis is conducted, the data of the original studies should be explicitly reported. Confounding variables such as location, gender percentages of patients/controls, and ages should be extracted from the original studies and recorded in the review (or appendix of the review) for access to readers. The full details on the numerical data should also be recorded (i.e. unit of exposure with SD values, type of measurement).

7) Assessment of heterogeneity

Future systematic reviews should assess the heterogeneity among the included studies (heterogeneity that arise from the differences in study design, methodology, PICOS framework of the research questions, etc.) and discuss it in the discussion section.

3.4.4 Shortcomings and Future Research

This study has some limitations. First, a very small number of systematic reviews was included. These reviews were on a very narrow subject area (neurodevelopment and Hg exposure); not all systematic reviews published in the field of environmental epidemiology was covered in this study. A review of all systematic reviews in environmental epidemiology should be conducted; reviews should be categorized according to chemical category (metals, air pollution, etc.) and also according to the presence of meta-analysis.

Moreover, there are other commonly used reporting guidelines such as MOOSE that should be used to assess the quality of reporting of systematic reviews in environmental epidemiology journals. The adherence levels for items of the different guidelines should be compared to determine which of the guidelines are more suitable for use in this field and what are the limitations found in the guidelines that should be adjusted for use in this study field specifically.

3.5. Conclusion

In summary, the most common reporting items that the systematic reviews were lacking were the development of a proper protocol before conducting the systematic review, the availability of a full literature search strategy, a detailed reporting of the process in study selection and data extraction/collection, the assessment and reporting of RoB in relation to the findings of the review and the assessment of heterogeneity among the included literature. In the case of meta-analysis, the details on the primary data were often missing.

These limitations found in the systematic reviews were observed most in systematic reviews without meta-analysis, while meta-analysis papers had relatively high affinity to the items of both PRISMA and AMSTAR-2.

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Chapter 4: Conclusion

This thesis assesses the quality of the systematic reviews of environmental epidemiology studies regarding their methodology and reporting. In the first research project (Chapter 2), I conducted a systematic review of systematic reviews on the relationship between mercury exposure and ASD diagnosis. In the process, I provided an overview of the findings of the published systematic reviews. I conducted my own meta-analysis by extracting the primary data from each included review paper. In the second research project (Chapter 3), I evaluated the quality of the systematic reviews included in the first project (Chapter 2) using the PRISMA statement (reporting quality) and the AMSTAR-2 checklist (conducting/methodology). With the limitations in the reporting quality of the included systematic reviews I have noticed during my meta-analysis process in Chapter 2, I summarized a list of common reporting items that the systematic reviews were lacking. In accordance, I also provided a list of recommendations on how to improve the reporting quality of systematic reviews on environmental epidemiology studies.

4.1 Main Findings

In the first project (Chapter 2), I included ten reviews for our systematic review; 7 of these papers were systematic reviews, and the rest were literature reviews with relevant data. The systematic reviews included 136 original studies in total, excluding the overlapping studies. These review papers reported the relationship between mercury exposure in various biomarkers such as hair, blood, urine and RBCs and other environmental exposure such as air pollution and ASD diagnosis of the exposed children. The overall trend in the findings of the systematic review was that there was no relationship observed between the mercury levels in the biomarkers and ASD

(urine mercury) or the scientific literature reported inconsistent results and the reviews have concluded that the relationship is “inconsistent” (hair mercury, blood mercury).

My own meta-analysis was conducted by extracting 39 individual studies with 4759 participants from the included systematic reviews. I calculated the SMD between the mercury levels in ASD patients and neurotypical controls. I converted all of the data values into one unit of exposure (hair mercury) to combine them into one summary estimate (SMD). My calculated SMD demonstrated that there is no significant difference between the hair mercury levels in ASD patients and neurotypical controls; hair mercury levels do not have any significant relationship with ASD diagnosis. I then conducted three subgroup analyses. The subgroup analysis showed that there was a significant difference between hair mercury concentrations between ASD patients and neurotypical controls in Asia and Africa but not in America and Europe. However, subgroup analysis showed no significant difference in SMD values of patients of different ages and different ASD disorders (ASD vs. autism).

In the second project (Chapter 3), I included seven additional systematic reviews with the seven systematic reviews from Chapter 2. I assessed the adherence levels of the systematic reviews to each item of the PRISMA statement and AMSTAR-2 checklist. I identified the particular items of the PRISMA and AMSTAR-2 that these reviews were missing (Items 5 and 10 of the PRISMA guideline and items 2, 5, 6, 7, 9, 13 and 14 of the AMSTAR-2). These items included the development of a proper protocol, the availability of a full literature search strategy, a detailed reporting of study selection and data extraction/collection, the assessment and reporting of RoB in relation to the findings of the review and the assessment of heterogeneity among the included literature. In the case of meta-analysis, the details on the primary data were often missing. The overall adherence level of the reviews to PRISMA was 64.3% and 46.45% for AMSTAR-2. Only

28.6% (4/14 studies) of the included systematic reviews stated to have followed the PRISMA statement.

4.2 Concluding Remarks

This thesis research not only contributes to the quality assessment of the systematic reviews of environmental epidemiology studies, but it also contributes to the understanding of the relationship between hair mercury levels and ASD diagnosis. This is the first systematic review of systematic reviews to combine the data for mercury levels in multiple biomarkers into one unit of measurement (hair mercury) to assess the relationship between mercury biomarker levels and ASD. However, for both of our projects, a very small number of systematic reviews was included. Not all systematic reviews published in the field of environmental epidemiology was covered in this study, and therefore, my project's findings may not be generalized to all the environmental epidemiology systematic reviews. However, I hope that this study can give a benchmark for the future research of systematic reviews in this field. For future studies, a review of all systematic reviews in environmental epidemiology should be conducted to increase the credibility of the quality assessment. Reviews should be separately assessed according to the chemical category (metals, air pollution, etc.) and also according to the presence of meta-analysis.

Appendix

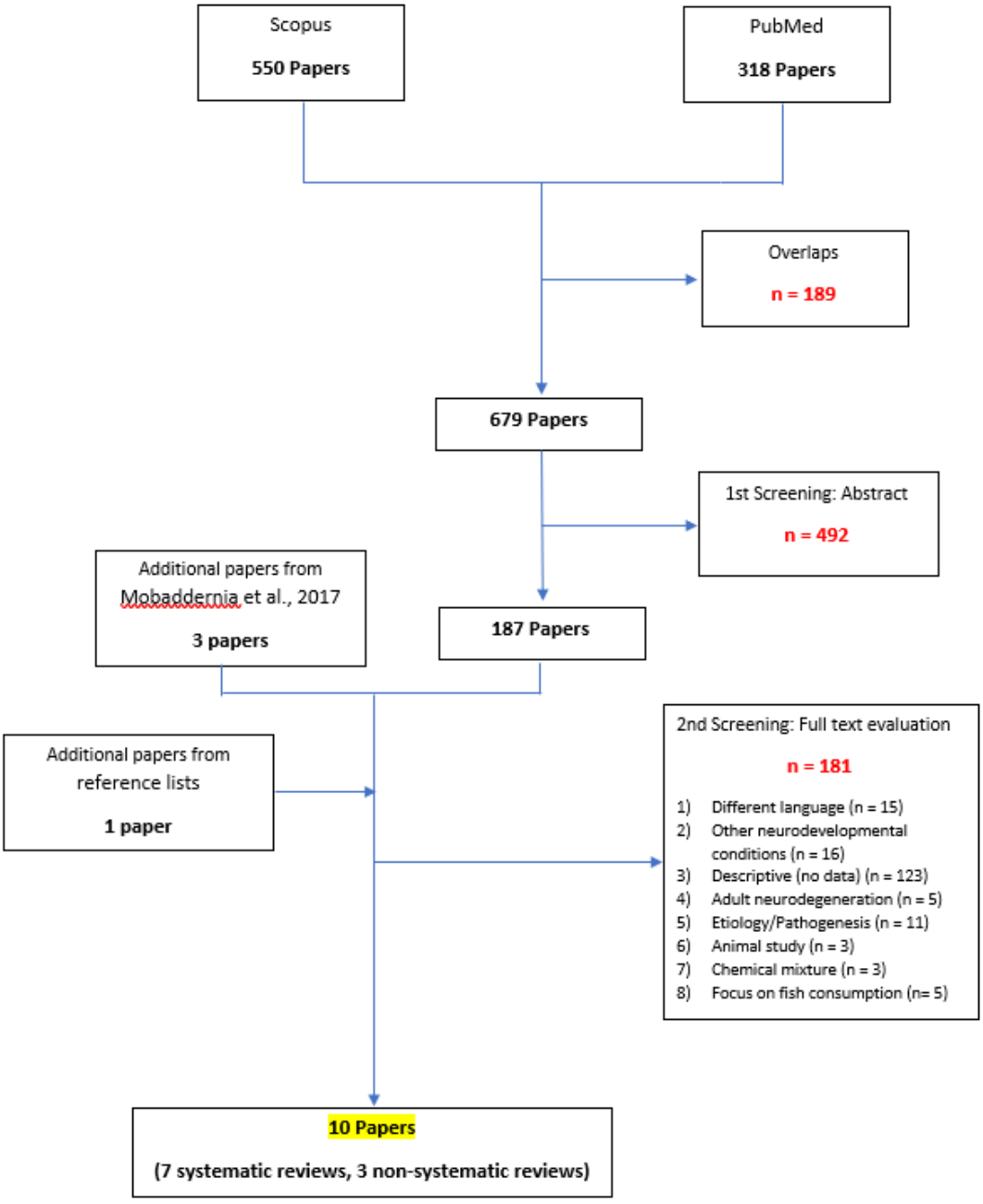

Figure A1. Flow diagram of study selection (systematic reviews).

