2025

Vaccines, thimerosal and autism spectrum disorder

Evidence review 2010 to 2025

World Health Organization

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Acknowledgement

This report is the result of a review carried out by Daniels BA, Davis R, Ring E (Davis Pier Consulting) and Deeks S (Dalhousie University) on behalf of the Pharmacovigilance (PVG) Team, Department of Regulation and Prequalification (RPQ), World Health Organization (WHO). The review was guided by the PVG Team: Shanthi Pal and Xinyu Weng shaped the research questions and coordinated the review process; Madhav Ram Balakrishnan, EunMi Kim, Smaragda Lamprianou, Sujeet Jain provided technical inputs and reviewed draft versions of the report. Special thanks are due to Kristine Macartney, co-chair of the WHO Global Advisory Committee on Vaccine Safety, for her expert advice and significant technical contributions in the draft report.

Acronyms

ASD: Autism Spectrum Disorder

NDD / NDDs: Neurodevelopmental Disorder(s)

SoE: Strength of Evidence

RoB: Risk of Bias

WHO: World Health Organization

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PHAC: Public Health Agency of Canada

AEFI: Adverse Events Following Immunization

ADHD: Attention-Deficit/Hyperactivity Disorder

SSRIs: Selective Serotonin Reuptake Inhibitors

BCG: Bacille Calmette–Guérin vaccine

DTaP: Diphtheria, Tetanus, acellular Pertussis vaccine

Hib: Haemophilus influenzae type b vaccine

HepB: Hepatitis B vaccine

IPV: Inactivated Polio Vaccine

IJEV: Inactivated Vero Cell Culture–Derived Japanese Encephalitis Vaccine

Influenza H1N1: Influenza A (H1N1) vaccine

JEV: Japanese Encephalitis Vaccine

LJEV: Live Attenuated Japanese Encephalitis Vaccine

MMR: Measles, Mumps, Rubella vaccine

MMRV: Measles, Mumps, Rubella, Varicella vaccine

PCV: Pneumococcal Conjugate Vaccine

RV: Rotavirus vaccine

Td: Tetanus, Diphtheria vaccine

VAR: Varicella vaccine

OR: Odds Ratio

CI: Confidence Interval

Executive Summary

Background

Autism spectrum disorder (ASD) is a diverse group of neurodevelopmental conditions characterized by varying degrees of difficulty with social interaction and communication, with onset typically occurring in early childhood. Scientific evidence suggests that multiple factors, including both genetic and environmental influences, contribute to the development of ASD.

Concerns about a potential link between vaccines and ASD gained significant attention in the late 1990s, following the publication of a fraudulent study that proposed connections between natural or vaccine-derived measles virus, inflammatory bowel disease, and ASD—particularly in relation to the measles–mumps–rubella (MMR) vaccine (Wakefield et al., 1998; see retraction notice, Editors of the Lancet, 2010). Additionally, vaccines that contain the organomercury preservative thiomersal have gained focused attention as an exposure pathway for mercury that could contribute to the development of ASD and neurodevelopmental disorders (NDDs) more broadly.

Concerns about thiomersal-containing vaccines and MMR vaccines (which do not contain a preservative) have persisted over time, despite no strong evidence to support a causal relationship.

The Global Advisory Committee on Vaccine Safety has reviewed the evidence regarding a potential link between vaccines and autism multiple times between 2002 and 2012, consistently concluding that the evidence does not support a causal relationship. This updated review was commissioned by the World Health Organization (WHO) to provide a comprehensive overview of the published scientific literature on these topics since 2010.

Objectives

To complete a systematic review that answers the following research questions. based on literature that has been published from 2010 to August 2025:

- What is the relationship between thiomersal-containing vaccines, and ASD?
- More broadly, what is the relationship between vaccines and ASD?

As a secondary objective, we explored the relationship between vaccines and broader NDDs by including and analyzing studies returned by the search strategy that specified NDD outcomes (regardless if ASD was also included).

Summary of Methods

Briefly, systematic searches for scientific literature were conducted in PubMed, Ovid, MEDLINE, Embase, and PsychINFO during the month of August 2025, with search dates from 2010 to August 2025. Eligible studies were human studies that assessed any vaccine exposure and/or specific exposure to thiomersal, with outcomes related to ASD or NDDs. All studies were peer-reviewed; pre-prints were excluded. Data extraction was performed by three review authors, and the methodological quality was assessed by two review authors, and

screened for validity by the senior review author. A qualitative assessment of the Strength of Evidence (SoE) and Risk of Bias (RoB) was performed by at least two reviewers for each article and if there was no consensus, the more conservative value was used. Studies were grouped by the two research questions, and sub-grouped based on key themes (Ahmed et al., 2025) including study population (e.g., prenatal, childhood), and the direction of the study findings (i.e., no association, positive association). Narrative reviews were also captured in this process and were used as resources for the discussion section, but not analyzed for SoE and RoB.

Summary of Findings

The search strategy yielded a total of 36 research articles, 31 primary research studies and 5 meta-analyses. Of those, 17 were related to the thiomersal research question, 18 were related to the general vaccine research question and 1 meta-analysis investigated both thiomersal containing and MMR vaccines). An additional 21 narrative reviews were captured in the selection process and were used to provide context for how primary research articles contributed to the broader scientific narrative on pharmacovigilance related to vaccines, ASD and NDDs.

Thiomersal and ASD

Of the 18 studies included for the first research question (16 primary research studies and 2 meta-analyses), findings were evenly divided between those reporting no relationship between thiomersal and ASD and/or NDDs (*n = 9, including 2 meta-analyses*) and those that reported that there was a positive association (*n = 9*). Briefly, the studies were thematically categorized based on the study population at time of vaccine exposure including prenatal and childhood and they reported outcome measures by calculating odds ratios, hazard ratios, or regression analysis.

No increased risk of neurodevelopmental delay or ASD in children were found in two studies investigating prenatal exposure to thiomersal containing vaccines. In the ASD study, neonatal vaccines up to 20 months of age did not result in an increased risk of ASD development. This was consistent with an additional five studies that investigated childhood vaccines and provided evidence that thiomersal-containing vaccines are not associated with ASD (3 of the studies) or NDDs (3 of the studies). In total, nearly 27,000 children were assessed across these studies from multiple upper middle income (Brazil, Turkey) and high income (Canada, Japan, United States) countries. Conversely, nine studies indicated a positive association between childhood exposure to thiomersal containing vaccines and ASD (8 of the studies) and NDDs (4 of the studies), with some indicating a dose-dependent relationship. Across these studies, the data records of over 285,000 US children were used, although these records were disproportionately "control" data.

Despite the number of studies concluding opposing findings related to this question, these studies differed markedly in the overall SoE and RoB (*Figure 1*) largely due to methodological quality and consistency. In particular, the studies that concluded no association between thiomersal and ASD all had low[1] to moderate[2] SoE values. RoB values ranged from low to high, related to sample size and types of studies (e.g., ecological, cross-sectional), study limitations were described by the authors, and research findings came from a variety of different research groups and countries. In contrast, the studies that indicated that there was an association

[1] Weak study design. Findings provide little weight on their own but may be considered as part of the broader evidence base

[2] Reasonably strong study design but with some limitations. Results are robust but with reduced certainty of effect size

all came from one group of authors from the United States and all had very low[3] SoE and high RoB. Overall, the studies reporting an association were found to be less reliable, and lacking a certain level of precision, particularly in defining control groups and labelling and fully describing the study design and study limitations. Importantly, both of the two meta-analyses provide strong[4] supporting evidence in their combined analysis of 26 primary research studies (publication dates 2003 to 2012)[5] concluding that there was no association between thiomersal-containing vaccines and ASD.

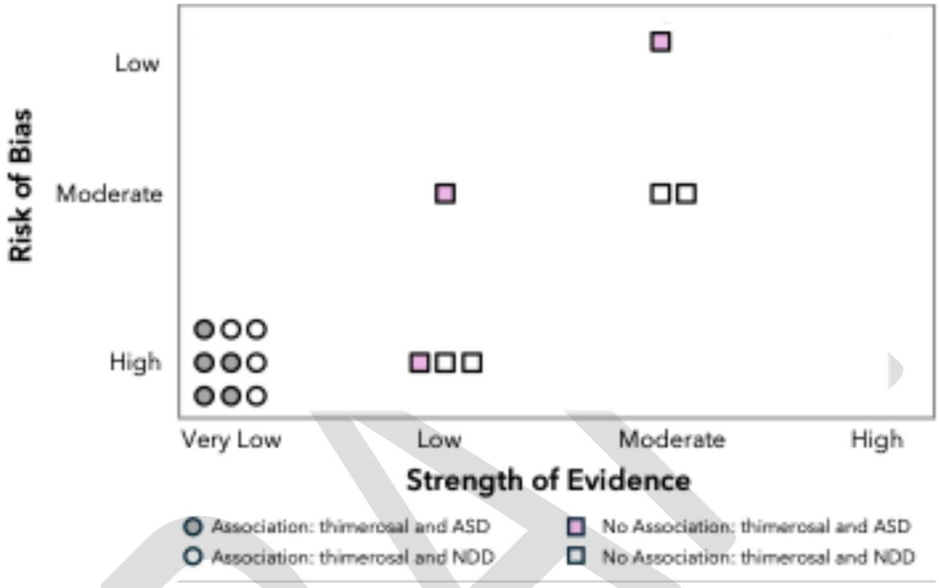

Figure 1. Summary of Strength of Evidence and Risk of Bias for Thiomersal and ASD / NDD.
Data points are categorized by their RoB and SoE values (adjusted so they are not overlapping). Three of the studies that assessed NDDs also assessed ASD.

Vaccines and ASD

Of the 19 studies (15 primary research studies and 4 meta-analyses) that were included for the second research question, the majority reported that there was no relationship between vaccines and ASD (*n = 17, including the 4 meta-analyses*) and only two reported that there was an association. These studies were also thematically categorized based on the study population at time of vaccine exposure including prenatal / neonatal and children, with an additional section on repeated exposure to vaccines in children.

No increased risk of ASD (4 studies) or NDDs (1 study) in children were found in the five studies investigating prenatal exposure to vaccines, regardless of trimester (as assessed in two of these studies). Similarly, no increased risk of ASD in eight studies that only investigated childhood vaccines, regardless of whether children received one or two MMR vaccine doses or a complete or incomplete set of childhood vaccines. In total, nearly 800,000 children were assessed across these studies from multiple high-income countries (Denmark, Italy, Japan, Poland, South Korea, United States). Conversely, two studies of records from at least 8,000 children

[3] Very weak or indirect evidence. Results are prone to bias, confounding and misinterpretation

[4] High-quality study design. Findings are robust to different assumptions, definitions, or analytic methods.

[5] One thiomersal-related study included this review (Price et al., 2010) was a part of both meta-analyses.

(United States) reported an association between vaccination exposures and increased odds of ASD following recommended childhood vaccines or the hepatitis B vaccine.

The SoE across the 13 primary research studies that indicated that there was no association between vaccines and ASD or NDD included several that were listed as "strong", owing to their overall study design (e.g., large retrospective cohort studies, control matching, adjusting for confounding variables). The remaining "no association" studies had low or moderate SoE. The two studies that showed a positive association between vaccines and ASD were both rated as very low SoE with high RoB. This included an ecological study that did not have individual exposure or outcome data, and a cross-sectional study that included a small case sample (*n = 30* ASD cases, *n= 7,044* controls) (*Figure 2*). Four meta-analyses also provide strong supporting evidence in their combined analysis of 32 primary research studies (publication dates 2001 to 2019) [6] concluding that there was no association between childhood vaccines and the development ASD.

Figure 2. Summary of Strength of Evidence and Risk of Bias for Vaccines and ASD / NDD.
Data points are categorized by their RoB and SoE values (adjusted so they are not overlapping).

Conclusion

The findings for each of the research questions are relatively consistent, underscoring that the most methodologically rigorous primary research studies do not support a causal association between vaccines, thiomersal-containing or otherwise, and ASD. Nine studies from a single author group did report an association with thiomersal-containing vaccines and ASD and/or other NDDS, and two studies from different author groups reported an association between MMR and ASD, however they all had very low SOE and high RoB. Moreover, none of the meta-analyses included in this review supported a causal association. These findings are also

[6] Collectively, four primary research studies on vaccines and ASD included in this review were also covered across the four meta-analyses.

consistent with the previous review conducted by WHO, as well as several other reviews that have been updated since 2012. The overwhelming weight of the evidence continues to strongly affirm the safety of current vaccines, showing no causal link with ASD or other NDDs.

Introduction

Vaccines are among the most effective public health interventions, preventing millions of deaths annually and significantly reducing infant and child mortality globally. Widespread vaccine use has led to the dramatic reduction of numerous fatal vaccine-preventable diseases and the eradication of smallpox. Despite these achievements, vaccine confidence has been challenged by real, presumed, and erroneously attributed vaccine safety concerns (Qian et al., 2020). These include issues that have been linked to the vaccine itself, as well as errors in administration. Understanding adverse events causally related to vaccines has been challenging for a myriad of reasons, with some examples being the difficulties of study design and data collection involved with the investigation of vaccine safety signals and by the spread of both misinformation and disinformation, amplified by social media and other online platforms. A number of factors have contributed to an erosion of trust in science and public health, decreasing vaccine acceptance including: (a) the challenges of proving a negative (i.e., there is no relationship between immunization and a negative outcome related to it); (b) the complexity of rigorously assessing causation when adverse effects occur after immunization (Canada, 2021); (c) the spread of disinformation; (d) the disconnect between lay understanding and scientific process and rigour; and, (e) the politicization of science.