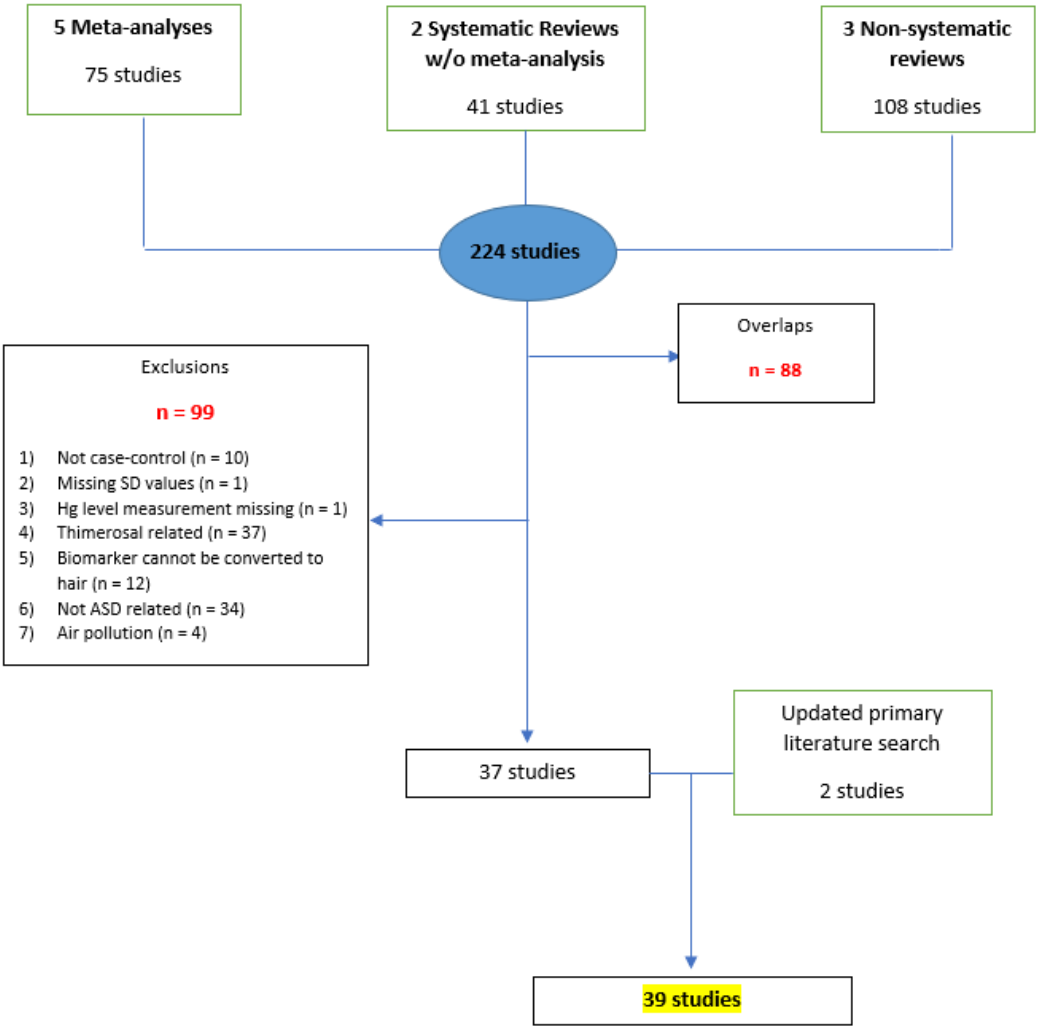

Figure A2-1. Selection of originalstudies for meta-analysis

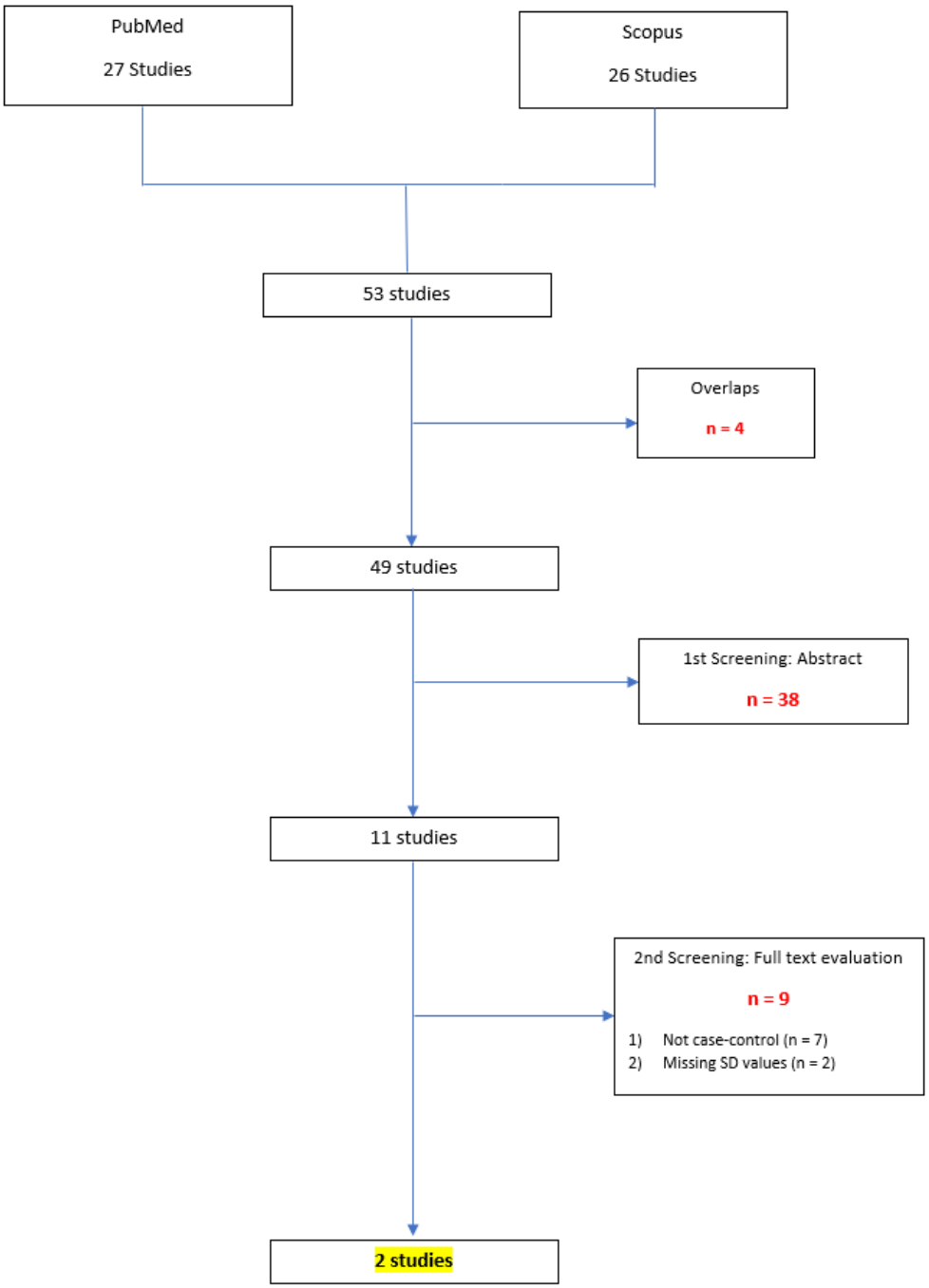

Figure A2-2. Flow diagram of study selection (originalstudies of year 2017-2020)