One of the most sustained and controversial purported vaccine safety associations that has negatively impacted vaccine confidence is the alleged link between vaccines and autism spectrum disorder (ASD), a claim that has been extensively studied and consistently refuted by scientific research since the late 1990s. This concern gained worldwide traction in 1998 when Wakefield and colleagues (Wakefield et al., 1998) hypothesized a link between the MMR vaccine and ASD. Early methodological flaws and overreaching assumptions in this study, together with existing and rapidly developed scientific lines of evidence refuting any causal association, allowed early refutation of link. By 2010, the Lancet formally retracted this study due to serious methodological flaws, undisclosed conflicts of interest, and as the lead author was found guilty of professional misconduct and removed from the United Kingdom's medical registry (Eggertson, 2010). Since this initial study, an array of primary research studies and systematic reviews and analyses have been conducted to assess whether there is a causal association between vaccines and ASD using epidemiological methods. The majority have reported that there is no association between vaccines, or their components, and ASD or other NDD, suggesting no causal link (Taylor et al., 2014; Di Pietrantonj et al., 2021). However, a few published studies have reported an association, with authors suggesting a causal relationship (DeLong, 2011; Geier et al., 2013).

ASD is a diverse group of neurodevelopmental conditions characterized by varying degrees of difficulty with social interaction and communication, with onset typically occurring in early childhood. The etiology of ASD is multifactorial and heterogeneous, involving both genetic and environmental components. Over past decades, there has been a continued rise in ASD diagnoses (Zeidan et al., 2022). Much of this increase can be attributed to factors such as improved case identification, expanded diagnostic criteria, and heightened community awareness, as opposed to a true increase in incidence (Zeidan et al., 2022). Nevertheless, the temporal association between increasing ASD diagnoses with increased use of vaccines in children, concurrent with increased global attention to vaccine safety (including following the deployment of novel mRNA vaccines during the COVID-19 pandemic) in combination with increasing information and misinformation dissemination through digital platforms has fueled ongoing public concern around the association between vaccines and ASD (Maroni, 2024). This concern has also included other neurodevelopmental disorders (NDDs) in addition to ASD. NDDs are conditions that emerge early in life due to atypical development of the brain, affecting areas such as cognition, communication, behavior, or motor function (e.g., ASD, ADHD, intellectual disability, learning disorders, and communication or motor disorders).

A multitude of factors can contribute to vaccine hesitancy, which collectively carry significant public health risks. For example, individuals who believe that vaccines are unsafe are generally less likely to immunize themselves or their children, contributing to reduced vaccine coverage ultimately leaving populations vulnerable to vaccine-preventable diseases (Wagner et al., 2020). UNICEF's State of the World's Children 2023 reported that the number of zero-dose children, those who have not received a single routine vaccine, rose by more than 35 percent between 2019 and 2021 This underscores the urgency of reinforcing public trust in vaccines through transparent and science-informed communication.

Previous reviews, including those commissioned by the WHO), have examined the evidence on potential associations between vaccine components, such as thiomersal and aluminum adjuvants, and adverse health outcomes including ASD (Di Pietrantonj et al., 2021). These reviews, conducted between 2002 and 2012, consistently concluded the lack of credible scientific evidence supporting a causal association between vaccines (and components) and ASD. During this time period, the Global Advisory Committee on Vaccine Safety has also repeatedly concluded that the evidence does not support a causal relationship. However, given the expanding body of scientific literature since 2012, the emergence of new vaccine technologies, and the evolving public discourse, an updated review of the evidence is timely and necessary.

The objective of this systematic review is to provide a current assessment on the state of peer-reviewed studies published from 2010 to 2025 that investigate potential associations between vaccines and ASD, with a particular focus on the specific organomercury vaccine component thiomersal. Additionally, this review will highlight those studies that report on other NDDs. By reviewing the most recent evidence, this review seeks to provide clinicians, policymakers, and public health professionals with clear, reliable, and balanced information to support evidence-based decision-making, strengthen vaccine confidence, and contextualize ongoing scientific and public dialogue.

Methods

Research questions

This systematic review was designed to provide an updated understanding of the evidence that has accumulated since 2010 on two primary research questions:

1. What is the relationship between thiomersal-containing vaccines and ASD?
2. More broadly, what is the relationship between vaccines and ASD?

As a secondary objective, we explored the relationship between vaccines and broader NDDs. We searched broadly for the research questions and did not apply specific search filters for NDDs. Instead, all studies on vaccines and ASD were reviewed in full text, and findings on NDDs were extracted where available. A further targeted search for articles on NDDs and ASD was also completed.

Protocol

This review closely followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. All search results were imported into Covidence for de-duplication, screening, and documentation management.

Eligibility criteria

The review included peer-reviewed studies, published in English between January 2010 and August 2025 (inclusive), that involved human populations of any age, sex, gender, ethnicity, or with any underlying conditions. Eligible studies were required to assess either general vaccine exposure or specific exposure to thiomersal, with outcomes related to ASD. Studies were also deemed eligible if the study did not investigate ASD specifically, but did examine outcomes related to NDDs more broadly (e.g., ADHD, intellectual disability, learning delays and disorders, and communication or motor disorders). Accepted study designs included single-arm trials, randomized and non-randomized controlled trials, cohort studies (controlled or uncontrolled; prospective, retrospective, or non-concurrent), case-control studies, quasi-experimental studies (such as interrupted time series, controlled before-after studies), cross-sectional and ecological studies, etiological/mechanistic pathway studies, case series and meta-analyses.

Research that was only related to non-human (animal) studies (but included studies where human and non-human populations were assessed together), studies investigating vaccine adjuvants (e.g., aluminum) or preservatives other than thiomersal, case reports, pre-prints, clinical practice guidelines, editorials, opinions, commentaries, conference abstracts, letters, and news articles were excluded. Studies of candidate or investigational vaccines not authorized or marketed, publications prior to 2010, and non-English publications were also excluded.

Search strategy

The search strategy combined vaccine-related terms (“vaccin*”, “thiomersal”, “thiomersal”) with ASD-related terms (“autism”). Vaccine-related terms were combined with OR, and they were combined with ASD-related terms using AND. For the secondary objective, studies addressing NDDs that emerged during screening were

retained. Lastly, reference lists of included articles and citation tracking were also reviewed to capture any additional eligible studies.

Searches were conducted in PubMed, Ovid, MEDLINE, Embase, and PsychINFO. The search strategy was adapted for each database to maximize retrieval and ensure compatibility with Covidence.

Study selection

All results were uploaded into Covidence, where any duplications were removed automatically. Two independent reviewers screened the titles, abstracts, and full texts against the inclusion criteria. Conflicts between reviewers were resolved by a third reviewer at the end of each stage of the study selection process. Where uncertainties remained, cases were discussed with a senior public health physician and vaccine expert. Reasons for exclusion at the full-text stage were documented and reported in the PRISMA diagram (*Figure 3*). During this process several peer-reviewed narrative reviews were also selected if they explicitly addressed the primary or secondary research topics listed above. While they were not analyzed in the same manner as the primary research, they were used to help provide context on how primary research assessing vaccines and ASD has been interpreted and discussed more broadly (see Discussion).

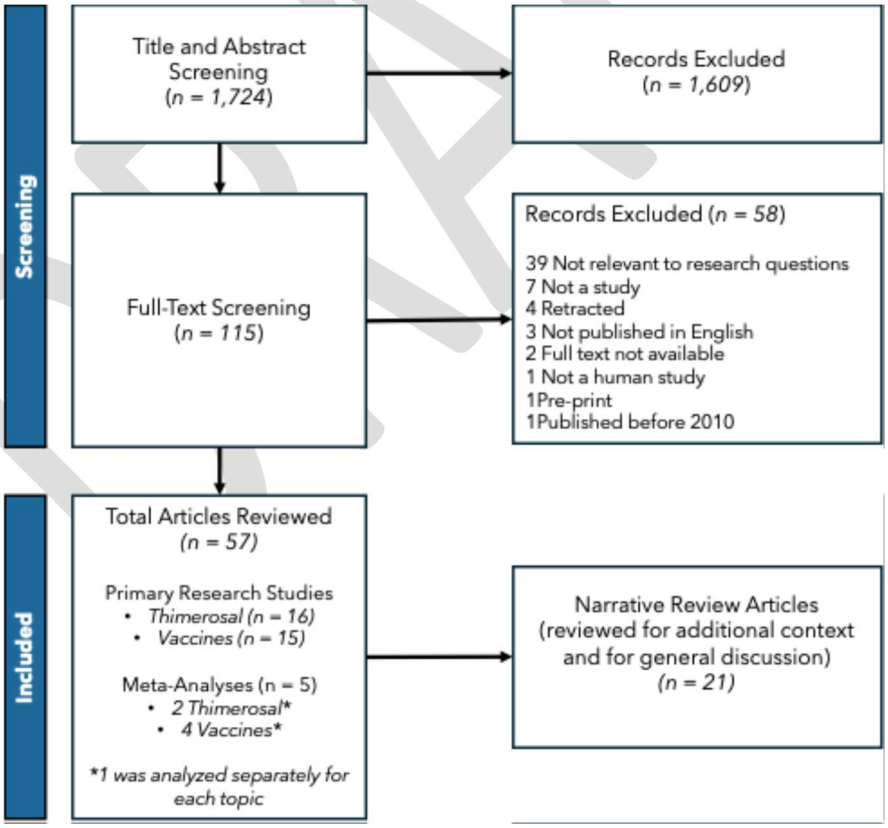

Figure 3. PRISMA diagram for study selection

Data extraction and analysis

Data extraction was performed independently by three reviewers using standardized criteria to capture study characteristics, populations, methods, findings, limitations, and gaps in evidence. Note that in some articles the n values differ between the abstract and methods, which summarized data cleaning processes and therefore provided the true study sample size. We chose to use the *n values* reported in the methods section of each article.

Findings were assessed for relevance, reliability, validity, and applicability, and thematic analysis was completed. To guide the qualitative assessment of the relevance, reliability, validity, and applicability of each article, we drew on elements of established critical appraisal tools, including the MetaQAT Tool from Public Health Ontario, RoB 2, and the Newcastle-Ottawa Scale. Principles from the Institute of Medicine Framework for Assessing Causality were also considered, where applicable, to help evaluate the strength of the evidence (SoE) and risk of bias (RoB of the included studies. At least two reviewers assessed SoE and RoB for each article and if there was no consensus, the more conservative value was used.

The following criteria were used to qualitatively assess the SoE and RoB for the included articles.

Strength of evidence (SoE)

SoE was assessed as the overall confidence that the study's findings reflect a true association (or lack thereof) between vaccines and ASD, based on review of study design, sample size[7], exposure/outcome measurement, analytic rigor, and consistency with the broader literature.

- **Strong:** High-quality design and analyses (large cohort, case–control) robust exposure and outcome measures (e.g., registries, biospecimens, gold-standard diagnostic tools), large sample size, appropriate adjustment for confounders, and findings consistent across sensitivity analyses. Additionally, results consistent with other high-quality studies can also demonstrate findings are robust to different assumptions, definitions, or analytic methods.
- **Moderate:** Reasonably strong design but with some limitations (e.g., modest sample size, administrative diagnoses instead of gold-standard tools, partial adjustment for confounders). Results are broadly consistent with other studies, but certainty is reduced.
- **Low:** Weak study design (e.g., small case–control, hospital-based, or cross-sectional), limited sample size, indirect or proxy measures of exposure/outcome, incomplete adjustment, or narrow generalizability. Findings provide little weight on their own but may be considered as part of the broader evidence base.
- **Very low:** Very weak or indirect evidence (e.g., ecological correlations, analyses using passive surveillance datasets, highly heterogeneous outcomes, or reanalysis with major methodological concerns). Findings are prone to bias, confounding, and misinterpretation; cannot support causal inference.

[7] While sample size is a component of understanding the Optimal Information Size (considering statistical power analyses), sample size was approached more broadly in line with modified Newcastle-Ottawa Scale criteria considering samples of ≥400 participants as "justified and satisfactory"

Risk of bias (RoB)

RoB reflects the likelihood that a study's design, conduct, or analysis introduced systematic errors that could distort results.

- **Low risk of bias:** Study design minimizes bias through prospective data collection, validated diagnostic tools, reliable exposure measurement, large sample size, appropriate comparison groups, and thorough adjustment for confounders. Sensitivity analyses confirm stability of findings.
- **Moderate risk of bias:** Some concerns exist (e.g., reliance on administrative diagnoses, modest sample size, incomplete confounder control, recall-based exposure measures), but these are unlikely to fundamentally change conclusions.
- **High risk of bias:** Major methodological issues (e.g., small case counts, proxy or heterogeneous outcomes, passive surveillance data, selective analyses, unaddressed confounding) that substantially weaken reliability. Findings may reflect artifacts of study design rather than true effects.

See Appendices for summary of extracted article information including commentary on the SoE and RoB.

List of vaccine abbreviations from included articles

Abbreviation	Full Name
BCG	Bacilli Calmette-Guerin
DTaP	Diphtheria, Tetanus, acellular Pertussis
Hib	*Haemophilus influenzae* type b
HepB	Hepatitis B
IPV	Inactivated Polio Vaccine
IJEV	Inactivated Vero cell culture-derived Japanese Encephalitis vaccine
Influenza H1N1	Influenza hemagglutinin 1 and neuraminidase 1
JEV	Japanese encephalitis vaccine
LJEV	Live attenuated Japanese Encephalitis vaccine
MMRV	Measles, mump, rubella, varicella
MMR	Measles, mumps, rubella
PCV	Pneumococcal conjugate vaccine
RV	Rotavirus
Td	Tetanus, diphtheria
VAR	Varicella

Overview of Findings

The database search yielded 2,921 records. After import into Covidence and removal of duplicates, 1,724 unique records remained. Title and abstract screening excluded 1,609 records as they did not meet the inclusion criteria. Subsequent full text review of 115 articles excluded 58 additional studies, resulting in 57 articles meeting criteria for inclusion in this review including 17 related to thiomersal-containing vaccines (16 primary research studies, 1 meta-analyses), 18 related to all other vaccines in general (15 primary research studies, 3 meta-analyses), one meta-analysis that looked at both thiomersal-containing vaccines and MMR, and 21 narrative reviews relevant for the two research questions (see *Figure 3*, above).