AMSTAR-2 (Shea et al., 2017) Score

1. Did the **research questions and inclusion criteria** for the review include the components of **PICO**?
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?
3. Did the review authors explain their selection of the study designs for inclusion in the review?
4. Did the review authors use a comprehensive literature search strategy?
5. Did the review authors perform study selection in duplicate (in pairs)?
    a. Two reviewers selected a sample of eligible studies and achieved a good agreement with the remainder selected by one reviewer
6. Did the review authors perform data extraction in duplicate?
7. Did the review authors provide a list of excluded studies and justify the exclusions?
8. Did the review authors describe the included studies in adequate detail?
9. Did the review authors use a satisfactory technique for assessing the **risk of bias (RoB)** in individual studies that were included in the review?
10. Did the review authors report on the sources of funding for the studies included in the review?
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**TOTAL**
Yoshimasu 2014	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**15**
Taylor 2014	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	**13**
Saghazadeh and Rezaei, 2017*	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	**12**
Jafari 2017*	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	1	**13**
De Palma 2014*	0	0	0	1	1	1	1	0	0	0	1	0	0	0	1	0	**6**
Rossignol 2014	1	0	1	1	0	0	0	1	0	0	N/A	N/A	0	0	0	1	**5**
Parker 2004	1	0	1	1	0	0	0	1	0	1	N/A	N/A	0	0	0	1	**6**

Highlighted: Meta-analyses or meta-analysis related categories

*: meta-analysis papers included in the meta-analysis of meta-analyses

Table A1. Case-control studies included in our meta-analysis of ASD and hair mercury levels

Biomarker	Number of papers	Number of datasets	Number of samples	Studies
Hair	25	30*	2291 (1197 cases; 1094 controls)	Adams et al., 2006; Adams et al., 2008*; Al-Ayadhi, 2005*; Albizzati et al., 2012; Blaurock-Busch et al., 2011; De Palma et al., 2012; Domingues et al., 2016; El-Baz et al., 2010 ; Elsheshtawy et al., 2011 ; Holmes et al., 2003 ; Ip et al., 2004; Jung et al., 2008 ; Kern et al., 2007; Lakshmi Priya and Geetha, 2011 ; Majewska et al., 2010* ; Mohamed et al., 2015 ; Obrenovich et al., 2011 ; Skalny et al., 2017a* ; Skalny et al., 2017b ; Wecker et al., 1985 ; Williams et al., 2008 ; Gil-Hernandez et al., 2020 ; Fido and Al-Saad, 2005; Hodgson et al., 2014; Yassa et al., 2014
Blood	9	9	1759 (956 cases; 803 controls)	Hertz-Picciotto et al., 2010; Li et al., 2017; Macedoni-Luksic et al., 2015; McKean et al., 2015; Mostafa and Refaei, 2007; Mostafa et al., 2016; Stamova et al., 2011; Yau et al., 2014 ; Qin et al., 2018
Urine	5	6*	709 (387 cases; 322 controls)	Adams et al., 2013; Bradstreet et al., 2003; Woods et al., 2010*; Wright et al., 2012 ; Metwally et al., 2015
NET	39	45*	4759 (2540 cases; 2219 controls)	Adams et al., 2013; Adams et al., 2006; Adams et al., 2008*; Al-Ayadhi, 2005*; Blaurock-Busch et al., 2011; Bradstreet et al., 2003; De Palma et al., 2012; Domingues et al., 2016; El-baz et al., 2010; Elsheshtawy et al., 2011; Hertz-Picciotto et al., 2010; Holmes et al., 2003; Ip et al., 2004; Jung et al., 2008; Kern et al., 2007; Lakshmi Priya and Geetha, 2011; Li et al., 2017; Macedoni-Luksic et al., 2015; Majewska et al., 2010*; McKean et al., 2015; Mohamed et al., 2015; Mostafa et al., 2016; Mostafa and Refaei, 2007; Obrenovich et al., 2011; Skalny et al., 2017a*; Skalny et al., 2017b; Stamova et al., 2011; Wecker et al., 1985; Williams et al., 2008; Woods et al., 2010*; Wright et al., 2012; Yau et al., 2014 ; Qin et al., 2018; Gil-Hernandez et al., 2020 ; Fido and Al-Saad, 2005 ; Hodgson et al., 2014; Yassa et al., 2014 ; Metwally et al., 2015

* Adams et al., 2008 has three datasets; Al-Ayadhi, 2005 has two datasets; Majewska et al., 2010 has two datasets; Skalny et al., 2017a has two datasets; Woods et al., 2010 has two datasets; other studies have one dataset each

Table A2. Characteristics of the originalstudies (39 studies; 45 data sets)

Study	Population	Ne**	Nc**	Age case (yrs)*	Age control (yrs)*	Male % (case)	Male % (control)	Biomarker	Health Outcome	Measurement of health outcome
Adams et al., 2006	Arizona, USA	51	40	7.10 (3-15)	7.10 (3-15)	76.48	N/A	Hair	ASD	Diagnosis by psychiatrist/ developmental pediatrician
Adams et al., 2008	Arizona, USA	67	21	1.38 (1-2)	1.38 (1-2)	100	100	Hair	ASD	DSM IV **
		11	11	1.38 (1-2)	1.38 (1-2)	0	0	Hair	ASD	DSM IV
		11	11	1.38 (1-2)	1.38 (1-2)	N/A	N/A	Hair	ASD	DSM IV
Adams et al., 2013	Arizona, USA	54	44	10 (5-16)	10 (5-16)	89	89	Urine	ASD	Diagnosis by psychiatrist/ developmental pediatrician
Al-Ayadhi, 2005	Riyadh area, Saudi Arabia	2	80	10.2	7.2	100	100	Hair	Asperger's Syndrome	E-2 criteria (Rimland 1968)
	Riyadh area, Saudi Arabia	65	80	9	7.2	100	100	Hair	Autism	E-2 criteria (Rimland 1968)
Albizzati et al., 2012	Italy	17	20	11.5 (6-16)	10.4 (6-16)	88.24	65	Hair	ASD	DSM IV, ADOS**
Blaurock-Busch et al., 2011	Saudi Arabia	25	25	5.29 (3-9)	6.25 (3-9)	88	76	Hair	ASD	DSM IV
Bradstreet et al., 2003	Arizona, USA	221	18	6.25 (3-15)	8.85 (3-16)	82.8	77.8	Urine	ASD	DSM IV
De Palma et al., 2012	Verona, Italy	44	61	9 (2-17)	8.4 (2-15)	84.10	40.10	Hair	Autism	DSM IV
Domingues et al., 2016	Bologna, Italy	29	36	7.3 (5-13)	8.4 (2-15)	89.7	72.2	Hair	ASD	DSM IV, ADOS, CARS**
El-Baz et al., 2010	Egypt	32	15	6.75 (2-13)	5.53 (2-11)	68.8	60	Hair	Autism	DSM IV

Elsheshtawy et al., 2011	Egypt	32	32	4.1 (3.8-4.1)	4 (3.8-4.1)	75	75	Hair	Autism	DSM IV
Hertz-Picciotto et al., 2010	California, USA	249	143	2-5	2-5	89	81	Blood	Autism	ADOS, ADI-R**, MSEL**, VABS**
Holmes et al., 2003	USA	94	45	1.5 (0.917-2)	1.5 (1-2)	77.66	75.56	Hair	Autism	DSM IV
Ip et al., 2004	Hong Kong	82	55	7.2 (4-11)	7.8 (4-11)	89	83.7	Hair	Autism	DSM IV
Jung et al., 2008	Korea	28	22	7.77	7.63	67.9	68.2	Hair	Autism	Diagnosis by psychiatrist/ developmental pediatrician
Kern et al., 2007	Dallas, USA	45	45	3	3	77.8	77.8	Hair	ASD	DSM IV
Lakshmi Priya and Geetha, 2011	India	45	50	4-12	4-12	80	40	Hair	Autism	CARS
Li et al., 2017	China	180	184	5.06 (3-8)	6.12 (3-8)	83.3	79.3	Blood	ASD	DSM IV, ICD-10**
Macedoni-Luksic et al., 2015	Slovenia	42	14	6.2	6.6	88.7	52.2	Blood	ASD	DSM IV
Majewska et al., 2010	Poland	55	38	3.69 (3-4)	3.55 (3-4)	54.55	50	Hair	Autism	DSM IV, CARS (at least score of 30 points)
		36	37	8.09 (7-9)	8.4 (7-9)	63.89	48.65	Hair	Autism	DSM IV, CARS (at least score of 30 points)
McKean et al., 2015	California, USA	164	58	0	0	91	79	Blood	Autism	ADI-R**, ADOS-G**, MSEL, VABS
Mohamed et al., 2015	Egypt	100	100	6.24 (2.5-15)	6.8 (2.5-15)	84	74	Hair	Autism	DSM IV
Mostafa and Refaei, 2007	Egypt	40	40	5.38 (3-8)	5.25 (3-8)	77.5	77.5	Blood	Autism	CARS