The findings of the literature search are organized by the two research topics using peer-reviewed articles that applied statistical analyses to assess the relationship between an intervention (e.g., receipt of thiomersal-containing vaccine) and an outcome (e.g., ASD, NDD) as per the inclusion criteria. Firstly, studies that specifically focused on thiomersal (and mercury-related studies) are presented. Secondly, studies that looked more broadly at any vaccine are presented. Each of these sections are further segmented into subthemes related to the overall narrative of the research being conducted such as the timimg of vaccine expousre in the study population (e.g., prenatal vaccination, childhood/adolescent vaccination) and the direction of association with ASD (i.e., no association or a positive association). The narrative reviews were assessed and highlighted in the Discussion section to demonstrate how the scientific community has broadly incorporated the primary research related to thiomersal, vaccines and ASD/NDDs into its discord.

Topic 1: Association between thiomersal and ASD

Reviewing the evidence for and against an association between thiomersal and ASD

The search strategy returned 18 research articles (16 primary research studies, 2 meta-analyses) from the past 15 years related to the topic of thiomersal and ASD. Altogether, the articles were focused on two main populations (pregnant women, children), and their exposure to thiomersal-containing vaccines).

Assessment of prenatal and neonatal thiomersal exposure and ASD or NDD (n = 2)

There were limited studies and study participants that assessed prenatal thiomersal exposure and its impact on neurodevelopment or in relation to ASD. A cross-sectional observational study (Marques et al., 2010) retrospectively investigated 82 mother-child pairs who were recruited into a different study on fish mercury exposure in Brazil from 2000-2001. It was later identified that 77% of the mothers had received a thiomersal-containing Td vaccine during pregnancy, while the remaining did not. The children, who all exclusively breastfed for 6 months, were assessed at this time for neurodevelopmental delays. Importantly, while neurodevelopmental delays (assessed by the Gesell Developmental Schedules) were associated with mercury levels in the child's hair samples, neither delays nor mercury levels were associated with vaccine administration. Additionally, a matched case-control studies concluded that thiomersal-containing vaccines or immunoglobins was not associated with increased ASD risk (256 children with ASD, 752 matched controls; Price et al., 2010). Logistic regression found no increased risk of ASD associated with higher thiomersal exposure at any assessed period (prenatal, birth–1 month, birth–7 months, birth–20 months. While both studies' findings support rejecting any association between prenatal thiomersal containing vaccine exposure and ASD or other NDDs, the overall strength of evidence (*Table 1*) is limited with the Marques et al., study due to a small sample size and study design, including the use of the Gessell Developmental Schedules which have been largely replaced by more modern developmental tests (e.g., the Denver Developmental Screening Test).

Table 1. Prenatal and neonatal thiomersal exposure and ASD or NDD

Author(s)	ASD	NDD	Study Type	*n (total)*	SoE	RoB
Marques et al., 2010	-	+	cross-sectional	82	low	high
Price et al., 2010	+	-	case-control	1,008	moderate	low

(see Appendix 1.1 for additional study details)

Assessment of childhood thiomersal exposure and ASD or NDD (n = 5)

There were four studies that investigated thiomersal-containing vaccines and their relationship to ASD specifically or NDDs more broadly, and a fifth study that examined vaccination history and blood mercury levels of children with NDDs. The lone ASD-specific study was a case-control study of 189 children with ASD and 224 age and sex matched controls in Japan that looked at the historical administration of six childhood vaccines (MMR, and thiomersal-containing vaccines: IPV, DTaP, JEV, influenza, HepB vaccines) over the first three years of life (Uno et al., 2015). The cumulative dose of thiomersal from the five thiomersal-containing vaccines was estimated based on vaccine records at 1, 3, 6, 12, 18, 24 and 36 months of age. The authors reported that neither MMR vaccines nor increasing dosage of thiomersal-containing vaccines was associated with ASD, with odds ratios ranging from 0.72 to 1.34.

A retrospective cohort study assessed the neuropsychological outcomes of 1,047 children in the United States (U.S.) aged 7 to 10 years who were born between 1993 and 1997 (Barile et al., 2012). The study used structural equation modeling of thiomersal exposure based on the clinical records of measured thiomersal contained in immunoglobulins and calculations of thiomersal in vaccines administration from birth to 7 months of age to determine if there was any relationship with impaired development of several neuropsychological constructs (intelligence, language, memory, executive function, fine motor skills, tics, behavior regulation). Overall, the study reported no evidence that thiomersal in vaccines was associated with negative long-term neuropsychological outcomes. While a small, statistically significant association with tics was observed, it was only evident in boys and was considered by the authors as likely spurious given challenges in measuring tics and weak biological plausibility.

A prospective longitudinal cohort study followed 535 mother-child pairs in 2009-2011 with pregnant women up to 19 years old who received prenatal care through the public health system in Brazil (da Cunha et al., 2020). Of those, 17% of children did not receive thiomersal-containing vaccines (listed in study as HepB, TripleViral and Tetravalent[8]), based on official immunization cards. The authors used linear regression with bivariate and multivariate analyses and reported that while there were some statistically significant relationships associated with motor, language, and cognitive development in the bivariate analysis, after controlling for confounding variables (e.g., maternal anxiety, education levels, childhood hospitalization), there was no relationship with thiomersal exposure in the multivariate analysis.

A cross-sectional prevalence study of 23,635 students up to grade 11 in Canada, compared the prevalence of pervasive developmental disorders[9] (PDDs) in children born before and after thiomersal-containing vaccines were discontinued in 1996 (Lazoff et al., 2010). A total of 187 children were identified with PDD and while the prevalence of PDD increased linearly across successive birth cohorts (OR 1.17 per year, 95% CI 1.12–1.23), this upward trend was unaffected by the removal of thiomersal in vaccines. The authors concluded that discontinuation of thiomersal in vaccines did not alter underlying PDD risk, suggesting that other factors (e.g., diagnostic broadening, awareness) accounted for rising prevalence.

The final article was a small-scale, cross-sectional study measuring the blood mercury and lead levels in 59 children with clinically diagnosed chronic NDDs (including 15 with ASD) and 59 age and sex matched controls (Dikme et al., 2013). The authors found that reported vaccination history and maternal dental fillings (potential

[8] It was not identified in the study which specific vaccines TripleViral and Tetravalent refer to

[9] The term PDDs has generally been replaced in scientific and other literature with ASD

mercury sources) were not associated with higher blood mercury in children. Furthermore, using atomic absorption spectrophotometry, no significant differences in blood mercury or lead levels between the two groups of children were identified. The research team concluded that blood mercury and lead levels of children with NDDs were not different from those without.

Together, these studies contribute to the epidemiological evidence that thiomersal-containing vaccines are not associated with ASD and NDDs. Across large prospective cohorts, case-control designs, and biomarker analyses, no relationship has been found, when other confounding factors were accounted for. As shown in *Table 2* there is a mix of SoE (low and moderate) and RoB (moderate and high) across these studies. Some of the key factors associated with the quality of these studies include robust statistical analyses that enabled removal of some confounding factors, prospective cohort design, the use of official vaccination records rather than parental recall, and the use of validated assessment tools. On the other hand, some limitations include small sample size, lack of detailed exposure history, and ecological study design.

Table 2. Childhood thiomersal exposure and ASD or NDD

Author(s)	ASD	NDD	Study Type	*n (total)*	SoE	RoB
Uno et al., 2015	+	-	case-control	413	low	moderate
Barile et al., 2012	-	+	retrospective cohort	1,047	moderate	moderate
da Cunha et al., 2020	-	+	prospective cohort	535	moderate	moderate
Lazoff et al., 2010	+	-	ecological	23,635	low	high
Dikme et al., 2013	+	+	cross-sectional	108	low	high

(see Appendix 1.2 for additional study details)

Reported association between thiomersal and ASD / NDDs (n =9)

The search strategy returned nine primary research studies, all from the same research group (Geier and colleagues), that describe positive associations, sometimes reporting dose-response relationships, between thiomersal containing vaccines, mercury exposure, and ASD or other NDDs. The Geier studies used variously accessed data from the U.S. Vaccine Safety Datalink (VSD), the Vaccine Adverse Event Reporting System (VAERS; a passive surveillance system database), the National Health and Nutrition Examination Survey (NHANES) to assess the associations between thiomersal exposure and outcomes such as ASD, atypical ASD, and emotional disturbances.

While this is not intended to be an overview of the work of the Geier research group, given the similarities across their studies, it is helpful to consider the included studies collectively (see Appendix 1.3 for specific details on each study). In general, this work relies heavily on the VSD dataset looking at populations of children and adolescent data records from 1990 to 2007. The group typically defined specific outcomes or cases of interest identified by selecting individual or collective International Classification of Diseases, 9th Edition (ICD-9) diagnostic codes, without clinical validation, such as ASD (Geier et al., 2017a), atypical ASD (Geier et al. 2017b), emotional disturbances (Geier et al. 2017c), PDD (Geier et al. 2015), and NDDs (Geier et al. 2014). Each of these studies then defined controls and exposure based exclusively on which type of Hib or HepB vaccine was reported in the VSD. Controls were defined as either those that did not receive a vaccine or that received a vaccine that was thiomersal-free. The comparator group was those that received the same type of vaccine except it contained or had a higher dosage of thiomersal.

Although not explicitly addressed in the studies, the distinction between thiomersal-containing and thiomersal-free vaccines often corresponded to differences in birth cohorts. Case and control groups were frequently

drawn from different time periods and different populations leading to a high degree of confounding. Children in earlier birth cohorts within the VSD dataset were more likely to have received thiomersal-containing vaccines, while those in later cohorts were more likely to have received thiomersal-free formulations. This temporal separation not only affects the probability of thiomersal exposure but may also influence the likelihood of receiving an ASD diagnosis (and other neurodevelopmental diagnoses and symptoms explored), given the documented increase in ASD prevalence and changes in diagnostic practices over time. In each of the studies highlighted above, Geier and colleagues found that there were significant differences in the odds ratios between comparators and controls, showing a positive association between thiomersal exposure and the type of diagnostic outcome under investigation.

Two other databases, NHANES (Geier et al., 2018a) and VAERS (Geier et al., 2013; 2016; 2018b) were also used to identify cases-control groups; however, in these databases the cases (e.g., ASD, NDDs) and exposures (e.g., thiomersal-containing or thiomersal-free vaccine receipt) were unverified reporting (e.g., parent reporting) and the studies relied on the vaccine administration date, based on when thiomersal was broadly removed from these childhood vaccines in the U.S. (around 2000). Again, as with the VSD-based datasets, Geier and colleagues found a positive association between thiomersal-containing vaccines and the case under investigation (e.g., ASD, neurodevelopmental outcomes). Importantly, these analyses rely on administrative coding, passive reporting, or narrow sub-populations (e.g., use of the VAERS database) which are vulnerable to confounding, exposure misclassification, selection biases, and temporal artifacts.

While these nine studies report an association between thiomersal-containing vaccines and ASD and other NDDs, several important limitations greatly affect the overall strength and reliability of the evidence (*Table 3*). Notably, the majority of studies from Geier et al. are stated by the authors to be case-control studies; however, the definition and selection of control groups are unclear, matching procedures are either not described or insufficiently detailed, and in some cases clearly do not meet the methodological criteria for a case-control study (e.g., comparable cases and controls). It appears from the total number of childhood records that were included that they are large studies (see *Table 3*). However, the number of cases relative to controls is quite low, representing less than 2% of the total numbers in most studies. Additionally, case and control data were often derived from different time periods, raising concerns about temporal comparability. Although it is impossible to account for all confounding variable, these studies generally do not account for any potential confounding variables, which, as highlighted above by Barile et al., (2012), can significantly distort associations and lead to misleading conclusions. Collectively, these studies suffered from substantial methodological flaws resulting in very low SoE and high RoB measures.

Table 3. Positive associations between thiomersal containing vaccines and ASD / NDDs

Author(s)	ASD	NDD	Study Type[10]	*n (total)*	SoE	RoB
Geier et al., 2017a	+	-	case-control	15,380	very low	high
Geier et al., 2017b	+	-	case-control	28,008	very low	high
Geier et al., 2017c	+	+	case-control	42,455	very low	high
Geier et al., 2015	+	-	case-control	26,166	very low	high
Geier et al., 2014	+	+	case-control	131,514	very low	high
Geier et al., 2018a	-	+	cross-sectional	1,192	very low	high
Geier et al., 2013	+	-	case-control	25,669	very low	high
Geier et al., 2016	+	-	case-control	11,856	very low	high
Geier et al., 2018b	+	+	case-control	3,346	very low	high

(see Appendix 1.3 for additional study details)

Results from peer-reviewed meta-analyses (n = 2)

Over the past fifteen years, two meta-analyses have statistically assessed vaccine administration, components, and specifically, thiomersal, and their potential relationship with ASD (Yoshimasu et al., 2014; Taylor et al., 2014) and Attention Deficit Hyperactivity Disorder (ADHD) (Yoshimasu et al., 2014). These meta-analyses calculate the odds ratios across studies to determine whether the odds of ASD (or ADHD) were altered following exposure to thiomersal-containing vaccines. Both meta-analyses concluded that there is no credible evidence of an association between thiomersal-containing vaccines and ASD. A similar conclusion was also reached for ADHD (Yoshimasu et al., 2014).

Yoshimasu and colleagues (2014) conducted a meta-analysis examining both thiomersal-containing vaccines and environmental mercury exposures during prenatal and early infancy. Drawing from 20 eligible studies (10 on thiomersal, 10 on environmental mercury exposures such as air pollution and dietary exposure), they reported no association between thiomersal exposure and ASD (summary OR 0.99, 95% CI 0.80–1.24). Similarly, no significant association was found for thiomersal and ADHD (OR 0.91, 95% CI 0.70–1.13). By contrast, significant associations were observed between environmental mercury exposures from air pollution for ASD (OR 1.66, 95% CI 1.14–2.17) and dietary exposure for ADHD (OR 1.60, 95% CI 1.10–2.33). The authors emphasized that these environmental findings should be interpreted cautiously given limited study numbers, methodological heterogeneity, and potential confounding. This meta-analysis provides clear and concrete evidence that vaccine-derived thiomersal exposure is not associated with ASD. Notably, three papers from Geier et al., (Geier and Geier, 2005; 2007; Geier et al., 2008) were captured in the meta-analysis by Yoshimasu and colleagues (2014); which still resulted in calculations that supported rejecting any causal association.

Taylor and colleagues (2014) pooled data from five cohort studies (over 1.25 million children) and five case-control studies (approximately10,000 children) assessing vaccines, thiomersal, mercury, and ASD outcomes. Of these, one study was included in this current review (Price et al., 2010). Their analysis found no increased risk of ASD associated with vaccination overall (OR 0.99, 95% CI 0.92–1.06), nor with specific exposures: MMR vaccine (OR 0.84, 95% CI 0.70–1.01), thiomersal (OR 1.00, 95% CI 0.77–1.31), or mercury (OR 1.00, 95% CI 0.93–1.07). Case-control data similarly reported no significant associations, and subgroup analyses did not alter these results. Risk of bias was generally low to moderate, with the strongest evidence coming from large-scale

[10] Study described by authors as case-control but lack key methodological elements of case control studies such as matched controls, valid exposure assessment, temporal clarity and control for confounding. More accurately described as ecological or cross-sectional

cohort studies (*Table 4*). The authors concluded that vaccines and their components are not associated with ASD.

Topic 1 Conclusion

Taken together, the best available evidence over the past 15 years does not support a causal relationship between thiomersal-containing vaccines and ASD, or NDDs more broadly. Multiple primary studies with detailed exposure assessment reported null results across prenatal and early-life windows. Ecological analyses show that ASD prevalence trends did not reverse after thiomersal was removed from routine schedules, and laboratory checks of vaccine lots that detected only trace mercury do not translate into population risk.

By contrast, the set of positive association studies, all from the same author group, are from analyses that rely on administrative coding, passive reporting, or narrow sub-populations (e.g., use of the VAERS database). These designs are vulnerable to confounding, exposure misclassification, selection biases, and temporal artifacts. Their findings have not been independently replicated at scale and are inconsistent with the direction and magnitude of effects seen in higher-quality evidence.

Finally, two independent meta-analyses synthesizing data from cohort and case-control studies (one of which was included in this analysis, Price et al., 2010) found no association with ASD and NDDs. One of the meta-analyses had selection criteria that excluded studies (presumably due to RoB associated with use of VAERS) such as those by Geier et al, highlighted above, while the other included three articles from this research groups from 2000 to 2010 providing commentary on the problems with them .

Taken together, the totality of evidence supports continued confidence in immunization programs while reinforcing the value of transparent communications, ongoing pharmacovigilance, and research that addressed genuine uncertainties. Future work is likely to be most impactful when focused on gene–environment interactions, well-designed observational studies with accurate exposure assessment, and outcomes beyond ASD—such as febrile seizures—framed within the context of their rarity and the overall benefit–risk profile of vaccines.

Topic 2: Vaccines and Autism

Evidence rejecting an association between vaccines (and vaccine-related interventions) and ASD

The search strategy returned 15 primary research studies and 4 meta-analyses from the past fifteen years whose analyses support rejecting the hypothesis that there is an association between vaccines and ASD. Collectively, the primary research studies investigated two main populations including (a) children whose mothers received prenatal vaccines (influenza, influenza H1N1, Covid-19 and DTaP vaccines *n = 5 studies*) and (b) children that received vaccines directly (influenza, DTaP, HepB, Hib, IPV, MMR, VAR vaccines) (*n = 9 studies*). One study looked at a subset of children (group b), focusing on younger siblings of those with a confirmed ASD diagnosis.

Prenatal Vaccinations and ASD or NDD in children (n = 5)

Vaccination and infections during pregnancy can pose concerns for both the health of the mother and child, during and following pregnancy. Across three studies, a total of 350,739 mother-child pairs were assessed for whether vaccination or infection from the vaccine-preventable disease (i.e., influenza or influenza H1N1 vaccine) during pregnancy was associated with increased risk of ASD among children (Zerbo et al., 2017; Ludvigsson et al., 2020; Becerra-Culqui et al., 2022). Another study investigated 81,993 mother-child pairs and whether DTaP vaccination during pregnancy was associated with increased risk of ASD in their offspring (Becerra-Culqui et al., 2018). Each of these four retrospective cohort studies followed children in the U.S. or Sweden for at least 6.5 years, collectively ranging from 2000 to 2018. The authors each used a Cox proportional hazard regression model to assess the risk of developing autism as a function of time, assessing any differences associated with the trimester that the mother developed the infection or received the vaccine. In all studies, hazard ratios ranging from 0.85 to 1.2 indicated that the children of mothers who were vaccinated during pregnancy (during any trimester) had no increased risk of developing ASD when compared to control groups of children of mothers who were not reported to have been infected or vaccinated during pregnancy.

The final study in this group was a smaller retrospective cohort study of 24,919 mother–child pairs in Scotland where mothers were infected with or vaccinated against Covid-19 during pregnancy and the child was assessed for neurodevelopmental concerns at 13 to 15 months (Hardie et al., 2025). Using this time point, the odds ratio was calculated and showed no increased risk in infants of mothers who received vaccine compared to infants of mothers who did not. The authors found a reduced odds (0.67 to 0.78) of problem-solving, personal–social, and emotional-behavioural concerns among infants compared to infants whose mothers did not have an infection or were not vaccinated during pregnancy.

Taken together, these articles provide robust evidence that supports rejecting an association between maternal vaccination as a cause of ASD in children (*Table 4*), given their strong SoE and correspondingly low RoB. Highlights associated with these ratings included the study type (i.e., respective cohorts) and larger sample sizes.

Table 4. Prenatal vaccinations and ASD / NDD in children

Author(s)	ASD	NDD	Study Type	*n (total)*	SoE	RoB
Becerra-Culqui et al., 2022	+	-	retrospective cohort	84,739	strong	low
Zerbo et al., 2017	+	-	retrospective cohort	196,929	strong	low
Ludvigsson et al., 2020	+	-	retrospective cohort	69,019	strong	low
Becerra-Culqui et al., 2018	+	-	retrospective cohort	81,993	strong	low
Hardie et al., 2025	-	+	retrospective population-level cohort	24,919	strong	low

(see Appendix 2.1 for additional study details)

Childhood vaccination and ASD (n = 5)

The five studies included in this section all included an assessment of the MMR vaccine, while two of them also investigated a broader range of vaccines including DTaP, HepB, Hib, IPV, VAR, BCG and IJEV / LJEV. Of these, three were retrospective cohort studies that included a total of 792,571 children from the U.S. (Jain et al.,

2015), Denmark (Hviid et al., 2019), and South Korea (Kim et al., 2022). A fourth study was a small sample case-control study of 96 children with ASD and 192 matched controls from Poland (Mrożek-Budzyn et al., 2010). Collectively the participants of this study were vaccinated (or not) from 1999 onwards and the association with ASD was assessed using either the hazards risk or the odds ratio. Each study consistently showed that there was neither increased risk or odds of receiving any of the vaccines administered and an ASD diagnosis in the children.

One of the strongest studies (strong SoE, low RoB) was by Hviid et al., (2019) who completed a nationwide cohort study in Denmark including 657,461 children born between 1999 and 2010 with follow-up through 2013, identifying 6,517 ASD cases. In addition to the overall findings showing no association between vaccination and ASD (aHR=0.93, 95% CI 0.85–1.02), subgroup analyses also found no association among children with autistic siblings, those with high autism risk scores, or across different time intervals since vaccination. Jain et al., (2015) focused on familial history of ASD and the likelihood of a younger sibling being diagnosed with ASD after receiving MMR vaccine. The authors found that there were no increased odds of ASD diagnosis for any group regardless of whether they had an older sibling with ASD or not. Kim et al., (2020) concluded that vaccination not only prevents against the vaccine preventable disease but may also confer protective effects against ASD-related traits and broader developmental psychopathology. Altogether these four studies had moderate to strong SOE and low to moderate ROB, with several large cohorts providing representative study populations and reliable information on vaccine status and clinical diagnoses based on national registry data, medical records, insurance claim data, and detailed clinical reporting.

The final study in this section provides a slightly different view into the research question because the entire study population had a confirmed diagnosis of ASD. Goin-Kochel and colleagues (2016) analyzed the parent-reported vaccine receipt among 2,755 children from the U.S.[11] with ASD enrolled in the Simons Simplex Collection (SCC) using pairwise comparisons across six vaccines (exposure) and four types of ASD onset (outcomes). They reported that there was no relationship between the receipt of any of the vaccine types and any of the patterns of onset of ASD. The SSC[12] serves as an important database in the ASD ecosystem. It is a data repository of nearly 3,000 children and adolescents (U.S. and Canada) who were a single child and diagnosed with ASD and with no other ASD or suspected ASD diagnoses in any second- or third-generation relative. Between 2007 and 2011, detailed clinical and genetic data were collected in the children who ranged in age from 4 to nearly 18 years, with the earliest participants born in 1991. Some researchers have used the repository to explore vaccine hesitancy (Fischbach et al., 2016), parental beliefs about causes and the relationship between different types of ASD (e.g., regression, late onset), and the child's vaccine history (mostly parent-reported). The vaccine coverage for core vaccines amongst the participants in the repository was high, with greater than 90% for DTaP, HepB, Hib, MMR, and IPV vaccines and greater than 80% for VAR. The study's large, well-characterized study population and use of standardized ASD diagnostic tools strengthen its SoE and RoB (*Table 5*), though reliance on parental report for vaccination limits precision.

[11] The authors excluded Canadian data from SSC in case there were any differences due to different vaccine schedules

[12] https://www.sfari.org/resource/simons-simplex-collection

Table 5. Childhood vaccination and ASD

Author(s)	ASD	NDD	Study Type	*n (total)*	SoE	RoB
Kim et al., 2020	+	-	cross-sectional population-based	39,383	moderate	moderate
Hviid et al., 2019	+	-	nationwide cohort	657,461	strong	low
Mrożek-Budzyn et al., 2010	+	-	case-control	288	moderate	moderate
Jain et al., 2015	+	-	retrospective cohort	95,727	strong	low
Goin-Kochel et al., 2016	+	-	cross-sectional	2,755	low	moderate

see Appendix 2.2 for additional study details)

Repeated vaccine exposure, antigen exposure, antibody response and ASD in children (n = 3)

The final primary research studies in this section are all case-control studies focused on repeated exposure to childhood vaccines and the corresponding increased antigen exposure, and subsequent antibody response to vaccine administration among children with ASD (cases) and those without (controls). Uno and colleagues (2012) performed a case–control study in Japan to examine MMR and other childhood vaccines in relation to ASD including 189 children with ASD (cases) and 224 matched controls. Based on the odds ratio there was no significant associations between either ASD in children and receipt of MMR vaccine (OR 1.10, 95% CI 0.64–1.90) or the total number of vaccine doses and ASD (OR 1.10, 95% CI 0.95–1.26). DeStefano et al., (2013) analyzed data from 256 children with ASD (cases) and 752 matched controls enrolled in three managed care organizations in the U.S. Using antigen counts from vaccine registries and records, they assessed cumulative and peak exposures during the first two years of life. Across multiple models, including ASD subtypes (e.g. ASD with regression) no significant associations were observed (adjusted ORs ≈ 1.0 per 25-antigen increase). Finally, Gentile et al., (2013) assessed immune responses to MMR vaccine antigens in 31 children with ASD and 29 controls. Seropositivity rates and antibody titers were comparable across groups, and no associations were found between antibody levels and ASD severity scores. Altogether, these studies had a mix of SoE from low to strong, with low to moderate RoB (*Table 6*). Each of the studies verified assessments of clinical diagnoses, had well-matched controls, and small to medium sample sizes. In particular, the rigorous exposure and outcome measurement, combined with null findings across multiple definitions by DeStefano et al., (2013), provide strong evidence that immunologic stimulation from vaccines does not increase ASD risk.

Table 6. Repeated exposure, antigen exposure, antibody response, and ASD in children

Author(s)	ASD	NDD	Study Type	*n (total)*	SoE	RoB
Uno et al., 2012	+	-	case-control	413	moderate	moderate
DeStefano et al., 2013	+	-	case-control	1,008	strong	low
Gentile et al., 2013	+	-	case-control	60	low	moderate

(see Appendix 2.3 for additional study details)

Evidence in support of a relationship between vaccination and ASD (n = 2 articles)

Two U.S.-based studies supported an association between vaccination exposures and increased odds of ASD or related developmental outcomes. DeLong (2011), using an ecological study design at the U.S. state-level,

examined the association between aggregate childhood vaccine coverage at 2 years of age in 1995-2001 with the prevalence of ASD and speech language impairment (SLI) among 8-year-olds in 2001-2007. The study reported a positive association such that a 1% increase in vaccination coverage correlated with a 1.7% rise in ASD or SLI prevalence. Gallagher and Goodman (2010) used U.S. National Health Interview Survey data (1997–2002) in a cross-sectional study design to assess whether HepB vaccination in the first month of life was associated with parental reports of an ASD diagnosis in boys aged 3 to 17 years. Among boys born before 1999, neonatal vaccination was associated with a threefold higher odds of ASD compared to later- or never-vaccinated boys, even after adjusting for race, maternal education, and family structure. Both studies provide very low SoE due to low methodological quality and carry a high risk of bias (*Table 7*), particularly related to confounding, misclassification, and inappropriate causal interpretation for DeLong (2011). Additionally, the study by Gallagher and Goodman (2010) acknowledged limitations to SoE including reliance on parental reporting of ASD, small sample size (n = 30 autism cases), and lack of mechanistic data.

Table 7. Evidence in support of a relationship between vaccination and ASD

Author(s)	ASD	NDD	Study Type	*n (total)*	SoE	RoB
DeLong, 2011	+	-	ecological	n/a	very low	high
Gallagher and Goodman 2010	+	-	cross-sectional	7,074	very low	high

(see Appendix 2.4 for additional study details)

Results from peer-reviewed meta-analyses (n = 4)

Over the past 15 years, there were four distinct meta-analyses that included a statistical assessment of vaccine administration in children and its relationship to ASD (Di Pietrantonj et al., 2021; Gidengil et al., 2021; Taylor et al., 2014; and Hobson et al., 2012). Generally, these analyses included studies that examined childhood vaccination up to five years of age. Two of these reports were updates to previously published analyses (Di Pietrantonj et al., 2021 and Gidengil et al., 2021), which were also considered in our review. All these studies primarily used the odds ratio to determine whether the odds of a child developing ASD after receiving or not receiving an MMR vaccine were equivalent. Other types of vaccines (e.g., VAR), vaccine-related components (e.g., thiomersal), and NDDs (e.g., speech delay) were also reported on in some of these meta-analyses. Importantly, none of the meta-analyses included any studies that individually reported an association between vaccine receipt and ASD. These studies were screened out of the meta-analyses based on small sample size and other methodological concerns. Two studies, which assessed a broad range of vaccine safety outcomes beyond ASD, reported safety concerns based on the odds ratio for aseptic meningitis, febrile seizures and idiopathic thrombocytopenic purpura. It is important to note that there was considerable, but not complete, overlap between the source primary research articles that met the search criteria across the four meta-analyses.