Mostafa et al., 2016	Egypt	84	84	6.8 (3-10)	7 (3-10)	73.81	63.5	Blood	ASD	CARS
Obrenovich et al., 2011	USA	26	39	N/A	N/A	N/A	N/A	Hair	ASD	DSM IV
Skalny et al., 2017a	Moscow, Russia	16	16	3.2 (3-4)	3.17 (3-4)	100	100	Hair	ASD	Diagnosis by psychiatrist/ developmental pediatrician
		17	17	6.85 (5-8)	6.85 (5-8)	100	100	Hair	ASD	Diagnosis by psychiatrist/ developmental pediatrician
Skalny et al., 2017b	Moscow, Russia	74	74	5.12 (3-8)	5.11 (3-8)	N/A	N/A	Hair	ASD	DSM IV, ICD-10
Stamova et al., 2011	USA	33	51	3.8 (2.3-4.75)	3.6 (2.3-4.75)	100	100	Blood	Autism	ADI-R, ADOS
Wecker et al., 1985	New Orleans, USA	12	22	5.67 (2-11)	4.18 (2-11)	100	100	Hair	Autism	DSM III**
Williams et al., 2008	USA	15	16	2-6	2-6	100	75	Hair	Autism	DSM IV
Woods et al., 2010	Washington, USA	59	59	6.01	6.39	100	100	Urine	Autism	DSM-IV, ADOS, ADI-R, CARS
		5	55	4.6	6.93	0	0	Urine	Autism	DSM-IV, ADOS, ADI-R, CARS
Wright et al., 2012	UK	47	115	9.6	12.6	79	55	Urine	ASD	RDC, ADI-R, ADOS-G
Yau et al., 2014	USA	76	147	0	0	N/A	N/A	Blood	ASD	DSM-IV, MSEL, VABS
Qin et al., 2018	Shenzhen, China	34	38	3-5	3-5	58.8	55.3	Blood	ASD	Diagnosis by psychiatrist/ developmental pediatrician

Gil-Hernandez et al., 2020	Spain	54	54	2-6	2-6	N/A	N/A	Hair	ASD	DSM-V, M-CHAT, ADOS-2, ADI-R, PDDBI, CARS, Battelle Developmental test, SDQ
Fido and Al-Saad, 2005	Kuwait	40	40	4.2 (4-7)	4.3 (4-7)	N/A	100	Hair	Autism	DSM-IV
Hodgson et al., 2014	Oman	27	27	5.3	5.5	81.5	74.1	Hair	ASD	CARS, DSM-IV
Yassa et al., 2014	Assiut city, Egypt	45	45	(3-10)	(3-10)	71.1	68.9	Hair	ASD	DSM-V
Metwally et al., 2015	Cairo, Egypt	55	75	4.56	N/A	N/A	N/A	Urine	ASD	DSM-V, CARS

* mean (range)

** Ne (Sample size of cases); Nc (Sample size of controls); DSM IV (Diagnostic and Statistical manual of Mental Disorders 4th Edition); DSM III (3rd edition); DSM V (5th edition); ADOS (Autism Diagnostic observation Schedule) (Le Couteuret al., 2003); CARS (Childhood Autism Rating Scale); ADI-R (Autism Diagnostic Inventroy-Revised) (Lord et al., 1994); ADOS (Autism Diagnostic Observation Schedules-Generic); ADOS-2 (2nd edition); ADOS-G (general); MSEL (Mullen Scales of Early Learning) (Mullen 1995); VABS (Vineland Adaptive Behavior Scales) (Sparrow et al., 1984); ICD-10 (International Classification of Diseases-10); RDC (Research Diagnostic Criteria from WHO International Classification of Diseases system version 10); M-CHAT (Checklist for Autism in Toddlers); PDDBI (The Pervasive Developmental Disorders Behavior Inventory); SDQ (Strengths and Difficulties Questionnaire)

Table A3. Meta-analysis calculations

Author	ASD Cases			Neurotypical Controls			N Total	SMD	SE	SMD (CI lower)	SMD (CI upper)
	Mean Hair Hg (µg/g)	SD	N	Mean Hair Hg (µg/g)	SD	N					
Adams et al., 2006	0.29	0.41	51	0.29	0.35	40	91	0	.20942085	-.41045733	.41045733
Adams et al., 2008	1.15	2.7	67	1.07	0.99	21	88	.03286371	.24791384	-.45303848	.5187659
Adams et al., 2008	0.9	1.3	11	0.7	0.55	11	22	.19276683	.41123707	-.61324301	.99877668
Adams et al., 2008	0.87	2.6	11	0.95	0.87	11	22	-.03969831	.41025263	-.84377869	.76438207
Adams et al., 2013	0.1875	0.1675	54	0.2175	0.19	44	98	-.16728472	.20185378	-.56291086	.22834143
Al-Ayadhi, 2005	1.941	0.065	2	0.713	0.228	80	82	5.3662151	.82370675	3.7517795	6.9806506
Al-Ayadhi, 2005	4.204	1.129	65	0.713	0.228	80	145	4.486223	.31143708	3.8758175	5.0966284
Albizzati et al., 2012	0.32	0.04	17	0.28	0.08	20	37	.60348174	.33030031	-.04389496	1.2508584
Blaurock-Busch et al., 2011	0.47	0.42	25	0.3	0.31	25	50	.45332025	.28206673	-.09952038	1.0061609
Bradstreet et al., 2003	7.14	15.09	221	2.28	2.72	18	239	.3328031	.24481033	-.14701633	.81262254
De Palma et al., 2012	0.54	0.5333	44	0.375	0.6407	61	105	.27380855	.19725288	-.1128	.66041709
Domingues et al., 2016	0.55	0.13	29	0.74	0.27	36	65	-.85676292	.25773555	-1.3619153	-.35161053
El-Baz et al., 2010	0.794	0.513	32	0.121	0.086	15	37	1.5443441	.34645977	.86529543	2.2233928
Elsheshtawy et al., 2011	4.1	0.8	32	4	0.8	32	64	.12348166	.24720462	-.36103049	.60799381
Hertz-Picciotto et al., 2010	0.1225	0.27	249	0.15	0.2575	143	392	-.10337229	.10478727	-.30875157	.10200699
Holmes et al., 2003	0.47	0.28	94	3.63	3.56	45	139	-1.547611	.20277211	-1.945037	-1.1501849
Ip et al., 2004	2.26	0.21	82	2.07	0.58	55	137	.4708579	.1756385	.12661276	.81510304
Jung et al., 2008	0.07	0.03	28	0.07	0.04	22	50	0	.28042655	-.54962594	.54962594
Kern et al., 2007	0.14	0.11	45	0.16	0.1	45	90	-.1886344	.209489	-.59922529	.22195649
Lakshmi Priya and Geetha, 2011	1.61	1.0849	45	0.37	0.04	50	95	1.646996	.23626037	1.1839341	2.1100578