Two of the meta-analyses did include analyses of the SoE and inherent study bias (Taylor et al., 2014; Gidengil et al., 2021), providing a systematic assessment of how readers should interpret their findings. Where available, they report low to moderate SoE[13] suggesting confidence in the validity of the findings, with some uncertainty

[13] There is consistency between the results for SoE and RoB reported our review

of the accuracy of the specific measures (e.g., odds ratios). Where RoB was explicitly highlighted, studies generally had low or moderate RoB, accepting that in observational population health studies there is inherent difficulty in having optimal research study design. For example, in studying differences between vaccinated and unvaccinated individuals, where vaccine coverage is high, the probability of having confounding factors across groups is also high. The articles also note potential limitations with the studies within their meta-analyses such as small sample sizes and selection bias due to participants screening. Each of the meta-analyses are explored in further detail below.

The Cochrane Library has maintained a review of the MMR and VAR vaccines since 2005, with updates in 2012 and 2021 (Di Pietrantonj et al., 2021). The Cochrane Review explores outcomes related to both (a) the effectiveness of vaccination and (b) vaccine safety, by assessing 138 articles, from any language, involving a total of nearly 23.5 million children. The search strategy for vaccine safety covered almost 60 years, from 1966 to 2019. Over this time the authors consistently reported that the vaccines under investigation were effective, with differences noted depending on vaccine type and dosage. There were reported safety concerns related to MMR/MMRV vaccines including some associations with aseptic meningitis, febrile seizures, and idiopathic thrombocytopenic purpura.

Importantly, based on 13 studies (including the four since 2010 that are included in this report), the Cochrane Review reported no association with ASD following receipt of childhood vaccines up to 5 years of age. Of these 13 studies, three were cohort studies totaling nearly 1.23 million children and could be assigned a Grade of Evidence, each with either low or moderate certainty about the outcome of no association. Similarly, no association between vaccine receipt in childhood was reported for another NDD (cognitive delay /developmental delay), with low certainty (337 children in a separate cohort study). Of the remaining studies, four were case-control, one was self-controlled case series, one was person-time cohort, and three were case-only ecological method, and were all published before 2010.

A review of the safety of vaccines administered in the U.S. was published in 2021 (Gidengil et al., 2021) and was an update to a 2014 AHRQ review and a 2011 Institute of Medicine report. This safety review included, among other outcomes, exploring the relationship between ASD and the and receipt of MMR vaccine in childhood. The review excluded studies of vaccines not currently in use in the U.S. and excluded non-English language studies. Similar to the Cochrane review, there was a clear association between MMR vaccine administration and the development of febrile seizures. The authors reported a high SoE to state there was no association between ASD and MMR across two studies. Of the 338 articles included in the study, only two new articles related to MMR vaccine and ASD were found for the updated report, both of which were returned in the search strategy for this report.

A 2014 meta-analysis of five cohort studies (~1.3 million children) and five case-control studies (~10,000 children) reported that there was no association between vaccines and/or vaccine-related components (i.e., mercury, thiomersal) and ASD based on calculating the odds ratios across the study outcomes (Taylor et al., 2014). This meta-analysis is also covered above in the Thiomersal and ASD section above.

Seven case-control studies and/or case series (~1,700 children) determined that there was no association between MMR vaccines and ASD specifically (Hobson et al., 2012). While six of the seven primary studies were all published before the search criteria for this review, the meta-analysis represents yet another published statistical analysis of those studies demonstrating that the odds of a child developing ASD after receiving or not receiving an MMR vaccine were equivalent.

Topic 2 Conclusion

The relationship between receipt of vaccines and development of ASD has been the subject of extensive research and debate over the past two decades. Between 2010 and 2025, the literature expanded to include multiple meta-analyses, large-scale epidemiological studies, ecological analyses, and mechanistic reviews.

From a public health perspective, the strongest and most methodologically rigorous evidence - meta-analyses of large cohort and case-control studies, and registry-based investigations - consistently demonstrates no causal link between vaccination and ASD. At the same time, cautionary papers highlight theoretical risks, ecological correlations, and mechanistic pathways for the development of ASD that remain incompletely understood. These have sustained public controversy, particularly in the context of widespread misinformation, disinformation and vaccine hesitancy.

Taken together, the cumulative findings from published research provides compelling evidence that vaccines, whether administered during pregnancy or childhood, do not increase the risk of ASD in childhood. Studies of maternal influenza, COVID-19, and DTaP vaccination show no elevated risks of ASD among children, while some even suggest protective associations with broader developmental outcomes. Large registry-based analyses confirm that MMR vaccine receipt in children does not increase ASD risk, even among children with familial vulnerability. This evidence-base supports the safety of vaccines with respect to ASD and underscores their broader role in protecting maternal, child, and population health.

Discussion

This updated literature review on the relationship between vaccines and thiomersal and their association with ASD and other NDDs is timely, as conflicting scientific evidence and general understanding continues to influence public discourse, personal decision-making, and public policy. The impact of this dissonance is still being realized, yet there are clear public health signals that can be seen due to sustained hesitancy around the role of vaccines in our daily lives. This review was conducted to support WHO's understanding and inform communications about the current state of the association between prenatal and childhood vaccines and neurodevelopmental outcomes, with a particular emphasis on thiomersal-containing vaccines and ASD. As highlighted in the results of this analysis, several organizations and researchers have put forward updated syntheses of the scientific literature on these topics, with the majority reaching a consistent conclusion that there is no evidence for a casual relationship between receipt of vaccines, whether or not they contain thiomersal, and development of ASD. This conclusion is strongly reinforced by the results of the current review, particularly when considering the methodological rigor, overall study designs, and statistical approaches employed across the studies finding no associations, many of which vary in population, data sources, and exposure/outcome definitions, yet yield consistent findings.

It is important to acknowledge that several high-quality studies published prior to 2010 were not included in this review but continue to contribute meaningfully to the broader body of evidence (e.g., Dales et al., 2001; Madsen et al., 2002; Institute of Medicine, 2004). From a methodological standpoint, definitively proving a null hypothesis, such as the absence of an association between vaccines and ASD, is inherently challenging. This uncertainty may leave room for lower-quality studies with methodological limitations to influence the discourse and perpetuate unsupported associations.

Beyond the primary research outlined above, there is a broader scope of narrative reviews from researchers around the world that are important to recognize because they may have a broader audience about the state of vaccines and ASD and, arguably, are more accessible than having to understand the mechanistic details of primary research papers. This is particularly relevant given that, at this stage of the scientific discourse, the quality and robustness of studies supporting claims of an association may be difficult to assess. In total, 13 reviews from a variety of sources including independent, university-based researchers and research-based organizations (e.g., RAND Corporation) highlight that there is no relationship between vaccines in general, and thiomersal-containing vaccines specifically, and ASD. Indeed, there is a consistent acknowledgment that MMR vaccine is a cornerstone of public health, preventing both immediate morbidity and long-term complications without contributing to ASD risk (Bester, 2016). Similarly, thiomersal-containing vaccines are safe for infants, children, and adults, and the public health benefits of vaccination far outweigh any theoretical risks (Gołoś and Lutyńska, 2015). There is an acknowledgement that minor adverse effects are more frequent with adjuvanted vaccines, but that both thiomersal and squalene remain safe for use in influenza vaccines (Montana et al., 2010). Garaparini et al., (2015) highlight how early, methodologically-flawed studies and media amplification fueled public concern, despite subsequent large-scale epidemiological research consistently showing no association. Those studies and ideas continue to be championed in the literature by a subset of researchers and organizations, such as Geier and colleagues who also had three narrative review articles that took these opposing views (Geier et al., 2010; 2015; Kern et al., 2016). The following paragraphs highlight some of the key narrative reviews that were returned in our search strategy, each offering a distinct perspective and proposing different approaches for advancing understanding of the seemingly conflicting evidence.

Gabis and colleagues (2022) reviewed the "persistent myth" that outlines childhood vaccination and ASD, even with decades of rigorous epidemiological and mechanistic studies showing no causal relationship. The authors highlighted that ASD diagnoses often emerge around the same age as routine immunizations, which, combined with occasional regression and uncertainty around ASD's etiology, has fueled parental concerns and conspiracy theories. The review addressed thiomersal, MMR vaccine, and immune system pathways, reaffirming that none are supported as causal factors in ASD. The paper emphasized that misinformation and vaccine hesitancy, amplified by social media, pose significant public health risks, including outbreaks of vaccine-preventable diseases. The authors stressed the importance of effective, empathetic communication and dissemination of evidence-based information to counter myths and reinforce vaccine confidence.

Conklin and colleagues (2021; funded by WHO[14]) reviewed six major vaccine safety concerns that shaped global debates between 1999–2019, including thiomersal, aluminum adjuvants, ASD, autoimmune disease, immune overload, and non-specific effects of vaccines. Drawing on multiple large-scale epidemiological studies, WHO's Global Advisory Committee on Vaccine Safety repeatedly concluded that thiomersal- and aluminum-containing vaccines do not increase the risk of ASD or other NDDs. Similarly, there was no credible evidence linking vaccines to autoimmune conditions or immune system "overload". While non-specific effects remain an area for further study, the authors concluded that available evidence does not support changes to immunization policy. The authors also emphasized that robust, transparent safety monitoring and effective public communication are critical to counter misinformation and sustain confidence in vaccination programs worldwide.

[14] https://stacks.cdc.gov/view/cdc/111639

Azevedo and colleagues (2023) reviewed evidence on neurotoxic effects of intrauterine and childhood exposure to organomercurials, particularly methylmercury and ethylmercury[15] (including thiomersal). The review summarized well established risks of methylmercury from fish consumption, highlighting its placental transfer and long-lasting developmental neurotoxicity. In contrast, for ethylmercury, the evidence base remains limited: few mechanistic or epidemiologic studies associate thiomersal exposure to adverse neurodevelopmental outcomes, and most large-scale human studies report no association with ASD. The authors emphasized the importance of distinguishing between methyl- and ethylmercury, noting that while both can cross the blood–brain barrier, ethylmercury is eliminated more rapidly. They concluded that childhood vaccination remains critical for disease prevention, though suggest that further mechanistic studies are warranted to clarify low-dose ethylmercury exposure effects.

Dórea (2017) reviewed evidence on the use of thiomersal-containing vaccines in young children, particularly in low- and middle-income countries where multi-dose vial use remain common. The review highlighted that ethylmercury, is neurotoxic at low doses, but its tolerance limits during sensitive developmental periods (pregnancy, infancy, early childhood) have not been rigorously defined. The author notes that experimental studies suggest potential hazards, and some observational work has linked exposure to neurodevelopmental outcomes such as tic disorders. However, population-level findings remain inconsistent, leaving the overall picture uncertain. The author stressed that while the benefits of thiomersal removal have been demonstrated in high-income settings, most of the world's children remain exposed due to cost considerations. The review concluded that evidence reflects a state of uncertainty, underscoring the need for safer alternatives and greater equity in access to thiomersal-free vaccines.

Bölte and colleagues (2019) provide a comprehensive review of environmental contributions to ASD, situating them alongside genetic and gene–environment interaction models. The review systematically assessed a wide array of exposures, including advanced parental age, prenatal metabolic and hormonal factors, perinatal complications, medications such as valproate and selective serotonin reuptake inhibitors (SSRIs), maternal infection and immune activation, nutrition, and toxicants (air pollutants, heavy metals, pesticides, organic pollutants). It also considered psychosocial stressors such as maternal migration, natural disasters, and institutional deprivation, as well as possible protective factors like folate and fatty acid intake. Vaccination was specifically reviewed, with the authors concluding that epidemiological studies consistently show no evidence of vaccines posing an ASD risk. Instead, the most consistently supported risks were advanced parental age, valproate exposure, maternal diabetes, and immune activation during pregnancy. The authors emphasized that while many environmental factors may play contributory or moderating roles, the overall evidence base remains heterogeneous and often confounded. They advocated for more refined research approaches, including twin studies, longitudinal cohorts, and multi-hit models, to disentangle causal from non-causal influences.

Just as in the primary research that was reviewed above, these representative narrative reviews consistently emphasize that there is no causal relationship between vaccines, whether or not they contain thiomersal, and ASD, and more broadly, NDDs. At the same time, they call for more targeted scientific investigation, improved global access to thiomersal-free vaccines and sustained efforts to understand and support the concerns of individuals and communities where hesitancy persist.

[15] a metabolite of thiomersal

In closing, the findings of this review, alongside the consistent conclusions of representative narrative reviews, underscore that the most methodologically rigorous studies do not support a causal association between vaccines, thiomersal-containing or otherwise, and ASD. The totality of credible scientific evidence strongly supports the safety of current vaccines in relation to both ASD and other NDDs. Moving forward, continued public trust will depend not only on the strength of scientific evidence but also on transparent communication, responsive research that addresses genuine uncertainties, and inclusive policies that ensure access to safe vaccines worldwide.