Li et al., 2017	1.265	0.3425	180	1.53	0.4225	184	364	-.68683971	.10766996	-.89786896	-.47581047
Macedoni-Luksic et al., 2015	1.55	0.75	42	1.65	0.925	14	56	-.12392891	.30452579	-.7207885	.47293068
Majewska et al., 2010	0.137	0.163	55	0.23	0.296	38	93	-.40685369	.21131752	-.82102842	.00732105
Majewska et al., 2010	0.211	0.312	36	0.14	0.085	37	73	.30909739	.23303093	-.14763485	.76582962
McKean et al., 2015	1.1825	1.69	164	1.1825	1.4025	58	222	0	.15224947	-.29840348	.29840348
Mohamed et al., 2015	0.39	0.37	100	0.25	0.16	100	200	.48928966	.14299333	.20902789	.76955143
Mostafa and Refaei, 2007	1.345	0.4625	40	1.3125	0.45	40	80	.07053919	.22152002	-.36363208	.50471046
Mostafa et al., 2016	4.522	0.9975	84	4.655	1.197	84	168	-.12016845	.15374498	-.42150306	.18116617
Obrenovich et al., 2011	0.3	0.529	26	0.571	0.486	39	65	-.53179591	.2544697	-1.0305474	-.03304447
Skalny et al., 2017a	0.1283	0.1015	16	0.2257	0.2526	16	32	-.49323002	.35011156	-1.1794361	.19297604
Skalny et al., 2017a	0.1047	0.0881	17	0.1513	0.1341	17	34	-.40103041	.33840753	-1.064297	.26223617
Skalny et al., 2017b	0.1407	0.0607	74	0.1993	0.0667	74	148	-.91419028	.17196817	-1.2512417	-.57713886
Stamova et al., 2011	0.16	0.1875	33	0.15	0.205	51	84	.04995222	.22139135	-.38396686	.48387129
Wecker et al., 1985	15.2	2.078	12	15.2	1.407	22	34	0	.35039303	-.68675772	.68675772
Williams et al., 2008	0.07	0.06	15	0.085	0.07	16	31	-.2234986	.35117105	-.91178122	.46478402
Woods et al., 2010	0.72	1.17	59	0.51	0.77	59	118	.21066086	.18343523	-.14886558	.57018731
Woods et al., 2010	0.23	0.26	5	0.38	0.86	55	60	-.17781465	.46131881	-1.0819829	.7263536
Wright et al., 2012	1.76	5.34	47	1.53	6.9	115	162	.03527265	.1723233	-.30247481	.37302011
Yau et al., 2014	1.6225	1.668	76	1.36	1.42275	147	223	.17319876	.14104068	-.10323589	.44963341
Qin et al., 2018	0.9725	0.205	34	0.2825	0.2625	38	72	2.878431	.33477278	2.2222884	3.5345736
Gil-Hernandez et al., 2020	8.26	10.57	54	13	12.68	54	108	-.40319317	.19304447	-.78155338	-.02483297
Fido and Al-Saad, 2005	4.5	0.2	40	0.3	0.04	40	80	28.840843	2.2907976	24.350962	33.330723

Hodgson et al., 2014	6.93	.36	27	.611	.033	27	54	24.361478	2.3594793	19.736983	28.985972
Yassa et al., 2014	5.21	.08	45	.11	.05	45	90	75.798796	5.6535737	64.717995	86.879597
Metwally et al., 2015	19.37	11.65	55	3.91	.98	55	130	2.0213382	.21647365	1.5970576	2.4456187

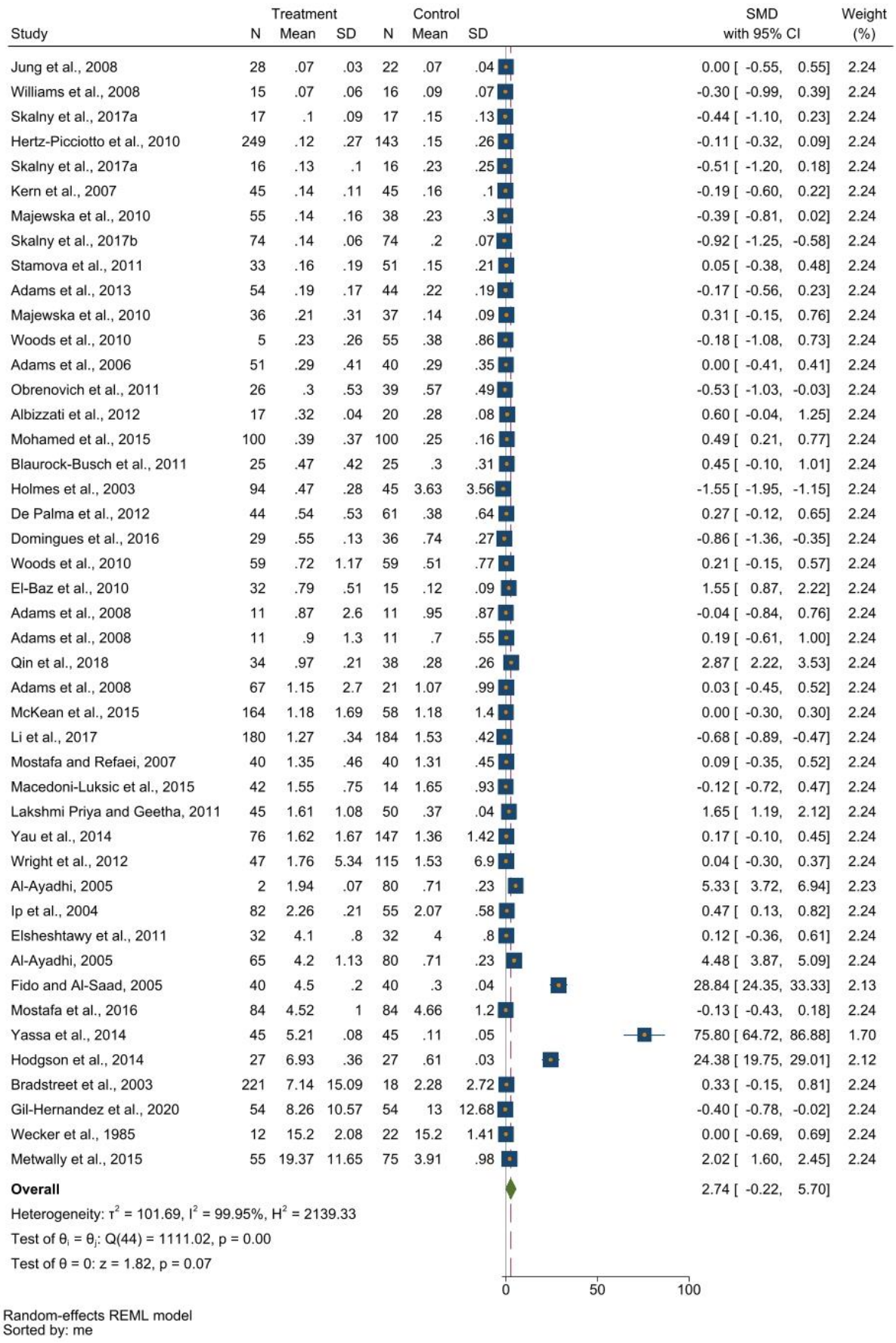

Figure A3. Forest plot of the association between hair Hg and ASD (39 studies; 45 datasets)

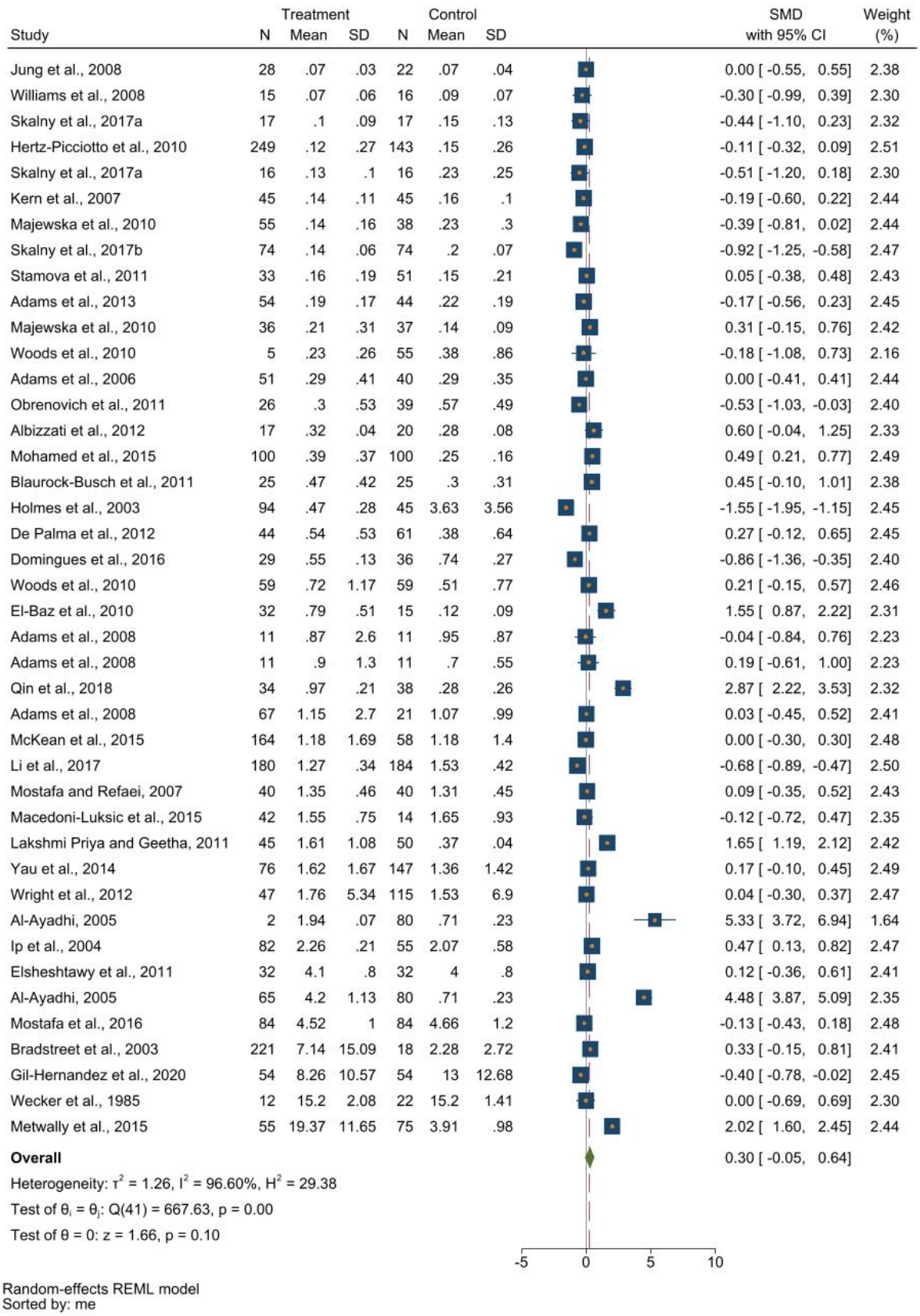

Figure A4. Forest plot of the association between hair Hg and ASD (w/o 3 outliers; Fido and Al-Saad, 2005; Yassa et al., 2014; Hodgson et al., 2014)

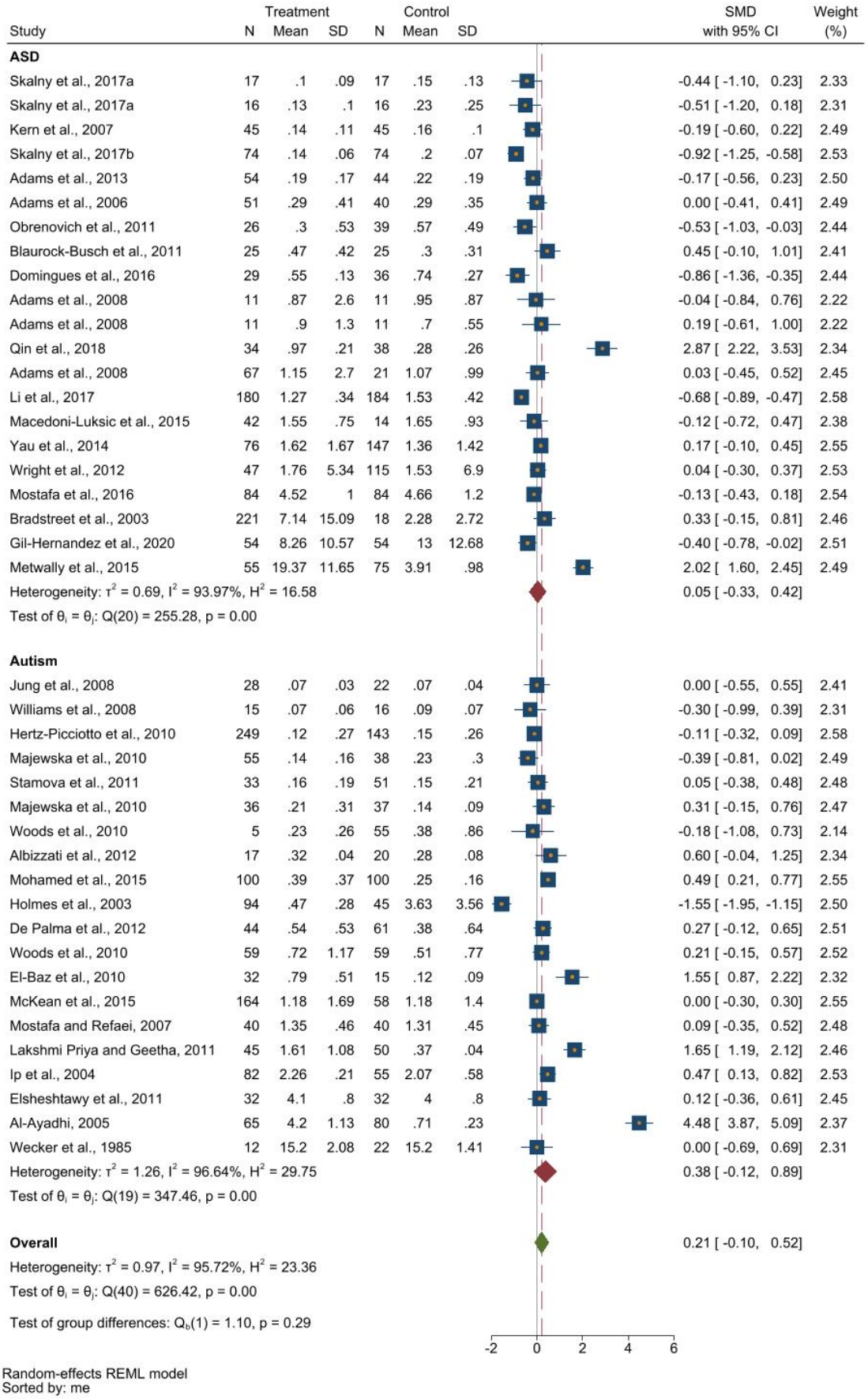

Figure A5. Forest plot of the association between hair Hg level and ASD patients and patients with "autism".

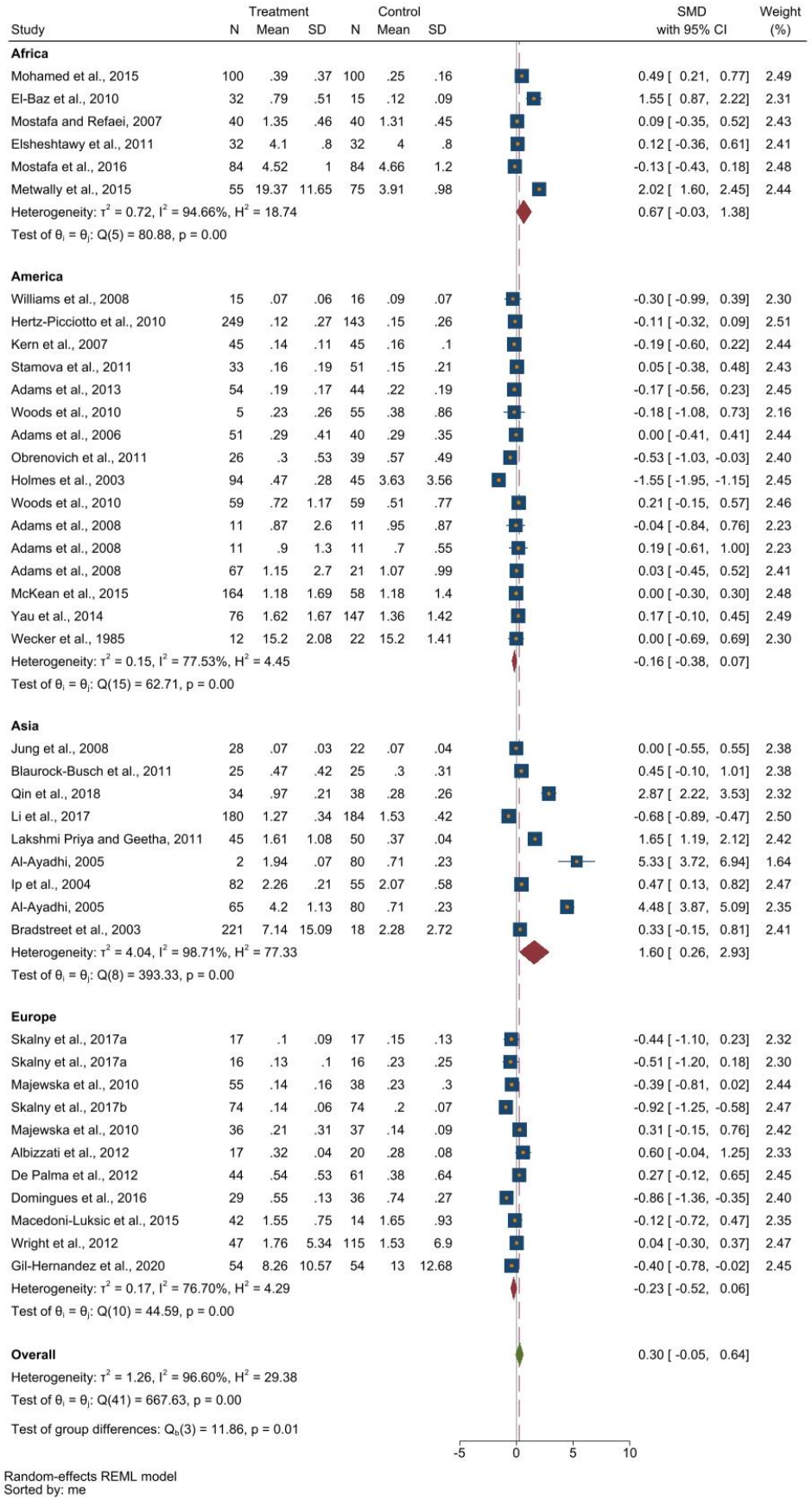

Figure A6. Forest plot of the association between hair Hg level and ASD in 5 different continents.