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Appendices: Study Summaries

Topic 1: Thiomersal and ASD

Appendix 1.1: Assessment of prenatal thiomersal exposure and ASD or NDD (Table 1)

1. *Marques et al., 2010: Thiomersal exposure (from tetanus-diphtheria vaccine) during pregnancy and neurodevelopment of breastfed infants at 6 months*

   A retrospective cross-sectional study (Brazil; Porto Velho, Amazon; 2000–2001 recruitment; n=82 mother–infant pairs with complete follow-up) examined whether maternal exposure to thiomersal-containing tetanus–diphtheria (Td) vaccines during pregnancy was associated with infant neurodevelopment at 6 months. Infants of exposed vs. unexposed mothers showed no significant differences in neurodevelopmental scores; regression models indicated infant hair mercury concentration at birth predicted GDS outcomes, but the number of maternal Td doses did not. Results suggest that prenatal thiomersal exposure from Td vaccines was not associated with measurable neurodevelopmental delay at 6 months, though follow-up was short and ASD was not directly assessed.

   Strength of evidence: LOW, **Risk of bias: HIGH**

   - Strengths include prospective data collection, biologic measures of mercury (maternal and infant hair), and standardized GDS developmental testing by trained professionals. Exposure was determined from maternal vaccination records (1–3 Td doses, each ~25 µg Hg), and outcomes were assessed with the Gesell Developmental Schedules (GDS).
   - Limitations include small sample size (n=82), reliance on GDS rather than gold-standard ASD diagnostic instruments, and follow-up only to 6 months (later-onset neurodevelopmental issues not captured). Exposure estimates assumed standard thiomersal content of Td vaccine batches; misclassification is

possible. The original dataset was designed for fish-mercury studies, not vaccines, raising concerns about secondary analysis bias

2. *Price et al. (2010) – Standardized diagnostic tools (ADOS, ADI-R) to confirm ASD diagnoses and medical records plus immunization registries were used to quantify ethylmercury exposure from thiomersal-containing vaccines and immunoglobulins*

   A matched case-control study conducted in three U.S. Managed Care Organization (MCO) using data from the MCO's computerized data files, maternal and child medical charts, and standardized telephone interviews (256 children with ASD - including 187 with autistic disorder and 49 with ASD with regression - 752 matched controls, born between 1994 and 1999. Logistic regression found no increased risk of ASD associated with higher thiomersal exposure at any assessed period (prenatal, birth–1 month, birth–7 months, birth–20 months). Subgroup analyses for autistic disorder and regression also found no association. Analyses found no increased risk of ASD, autistic disorder, or ASD with regression for prenatal or infant exposures; some adjusted models suggested decreased risk with higher exposure, interpreted as likely due to chance or residual confounding.

   Strength of evidence: MODERATE, Risk of bias: LOW

   - Strengths include independent diagnostic validation with standardized tools: ADOS, ADI-R), prospectively collected medical records and immunization registries used to quantify ethylmercury exposure from thiomersal-containing vaccines and immunoglobulins, and adjustment for a broad range of covariates (maternal, perinatal, and child health factors).
   - Limitations include moderate participation rates (48% of potential cases, 32% of potential controls), which raises concern for selection bias, though exposure levels did not differ between participants and nonparticipants. As an observational study, residual confounding cannot be entirely excluded, but overall risk of bias is low.

Appendix 1.2: Assessment of childhood thiomersal exposure and ASD / NDD (Table 2)

1. *Uno et al. (2015) – Early exposure to the combined measles–mumps–rubella vaccine and thiomersal-containing vaccines and risk of autism spectrum disorder*

   A case-control study of 189 children with ASD and 224 age and sex matched controls in Japan to assess whether early MMR vaccination or cumulative thiomersal exposure increased autism risk. Case data were obtained from clinical records in the Yokohama Psycho Developmental Clinic (YPDC) and vaccination data were obtained from the Maternal and Child Health (MCH) handbook. The thiomersal content in vaccines was calculated from the manufacturer, lot number, and vaccination information in the MCH handbook (maintained by health professionals, not parental recall). Vaccination histories were reviewed up to 36 months of age, and odds ratios were calculated. The ORs (95% CIs) of MMR vaccination and thiomersal dosage associated with ASD, respectively, were 0.875 (0.345–2.222) and 1.205 (0.862–1.683) at age 18 months, 0.724 (0.421–1.243) and 1.343 (0.997–1.808) at 24 months, and 1.040 (0.648–1.668) and 0.844 (0.632–1.128) at 36 months. Results showed no significant differences in MMR vaccine status or thiomersal

dose between cases and controls. Further, adjusted odds ratios showed no statistically significant association between vaccine exposures and ASD risk from 18 to 36 months. Despite the removal of thiomersal, autism prevalence continued to rise, leading the authors to conclude that early exposure to combined MMR vaccines was unlikely to be a primary cause of autism.

Strength of evidence: LOW, Risk of bias: MODERATE

- Strengths include use of population surveillance data and clear documentation of vaccine policy changes, objective exposure assessment and validated outcomes measures via official records, and comprehensive temporal analysis across critical early developmental periods (1-36 months).
- Limitations include small sample size, (no direct measurement of exposure), potential confounding from changes in diagnostic criteria and awareness over time, and lack of adjustment for other risk factors. The reliance on aggregate data means associations at the individual level cannot be confirmed.

2. *Barile et al. (2012) – Thiomersal Exposure in Early Life and Neuropsychological Outcomes 7–10 Years Later*

A secondary analysis of a retrospective cohort (U.S.; Vaccine Safety Datalink; children born 1993–1997; n=1,047 assessed at ages 7–10) reanalyzed data from Thompson et al. (2007) using structural equation modeling to group 25 neuropsychological tests into seven latent constructs. Thiomersal exposure from birth to 7 months was not associated with six constructs (intellectual functioning, language, verbal memory, executive function, fine motor, behavior regulation). A small association with the presence of tics was observed in boys (β=0.17, p=0.03), but not in girls. The null findings for all other outcomes, combined with more efficient modeling to reduce Type I error, suggest thiomersal exposure is not linked to adverse neuropsychological development. Tic findings should be interpreted cautiously due to crude assessment methods and limited biological plausibility. Overall, the study reported no evidence that thiomersal harms long-term neuropsychological outcomes.

Strength of evidence: MODERATE, Risk of bias: MODERATE

- Strengths include use of prospectively collected vaccination data, large sample size for a neuropsychological study, blinded standardized test administration, and adjustment for socioeconomic and maternal covariates.
- Limitations include low participation rate (30% of eligible families), exclusion of low birthweight and medically complex children (limiting generalizability to vulnerable groups), and crude measurement of tics (single 3-hour observation by testers with minimal training). Diagnostic tools for ASD were not assessed—this study examined neuropsychological constructs only—so it does not directly address autism risk. Residual confounding (e.g., genetics, environment) cannot be excluded.

3. *da Cunha et al., (2020) – Thiomersal-containing vaccines and deficit in child development: Population-based study in southern Brazil*

This longitudinal cohort study (Brazil; Pelotas; 2009–2011 recruitment, follow-up at 24–36 months; n=535 mother–child dyads) assessed whether exposure to thiomersal-containing vaccines (HepB, MMR, tetravalent) was associated with motor, cognitive, and language development using the Bayley Scales of Infant and Toddler Development III (Bayley-III). In bivariate analyses, children who received 4–8 thiomersal-containing vaccines (TCV) doses had lower mean motor and language scores than those who

received 0–3 doses ($p<0.05$), but these associations disappeared in multivariate models adjusting for socioeconomic, maternal, and child health factors. The study concluded that TCVs were not associated with impaired child development.

Strength of evidence: MODERATE, **Risk of bias: MODERATE**

- Strengths include prospective cohort design, use of vaccination cards for exposure ascertainment, gold-standard Bayley-III developmental assessment, and adjustment for maternal depression, anxiety, socioeconomic class, smoking, prematurity, and hospitalizations.
- Limitations include small to moderate sample size (n=535), grouping of exposure categories (0–3 vs. 4–8 doses), potential misclassification if vaccination cards were incomplete, and follow-up only to age 2–3 years (missing later-onset developmental issues). ASD was not directly assessed; outcomes were general developmental domains.

4. *Lazoff et al. (2010) – Prevalence of Pervasive Developmental Disorders among Children at the English Montreal School Board*

A cross-sectional prevalence study (n=23,635 children enrolled in the English Montreal School Board; 187 with PDD) assessed diagnostic records and compared prevalence trends before and after thiomersal was removed from vaccines in Quebec in 1996. Prevalence increased linearly across successive birth cohorts (OR 1.17 per year, 95% CI 1.12–1.23), but this upward trend was unaffected by the removal of thiomersal in vaccines. The authors concluded that discontinuation of thiomersal in vaccines did not alter underlying PDD risk, suggesting that other factors (e.g., diagnostic broadening, awareness) accounted for rising prevalence.

Strength of evidence: LOW, Risk of bias: HIGH

- Strengths include large population-based sampling across 71 schools, standardized administrative coding, and regression modeling of birth cohort trends. This study replicates earlier findings (Fombonne et al., 2006).
- Limitations include absence of clinical validation for all diagnoses, potential undercounting of children in special schools, and inability to adjust for changing diagnostic criteria, awareness, or service availability over time. As an ecological study, results cannot attribute risk at the individual level.

5. *Dikme et al. (2013) – The relation between blood lead and mercury levels and chronic neurological diseases in children including assessment of vaccine history*

A Turkish case–control study (n=59 children with neurodevelopmental disorders - autism n=15, ADHD n=17, epilepsy n=14, motor–mental retardation n=13 - n=59 matched healthy controls) measured blood mercury and lead levels using atomic absorption spectrophotometry. Importantly, reported vaccination history and maternal dental fillings (potential mercury sources) were not associated with higher blood mercury. No significant differences were observed between cases and controls in mean blood mercury (0.84 µg/L vs. 0.99 µg/L) or lead levels (1.91 µg/dL vs. 2.19 µg/dL). The authors concluded that blood and lead levels of children with NDDs were not different from those without.

Strength of evidence: LOW, Risk of bias: HIGH.

- Strengths include direct biological measurement of exposure, use of standardized diagnostic criteria (DSM-IV for autism/ADHD), and consideration of environmental exposures (vaccination history, fish consumption, maternal dental fillings).
- Limitations include small sample size, lack of prenatal or cumulative exposure data, reliance on non-specific retrospective self-reported data, potential misclassification of exposure due to reliance on single blood samples, and limited generalizability beyond the study's hospital-based Turkish population.

Appendix 1.3: Reported association between thiomersal and ASD / NDDs (Table 3)

1. *Geier et al. (2017a) – Increased risk for an atypical autism diagnosis following Thiomersal-containing vaccine exposure in the United States: A prospective longitudinal case-control study in the Vaccine Safety Datalink.*

   A prospective longitudinal case–control study (U.S.; Vaccine Safety Datalink; births 1991–2000; n=164 atypical autism cases vs. n=15,216 controls) assessed ethylmercury exposure from thiomersal-containing hepatitis B vaccines during the first 1–6 months of life using VSD medical records. The study assessed whether thiomersal-containing hepatitis B vaccines increased risk of "atypical autism" (ICD-9 299.80), including PDD not otherwise specified (PDD-NOS) and Asperger syndrome (Geier et al., 2017a). Cases were significantly more likely to have received higher mercury doses in the first 1–6 months of life, with ORs ranging from ~4.0 to >6.0 depending on exposure window. The authors report higher odds of atypical autism with greater early-life TM-HepB exposure and a dose–response trend (e.g., per-µg Hg OR≈1.14), including in sex-stratified analyses.

   Strength of evidence: VERY LOW, **Risk of bias: HIGH**

   - Strengths include prospective exposure capture from the VSD and a large control pool
   - Limitations included (1) Outcome measurement relies on administrative ICD-9 coding (299.80) without standardized diagnostic instruments; (2) Control selection/follow-up required ≥8.05 years of enrollment, producing non-overlapping birth years for cases (1991–1998) vs. controls (1991–1992), which can introduce bias from changes over time in how autism was diagnosed or how vaccines were given; (3) Analytic flexibility/sensitivity to exposure windows and follow-up rules; (4) limited adjustment for potential confounders (genetic, socioeconomic, environmental). The reported dose–response (per-µg Hg) further depends on these modeling choices.

2. *Geier et al. (2017b) – Thiomersal exposure and disturbance of emotions specific to childhood and adolescence: A case-control study in the Vaccine Safety Datalink (VSD) database*

   This retrospective case–control study (U.S.; Vaccine Safety Datalink; births 1991–2000; n=517 cases with ICD-9 code 313.xx "disturbance of emotions," n=27,491 controls) examined cumulative mercury exposure from thiomersal-containing hepatitis B vaccines within the first 6 months of life. The authors evaluated whether thiomersal-containing vaccines were associated with later diagnoses of "disturbance of emotions specific to childhood and adolescence" (ICD-9 313.xx) .Reported associations were significant: OR=1.34 for

12.5 µg Hg in the first month, OR=1.34 for 25 µg in the first 2 months, OR=2.37 for 37.5 µg in the first 6 months, and OR=1.03 per µg Hg (dose–response). Effects were observed for males but not females. The authors argued these findings extended the spectrum of thiomersal-associated outcomes beyond ASD.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include prospective vaccination data from the VSD and large control group size.
- Limitations include reliance on an administrative ICD-9 category ("disturbance of emotions") with poor specificity, absence of standardized diagnostic instruments (ADOS, ADI-R), selective restriction to hepatitis B vaccines (ignoring other thiomersal sources), and results that vanish under alternative analytic specifications (e.g., shorter control follow-up). The study reports dose–response findings, but these are likely inflated by outcome misclassification and analytic flexibility. Residual confounding from genetics, socioeconomic status, and environment was not addressed. The outcome definition was extremely broad (covering anxiety, shyness, selective mutism, oppositional defiance, identity disorder, academic underachievement, etc.), Findings appear highly sensitive to analytic choices such as control follow-up length.

3. *Geier et al. (2017c) – Abnormal Brain Connectivity Spectrum Disorders Following Thiomersal Administration: A Prospective Longitudinal Case–Control Assessment of Medical Records in the Vaccine Safety Datalink*

A prospective longitudinal case–control study (U.S.; Vaccine Safety Datalink; n=411 ASD cases, n=241 tic disorder cases, n=1,041 ADHD case groups from 1991-2000 vs. large control groups from 1991–1993 birth cohorts) examined thiomersal exposure from Hib vaccines within the first 15 months of life. The study reported dose-dependent associations between thiomersal exposure and ASD, tic disorder, and ADHD, with odds ratios of ~1.4–1.5 per 25 µg Hg.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include use of prospectively collected medical records and a large population database (VSD).
- Limitations include reliance on diagnostic codes without clinical validation, selective exposure/outcome windows, potential misclassification of vaccine thiomersal content, lack of adjustment for key confounders (socioeconomic status, family history, environmental exposures), and methodological concerns about analytic choices. Reported associations are likely driven by design and analytic bias rather than true effects.