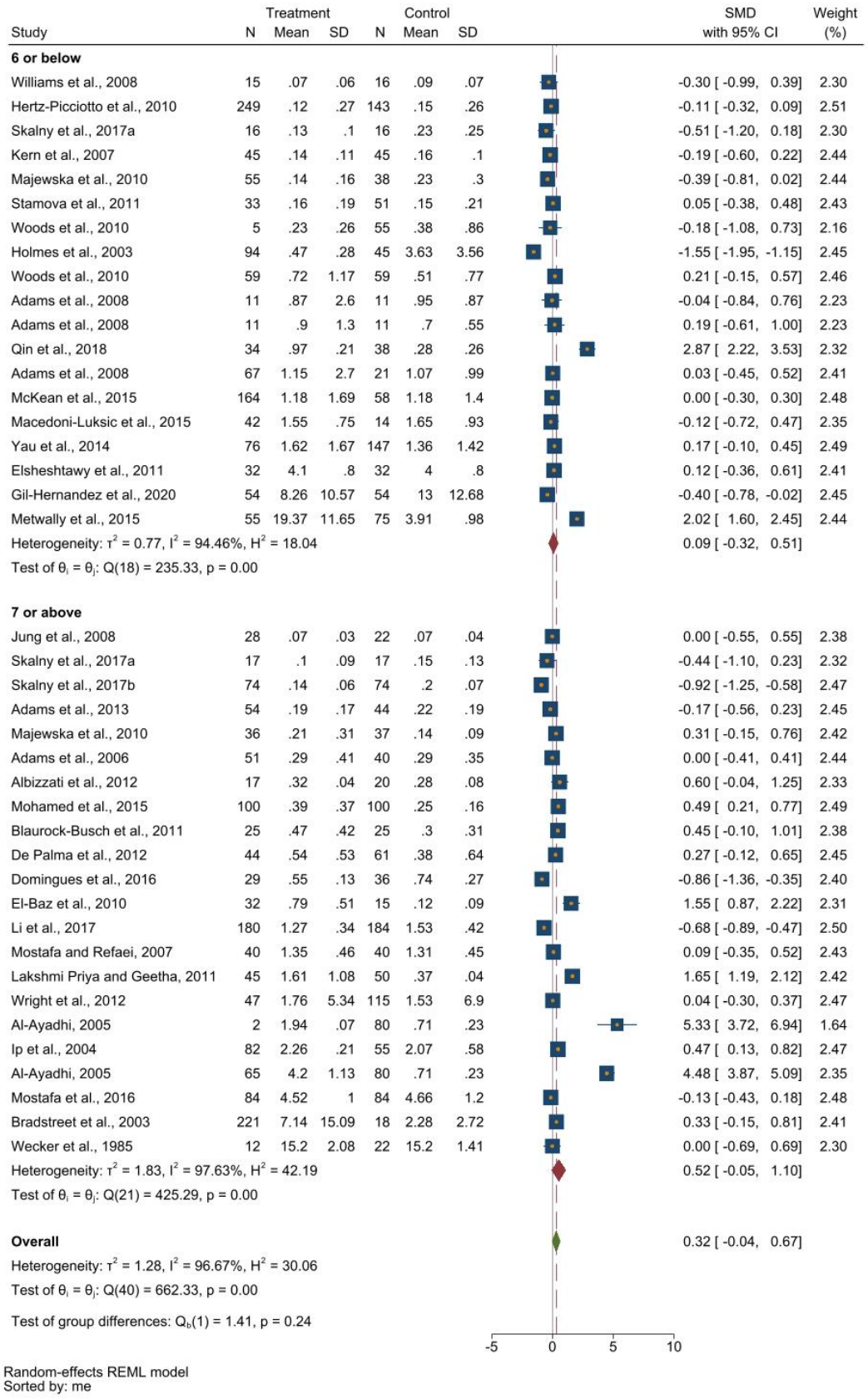

Figure A7. Forest plot of the association between hair Hg level and ASD in different age groups.

Table A4. Overall relationship trends found in the review papers

Type of Exposure	Number of review papers	Overall relationship
Thimerosal (vaccines)	5	No relationship
Air pollution	2	Positive
RBC Hg	3	Positive
Hair Hg	5	Inconsistent
Blood Hg	5	Inconsistent/positive
Urine Hg	4	No relationship

Table A5. Meta-regression data table (34 studies; 40 datasets)

```
. metareg _meta_es mc , wsse(_meta_se) graph noconstant

Meta-regression                                 Number of obs   =        40
REML estimate of between-study variance         tau2            =     1.399
% residual variation due to heterogeneity       I-squared_res   =    94.14%
With Knapp-Hartung modification
```

_meta_es	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
mc	.1019009	.1302872	0.78	0.439	-.1616299	.3654317

* 5 outliers were excluded (Gil-Hernandez et al., 2020; Wecker et al., 1985; Fido and Al-Saad, 2005; Yassa et al., 2014; Hodgson et al., 2014)

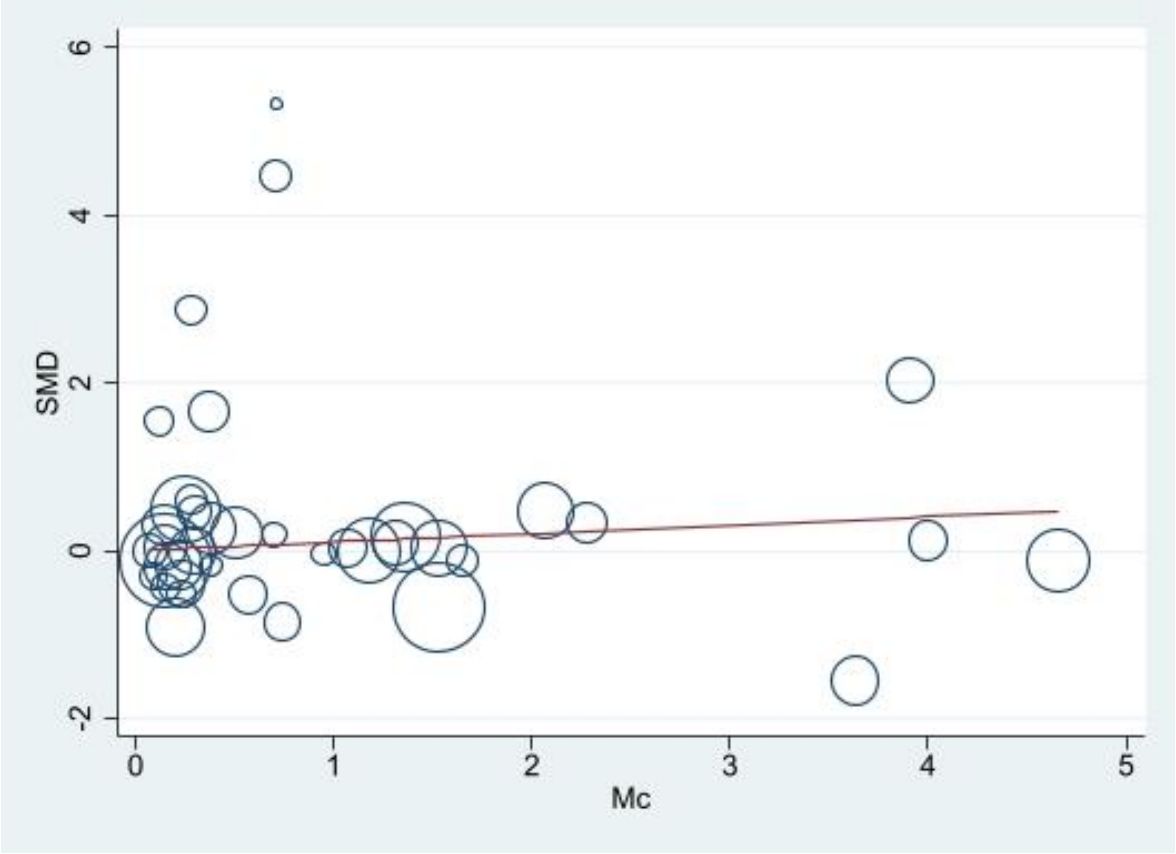

Figure A8. Meta-regression of SMD between hair Hg levels in ASD patients vs. neurotypical controls in relation to the mean Hg hair levels in the neurotypical controls