4. *Geier et al. (2015) – A Case-Control Study Evaluating the Relationship Between Thiomersal-Containing Haemophilus influenzae Type b Vaccine Administration and the Risk for a Pervasive Developmental Disorder Diagnosis in the United States*

A retrospective case–control study (U.S.; Vaccine Safety Datalink, 1991–2000 birth cohorts; n=534 PDD cases vs. n=25,632 controls) examined cumulative mercury exposure from thiomersal-containing Haemophilus influenzae type b (Hib) vaccines within the first 4, 6, and 15 months of life. The study reported significantly higher odds of PDD with increased thiomersal exposure (e.g., OR=1.97 for 75 µg vs. 25 µg at 6 months; OR=3.94 for 100 µg vs. 25 µg at 15 months), with a reported dose–response trend (OR per µg =

1.02). The authors interpreted these findings as evidence that early-life exposure to thiomersal contributed to PDD risk.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include use of a large administrative database (VSD), prospective capture of vaccination records, and attempts to use a non-biologically plausible outcome (febrile seizures) as a negative control.
- Limitations include reliance on ICD-9 diagnostic codes without standardized clinical validation, selective focus on Hib vaccines while excluding other thiomersal-containing vaccines, and potential exposure misclassification due to assumptions about vaccine formulation and reconstitution practices. Analytic choices (e.g., long control follow-up vs. short) substantially changed results, highlighting instability. Residual confounding from genetics, socioeconomic factors, and environmental exposures was unaddressed. Overall, the claimed dose–response association is likely driven by methodological weaknesses rather than true effects.

5. *Geier et al. (2014) – A Dose-Response Relationship between Organic Mercury Exposure from Thiomersal-Containing Vaccines and Neurodevelopmental Disorders*

A retrospective case–control study (U.S.; Vaccine Safety Datalink, 1991–2000 birth cohorts; n=492 PDD cases, n=5,699 specific developmental delay cases, n=344 tic disorder cases, n=1,485 hyperkinetic syndrome cases, each compared with tens of thousands of controls) examined cumulative mercury exposure from thiomersal-containing hepatitis B vaccines within the first six months of life. The study reported significant dose–response associations for all four neurodevelopmental disorders (e.g., OR per µg Hg = 1.05 for PDD, 1.03–1.05 for others; OR =2.3–3.8 at 37.5 µg cumulative exposure). Control groups were from birth cohorts from 1991 to 1993 or 1995, while comparator groups were from 1991 up to 2000. The authors concluded thiomersal exposure was dose-dependently associated with NDDs.

Strength of evidence: VERY LOW, **Risk of bias: HIGH**

- Strengths include use of automated medical records and large sample sizes.
- Limitations include reliance on ICD-9 diagnostic codes without clinical validation, selective restriction to hepatitis B vaccine exposure (ignoring other thiomersal or environmental sources), and analytic choices (specific exposure windows, per-µg modeling, shortened follow-up for controls) that may have biased results. Assumes thiomersal exposure based on vaccination schedules and the type of vaccine (e.g., hepatitis B vaccines containing thiomersal), without confirming. Diagnostic tools were administrative codes (ICD-9), not standardized instruments such as ADOS or ADI-R. Residual confounding from genetics, socioeconomic status, healthcare access, and environmental exposures was not addressed. The claimed dose–response relationship is likely inflated by these design issues, limiting the reliability of causal inference.

6. *Geier et al. (2018a) – A Cross-Sectional Study of the Association between Infant Hepatitis B Vaccine Exposure in Boys and the Risk of Adverse Effects as Measured by Receipt of Special Education Services.*

A cross-sectional study (USA; NHANES 2001–2014; boys aged 7–8 years; n=1,192, weighted n=24.5 million) examined whether infant hepatitis B vaccination was associated with receipt of special education services

(SES). The analysis compared boys who received three doses of hepatitis B vaccine assumed to be thiomersal-containing (n=524; weighted n=11.2 million) with unvaccinated boys (n=41; weighted n=0.7 million). The study reported large associations (adjusted OR=9.23, 95% CI 1.3–65.3) and a prevalence ratio of 8.96, concluding that thiomersal exposure increased risk of SES. However, exposure status was inferred indirectly from birth year (1994–2000 as thiomersal-containing; 2001–2007 as thiomersal-reduced) rather than measured, outcome was based on parental survey reports of SES, and unexposed groups were very small.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include use of a nationally representative dataset (NHANES) and application of survey-weighted regression models.
- Limitations include: (1) cross-sectional design, preventing causal inference; (2) exposure misclassification, as vaccine thiomersal content was assumed by era rather than verified for each child; (3) outcome misclassification, as SES is a broad category that includes many conditions not biologically linked to mercury; (4) very small unexposed sample size (n=41), leading to unstable odds ratios with extremely wide confidence intervals; (5) diagnostic tools were parental survey responses, not validated clinical instruments; and (6) results are highly sensitive to analytic assumptions.

7. *Geier et al. (2013) – A two-phase study evaluating the relationship between Thiomersal-containing vaccine administration and the risk for an autism spectrum disorder diagnosis in the United States*

In this two-phase study using data from VAERS (phase 1) and VSD (phase 2) , Geier et al. (2013) investigated whether thiomersal exposure from hepatitis B vaccine was associated with ASD risk. Phase I reported more ASD events following thiomersal-containing DTaP versus thiomersal-free DTaP in VAERS (38 vs. 17 cases). Phase II reported higher odds of ASD diagnosis with cumulative thiomersal exposure from hepatitis B vaccines (e.g., OR=2.18 for one dose in the first month; OR=3.39 for three doses by six months). The authors concluded that their results supported a causal association.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Phase I was based on VAERS, which is prone to underreporting, misclassification, and lacks denominators for incidence estimates. Phase II, though using VSD records, relied on retrospective coding without validated ASD diagnoses and imposed restrictive follow-up rules that may have biased case/control classification. Exposure to other thiomersal-containing vaccines and environmental mercury was not accounted for, increasing residual confounding. Overall, methodological weaknesses and selective analytic choices limit the reliability of the associations reported.

8. *Geier, et al. (2016) – A Two-Phase Case-Control Study of Autism Risk Among Children Born From the Late 1990s Through the Early 2000s in the United States.*

A two-phase study assessed whether reduced thiomersal exposure in U.S. vaccines after 1999 was linked to lower autism risk. In Phase I, analysis of VAERS reports from 1998–2003 (73 autism cases, 11,783 controls), comparing autism-related to non-autism related reports based on year of vaccination, showed decreasing odds of autism with more recent vaccination years (OR = 0.65 per year). Phase II, a matched case-control study in Dallas (40 ASD cases, 40 controls), using date of birth as a proxy for thiomersal exposure, also found autism cases had earlier birth years and lower odds of ASD with more recent birth (OR = 0.67 per year). The authors concluded autism risk declined with reduced thiomersal exposure.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Phase I is limited by VAERS' passive reporting, underreporting, misclassification, and lack of denominators. Phase II is limited by very small sample size (n=40 cases, n=40 controls), single geographic setting, potential selection bias, and reliance on CARS rather than gold-standard diagnostic tools (e.g., ADOS, ADI-R). Neither phase controlled for changes in diagnostic practices or awareness during 1998–2003, which could explain observed trends. The combined design amplifies weaknesses rather than mitigating them, leaving findings unreliable for causal inference.

9. *Geier et al. (2018b) – The risk of neurodevelopmental disorders following Thiomersal-containing Hib vaccine in comparison to Thiomersal-free Hib vaccine administered from 1995 to 1999 in the United States*

A case–control study (n=3,346 adverse event reports from VAERS, including 92 autism cases and other neurodevelopmental disorders) compared thiomersal-containing Hib vaccines (HIBTITER™) with thiomersal-free Hib vaccines (PEDVAXHIB™) administered between 1995–1999. The study reported significantly elevated odds ratios for autism (OR=2.75), developmental delay (OR=5.39), psychomotor disorder (OR=2.38), and neurodevelopmental disorders overall (OR=2.70) in the population study group that was determined to receive thiomersal-containing vaccines. No differences were observed for unrelated adverse outcomes (e.g., febrile seizures). The authors concluded this supported a causal association.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include attempt to compare two vaccine formulations with and without thiomersal, restricting analyses to Hib vaccines to reduce heterogeneity, and long follow-up (>15 years post-vaccination).
- Limitations include reliance on passive surveillance data prone to underreporting, reporting bias, and misclassification of both exposure and outcomes. Diagnostic categories were based on VAERS coding, not standardized clinical instruments (e.g., ADOS, ADI-R), and outcome definitions were heterogeneous (autism, developmental delay, psychomotor disorder grouped together). The small number of autism cases (n=58) and developmental delay cases (n=36) makes estimates unstable. Results are highly sensitive to analytic choices, and residual confounding from genetics, environment, and healthcare access was unaddressed.

Appendix 1.4: Results from peer-reviewed meta-analyses (Table 4)

1. *Yoshimasu et al. (2014) – A meta-analysis of the evidence on the impact of prenatal and early infancy exposures to mercury on autism and attention deficit/hyperactivity disorder in the childhood*

   A meta-analysis examining both thiomersal-containing vaccines and environmental mercury exposures during prenatal and early infancy. Drawing from 20 eligible studies (10 on thiomersal, 10 on environmental exposures), they reported no association between thiomersal exposure and ASD (summary OR 0.99, 95% CI 0.80–1.24). Similarly, no significant association was found for thiomersal and ADHD (OR 0.91, 95% CI 0.70–1.13). By contrast, significant associations were observed between environmental mercury exposures and both ASD (OR 1.66, 95% CI 1.14–2.17) and ADHD (OR 1.60, 95% CI 1.10–2.33). The authors emphasized that these environmental findings should be interpreted cautiously given limited study numbers, methodological heterogeneity, and potential confounding This comprehensive synthesis provides strong evidence that thiomersal exposure is not associated with increased ASD or ADHD risk, while certain environmental exposures may carry risk.

   Strength of evidence: STRONG, Risk of bias: LOW

   - Strengths include systematic literature search across multiple databases, independent data extraction, separate analyses by mercury type (ethyl, methyl, inorganic), and correction for publication bias.
   - Limitations include the small number of eligible studies, heterogeneity in diagnostic tools from those studies (ICD codes, DSM-IV, ADI-R, clinical scales), and variation in exposure measurement (vaccine records, maternal Rh status, air pollution models, hair/cord blood mercury). While some included primary studies (e.g., VAERS-based analyses) are lower quality, their inclusion did not materially affect pooled estimates. Overall, the risk of bias in this synthesis is low.

2. *Taylor et al. (2014) – Vaccines are not associated with autism: An evidence-based meta-analysis of case-control and cohort studies*

   A meta-analysis with pooled data from five cohort studies (over 1.25 million children) and five case-control studies (~10,000 children) assessing vaccines, thiomersal, mercury, and ASD outcomes. Their analysis found no increased risk of ASD associated with vaccination overall (OR 0.99, 95% CI 0.92–1.06), nor with specific exposures: MMR vaccine (OR 0.84, 95% CI 0.70–1.01), thiomersal (OR 1.00, 95% CI 0.77–1.31), or mercury (OR 1.00, 95% CI 0.93–1.07). Case-control data similarly reported no significant associations, and subgroup analyses did not alter these results. Risk of bias was generally low to moderate, with the strongest evidence coming from large-scale cohort studies. The authors concluded that vaccines and their components are not associated with autism, underscoring the importance of continued immunization programs.

   Strength of evidence: STRONG, Risk of bias: LOW

   - Strengths include a comprehensive PRISMA-guided search, inclusion of large population-based cohorts and well-designed case–controls, use of the Newcastle–Ottawa Scale for study quality, and sensitivity analyses showing stability of results.
   - Potential limitations include heterogeneity among included studies (e.g., diagnostic methods ranging from ICD codes to standardized tools like ADI-R), moderate bias risk ratings for some case–controls,

and possible publication bias in smaller studies (though Egger's and Begg's tests suggested no major impact). Overall, the large sample sizes, consistent null findings, and exclusion of VAERS-only studies make this a robust synthesis.

Topic 2: Vaccines and ASD

Appendix 2.1: Prenatal Vaccinations and ASD or NDD in children (Table 5)

1. *Becerra-Culqui et al. (2022) – Prenatal Influenza Vaccination or Influenza Infection and Autism Spectrum Disorder in Offspring*

A retrospective U.S. cohort study (Kaiser Permanente, 84,739 mother–child pairs, 46 257 women vaccinated, deliveries between 2011 and 2014, and followed until 2018) assessed whether mothers influenza vaccination or infection during pregnancy was associated with ASD diagnoses in the child. Electronic health records were used to ascertain prenatal influenza vaccination (n=46,257 exposed), laboratory/ICD-coded influenza infection, and ASD diagnoses in children aged one year and older via ICD-9/10 codes. Using Cox models with inverse probability of treatment weighting, the study found no increased risk of ASD with maternal vaccination in any trimester, and influenza infection likewise showed no association (Hazard Ratios from 1.04 to 1.13). Results were consistent across trimesters, birth-year strata, and nulliparous women. Exposure measured via EHR; outcome via standardized clinical coding within an integrated system; multiple sensitivity/stratified analyses showed stable null findings. The authors concluded that neither maternal influenza infection nor influenza vaccine during pregnancy are linked to ASD in the child, supporting vaccine safety in pregnancy.

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include a large, population-based cohort; prospective capture of vaccination, infection, and outcomes in linked records; covariate balance via IPTW; and consistency across subgroup and sensitivity analyses.
- Limitations include reliance on administrative ASD codes (no universal chart-level diagnostic validation), potential under-ascertainment of milder infections (few infections: n=571; only 15 ASD outcomes among the infected, yielding wide CIs), and modest average follow-up (~4.4–4.5 years). These issues are unlikely to overturn the robust null association for vaccination.