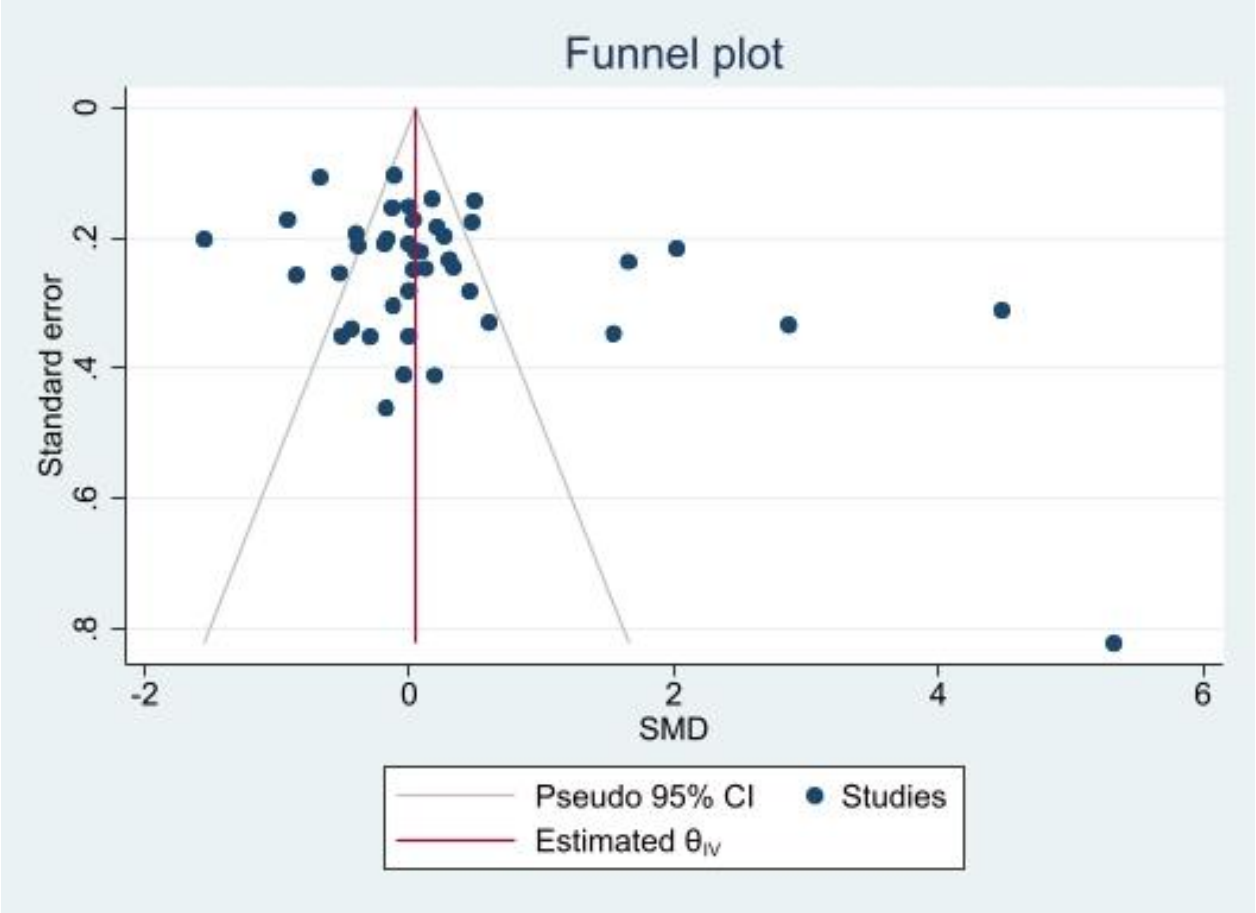

Figure A9. Begg's funnel plot of all the primary studies (36 studies; 42 datasets)

Table A6. PRISMA assessment (reporting quality) of the included systematic reviews.

Section/ Topic	#	Checklist item	Yoshimasu et al., 2014*	Taylor et al., 2014*	Saghazadeh and Rezaei, 2017*	Jafari et al., 2017*	De Palma et al., 2012*	Rossignol et al., 2014	Parker et al., 2004	Julvez and Grandjean, 2009	Kern et al., 2017	Koning et al., 2017	Pajewska-Szymyt et al., 2019	Wolowiec et al., 2013	Williams and Ross , 2007	Schoeman et al., 2015
Title																
Title	1	Identify the report as a systematic review, meta-analysis , or both	Y	Y	Y	Y	N	Y	N	N	Y	Y	N	N	Y	Y
Abstract																
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Y	Y	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	Y
Introduction																
Rationale	3	Describe the rationale for the review in the context of what is already known.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Methods																

Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N	N	Y	Y	N	N	N	N	N	Y	N	N	N	N
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Y	Y	Y	Y	Y	N	N	N	N	Y	N	Y	N	N
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Y	Y	Y	Y	N	Y	Y	Y	N	Y	N	N	N	Y
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	Y
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Y	Y	Y	Y	N	Y	Y	Y	N	N	N	N	N	Y
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Y	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	Y
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Y	Y	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Y	Y	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Y	Y	Y	Y	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A	N/A	N	Y	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Results																
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see Item 12).	Y	Y	Y	Y	Y	N	N	N	N	Y	N	N	N	Y
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot.	Y	Y	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Y	Y	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Y	Y	Y	Y	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Additional analyses	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A	N/A	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Discussion																
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers).	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias).	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N	N	Y
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Funding																
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Y	N	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N

* Systematic reviews with meta-analysis.

Table A7. AMSTAR-2 assessment (conducting/methodological quality) of the systematic reviews.

#	Checklist item	Yoshimasu et al., 2014*	Taylor et al., 2014*	Saghazadeh and Rezaei, 2017*	Jafari et al., 2017*	De Palma et al., 2012*	Rossignol et al., 2014	Parker et al., 2004	Julvez and Grandjean, 2009	Kern et al., 2017	Koning et al., 2017	Pajewska-Szymyt et al., 2019	Wolowiec et al., 2013	Williams and Ross , 2007	Schoeman et al., 2015
1	Did the research questions and inclusion criteria for the review include the components of PICO?	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
2	Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	N	N	Y	Y	N	N	N	N	N	Y	N	N	N	N
3	Did the review authors explain their selection of the study designs for inclusion in the review?	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
4	Did the review authors use a comprehensive literature search strategy?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
5	Did the review authors perform study selection in duplicate (in pairs)?	Y	Y	N	Y	Y	N	N	N	N	Y	N	N	N	Y
6	Did the review authors perform data extraction in duplicate?	Y	Y	N	N	Y	N	N	N	N	N	N	N	N	Y
7	Did the review authors provide a list of excluded studies and justify the exclusions?	Y	N	Y	N	Y	N	N	N	N	N	N	Y	N	N
8	Did the review authors describe the included studies in adequate detail?	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y
9	Did the review authors use a satisfactory technique for assessing the risk of bias	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	Y

	(RoB) in individual studies that were included in the review?														
10	Did the review authors report on the sources of funding for the studies included in the review?	Y	N	Y	N	N	N	Y	Y	N	Y	N	Y	Y	N
11	If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Y	Y	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	Y	Y	N	Y	N	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
13	Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?	Y	Y	N	Y	N	N	N	N	N	Y	N	N	N	Y
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Y	Y	Y	Y	N	N	N	N	N	Y	N	N	N	Y
15	If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Y	Y	Y	Y	Y	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Y	Y	Y	Y	N	Y	Y	N	N	Y	N	N	N	N

* Systematic reviews with meta-analysis.