2. *Zerbo et al. (2017) – Association Between Influenza Infection and Vaccination During Pregnancy and Risk of Autism Spectrum Disorder*

A retrospective cohort study (U.S.; Kaiser Permanente Northern California; births 2000–2010; n=196 929 children, 3,103 ASD cases; follow-up to 2015, median 8.3 years) examined associations between maternal influenza infection or vaccination during pregnancy and ASD risk in the child after birth. Maternal influenza infection (n=1,400) was not associated with ASD (aHR=1.04, 95% CI 0.68–1.58). Influenza vaccination at any point during pregnancy (n=45 231) was also not associated with childhood ASD (aHR=1.10, 95% CI

1.00–1.21). A modestly elevated risk was observed for first-trimester vaccination (aHR=1.20, 95% CI 1.04–1.39), but this finding was not statistically significant in the adjusted analysis after Bonferroni correction for multiple testing, suggesting chance.

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include population-based design, large sample size, prospectively recorded maternal influenza vaccination and infection data, and outcome ascertainment from medical records with validation showing >90% positive predictive value for ASD diagnoses. Analyses adjusted for a wide set of maternal and child covariates, including conception season, maternal comorbidities, education, and race/ethnicity.
- Limitations include potential under-ascertainment of subclinical influenza infections, lack of data on vaccinations given outside Kaiser facilities (though >89% captured), and instability of trimester-specific estimates due to relatively small case counts. Diagnostic tools were ICD-9 codes, validated against ADI-R/ADOS in a subset. Results were consistent across sensitivity analyses

3. *Ludvigsson et al. (2020) – Maternal Influenza A(H1N1) Immunization During Pregnancy and Risk for Autism Spectrum Disorder in Offspring: A Cohort Study*

A retrospective cohort study (Sweden; national registers; births Oct 2009–Sept 2010; n=69,019 children including n=39,726 exposed to maternal H1N1 vaccine, n=13,845 during first trimester; follow-up mean 6.7 years) examined whether prenatal exposure to the AS03-adjuvanted H1N1 vaccine (Pandemrix) was associated with ASD in children after birth. During follow-up (6.7 years), 394 vaccine-exposed children (1.0%) and 330 unexposed (1.1%) were diagnosed with ASD. Adjusted hazard ratios showed no association (adjusted HR=0.95, 95% CI 0.81–1.12), including for first-trimester exposure (aHR=0.92, 95% CI 0.74–1.16).

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include prospective exposure ascertainment through vaccination registers, population-based design, large sample size, and adjustment for maternal, infant, sociodemographic, and health covariates. ASD diagnoses were based on ICD-10 hospital and outpatient codes. Multiple sensitivity analyses (excluding incomplete regions, restricting to primary diagnoses, truncating follow-up at 6 years) showed consistent null results.
- Limitations include lack of data on maternal H1N1 infection, potential unmeasured paternal/genetic confounders, and restriction to one specific vaccine formulation (Pandemrix), which may limit generalizability to other influenza vaccines.

4. *Becerra-Culqui et al. (2018) – Prenatal Tetanus, Diphtheria, Acellular Pertussis Vaccination and Autism Spectrum Disorder*

A retrospective cohort study (U.S.; Kaiser Permanente Southern California; births 2011–2014; n=81,993 children, 39,077 exposed to prenatal Tdap vaccine) examined whether maternal Tdap vaccination during pregnancy was associated with ASD risk to the child after birth. During follow-up to 2017, ASD incidence was 3.78 per 1,000 person-years in the exposed group and 4.05 in the unexposed. Adjusted Cox models

with inverse probability of treatment weighting found no increased risk (HR=0.85, 95% CI 0.77–0.95). Subgroup analyses by birth year and parity were consistent.

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include population-based design within an integrated healthcare system, prospectively recorded vaccination and outcome data, use of propensity weighting to balance covariates, and consistency across stratified analyses. ASD diagnoses were based on ICD-9/10 codes in electronic medical records, made by qualified mental health specialists, and supported by California insurance law requiring standardized procedures for ASD diagnosis during the study period.
- Limitations include somewhat shorter average follow-up among vaccinated children (3.9 vs. 4.4 years), potential under-ascertainment of milder ASD cases in later birth cohorts, and lack of adjustment for family history of ASD. Results were robust across sensitivity checks.

5. *Hardie et al. (2025) – Early childhood developmental concerns following SARS-CoV-2 infection and COVID-19 vaccination during pregnancy: a Scottish population-level retrospective cohort study*

A retrospective population-level cohort study (Scotland; COPS dataset linked with child health reviews; n=24,919 mother–child pairs, births May 2020–Sept 2021; follow-up to 13–15 months) examined associations between maternal SARS-CoV-2 infection, COVID-19 vaccination during pregnancy, and early childhood developmental concerns. No associations were found between SARS-CoV-2 infection during pregnancy and any developmental concerns. COVID-19 vaccination during pregnancy was not associated with increased risk, and in adjusted models was linked to reduced odds of certain concerns (problem solving OR=0.78, personal–social OR=0.76, emotional–behavioural OR=0.67). The authors discussed the possibility of residual confounding for measure they could not account for such as level of social support, substance use, and medications.

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include population-based design, large sample size, linkage of multiple administrative datasets, and adjustment for a broad set of maternal, infant, and sociodemographic covariates. Developmental concerns were assessed using structured child health reviews, including the Ages & Stages Questionnaire (ASQ-3), rather than parental report alone.
- Limitations include restriction to early developmental concerns (13–15 months), exclusion of trimester 1 vaccinations due to low uptake, reliance on PCR-confirmed SARS-CoV-2 cases (missed asymptomatic/untested infections), and potential unmeasured confounding (e.g., maternal health behaviors, social support). Diagnostic tools were structured developmental reviews (ASQ-3 plus health visitor assessment), not full ASD diagnostic instruments.

Appendix 2.2: Childhood vaccination and ASD (Table 6)

1. *Kim et al. (2020) – Childhood vaccination as a protective factor for developmental psychopathology*

A cross-sectional, population-based study (South Korea; Discovery Sample n=10,002, Replication Sample n=29,381; school-aged children 7–13 years) examined parental report of completion of six recommended childhood vaccinations and risk of developmental psychopathology, including ASD. ASD likelihood was assessed using the Autism Spectrum Screening Questionnaire (ASSQ), while behavioral problems were measured with the BASC-2 Parent Rating Scale. In both the discovery and replication samples, incomplete vaccination was associated with higher odds of ASD risk (Discovery aOR=2.33 for <3 vaccines vs. 6 vaccines; Replication aOR=2.19) and with higher internalizing/externalizing scores, with dose–response trends. Fully vaccinated children had the lowest risk across both cohorts. The authors concluded that vaccination not only prevents infectious diseases but may also confer protective effects against ASD-related traits and broader developmental psychopathology.

Strength of evidence: MODERATE, Risk of bias: MODERATE

- Strengths include large representative cohorts, replication in an independent sample, use of validated screening tools (ASSQ, BASC-2), and adjustment for demographic and family psychiatric covariates.
- Limitations include that vaccination histories were parent-reported, introducing potential recall error, and ASD was based on a screening instrument (ASSQ) rather than standardized diagnostic assessments (e.g., ADOS, ADI-R, DSM-IV/5). Non-participation was substantial (34–38% of targets), raising the possibility of non-response bias. Residual confounding from unmeasured factors (e.g., infection history, healthcare access) could not be excluded.

2. *Hviid et al. (2019) – Measles, Mumps, Rubella Vaccination and Autism: A Nationwide Cohort Study*

A nationwide cohort study (Denmark; 1999–2010 births; n=657,461 children; follow-up through 2013 (5,025,754 person-years); 6,517 ASD cases) examined the association between MMR vaccination and autism using Danish population registries. Fully adjusted analyses showed no association (aHR=0.93, 95% CI 0.85–1.02). Subgroup analyses likewise found no increased risk among children with autistic siblings, high autism risk scores, or within specific time windows after vaccination.

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include nationwide coverage, prospective ascertainment of vaccinations and outcomes through linked registries, large sample size, and adjustment for multiple perinatal, familial, and sociodemographic covariates. Autism diagnoses were based on ICD-10 codes assigned by child psychiatrists, validated in Denmark with high positive predictive value (92.5%). Multiple sensitivity analyses (requiring ≥2 ASD diagnoses, stratifying by phenotypes, testing dose-response with two MMR doses, and varying adjustment methods) showed consistent null results, reinforcing robustness.
- Limitations include lack of individual chart review, possible diagnostic delay relative to symptom onset, and restriction to a single national setting.

3. *Mrożek-Budzyn, et al. (2010) – Lack of Association Between Measles-Mumps-Rubella Vaccination and Autism in Children: A Case-Control Study*

A case–control study (Poland; 2002–2006; n=96 autism cases, n=192 age- and sex-matched controls) examined associations between MMR vaccination, single measles vaccination, and autism. Vaccination status and timing were confirmed from physician records, and autism diagnoses were made by child psychiatrists using ICD-10. Logistic regression showed no increased autism risk for MMR vs. non-vaccinated (OR=0.17, 95% CI 0.06–0.52) or for MMR vs. single measles vaccine (OR=0.44, 95% CI 0.22–0.91). Results consistently suggested lower autism odds among vaccinated children.

Strength of evidence: MODERATE, Risk of bias: MODERATE

- Strengths include physician-confirmed vaccination history (minimizing recall bias), psychiatrist-confirmed autism diagnoses, and adjustment for maternal and perinatal covariates (e.g., maternal age, pregnancy medications, Apgar score).
- Limitations include relatively small case numbers (n=96), potential residual confounding (e.g., healthcare access, socioeconomic status), and restriction to a regional Polish population, which may limit generalizability. Diagnostic tools were ICD-10 codes assigned by psychiatrists, not gold-standard research instruments like ADOS or ADI-R.

4. *Jain et al. (2015) – Autism Occurrence by MMR Vaccine Status Among US Children With Older Siblings With and Without Autism*

This retrospective cohort study (U.S.; Optum Research Database, 2001–2012; n=95,727 children, including n=1,929 with older siblings with ASD; follow-up to ≥5 years) examined MMR vaccination status (0, 1, or 2 doses) and ASD risk. Among children with older siblings with ASD, 134 (6.9%) were diagnosed with ASD, compared with 860 (0.9%) of those with unaffected siblings. MMR receipt was not associated with increased ASD risk in either group: adjusted RR at age 5 for two vaccine doses was 0.56 (95% CI 0.30–1.04) among children with affected siblings, and 1.09 (95% CI 0.76–1.54) among those with unaffected siblings.

Strength of evidence: STRONG, Risk of bias: LOW

- Strengths include large, diverse cohort from a nationwide claims database, continuous enrollment to age ≥5 years, and adjustment for sociodemographic, perinatal, and health covariates. Exposure was captured from claims, minimizing recall bias. ASD diagnoses required ≥2 claims with ICD-9 codes, with validation studies showing approximately 87% positive predictive value. Sensitivity analyses (e.g., 1-claim definition, exclusion of children with missing sociodemographic data) did not change results.
- Limitations include reliance on administrative claims rather than direct clinical validation, possible under-ascertainment of vaccinations given outside insurance claims, and limited racial/ethnic diversity compared with the US population. Nonetheless, findings were consistent across subgroup and sensitivity analyses.

5. *Goin-Kochel et al. (2016) – Parental report of vaccine receipt in children with autism spectrum disorder: Do rates differ by pattern of ASD onset?*

A cross-sectional study (U.S.; Simons Simplex Collection, 2007–2011; n=2,755 children with ASD, assessed with ADOS and ADI-R, grouped by onset pattern: early onset, plateau, delay-plus-regression, regression) compared parent-reported vaccine receipt (DPT/DTaP, Hepatitis B, Hib, polio, MMR, varicella) across ASD-onset subtypes. Vaccination rates were very high (>90% for all except varicella) and equivalent across onset groups within a 10% margin, with only minor differences for varicella and DPT receipt. Findings do not support an association between regressive-onset ASD and vaccines.

Strength of evidence: LOW, Risk of bias: MODERATE

- Strengths include large sample size, rigorous ASD diagnosis using ADOS/ADI-R, and multicenter North American recruitment.
- Limitations include that vaccine receipt was primarily based on parental report, with no systematic validation against medical records. Recall bias and misclassification (e.g., uncertainty about schedule adherence or partial vaccine receipt) are possible. The study population exclusion criteria and demographic profile (predominantly White, higher-income families) limit generalizability.

Appendix 2.3: Repeated vaccine exposure, antigen exposure, antibody response and ASD in children (Table 7)

1. *Uno et al. (2012) – The combined measles, mumps, and rubella vaccines and the total number of vaccines are not associated with development of autism spectrum disorder: The first case–control study in Asia*

A case–control study (Japan; 1984–1992 births; n=189 ASD cases, n=224 age- and sex-matched controls) investigated associations between MMR vaccine, total number of vaccine injections, and ASD onset using records from the Maternal and Child Health (MCH) handbook. ASD diagnoses were made with DSM-IV criteria and validated using the Diagnostic Interview for Social and Communication Disorders (DISCO), a gold-standard tool. Vaccination history was documented prospectively by healthcare professionals in the MCH handbook. Analyses found no association between MMR vaccination and ASD (OR=1.04, 95% CI 0.65–1.68) or between number of vaccine injections and ASD (OR=1.10, 95% CI 0.95–1.26).

Strength of evidence: MODERATE, Risk of bias: MODERATE

- Strengths include validated ASD diagnosis with DSM-IV and DISCO, use of prospectively recorded vaccination data, and adjustment for key perinatal and neonatal covariates (e.g., maternal hypertension, Apgar score).
- Limitations include modest sample size (n=189 cases), relatively wide confidence intervals, and restriction to a genetically homogeneous Japanese population, which may limit generalizability. Some known ASD risk factors (e.g., parental age, prenatal medication use, comorbid conditions) were not captured in the MCH records.

2. *DeStefano et al. (2013) – Increasing Exposure to Antibody-Stimulating Proteins and Polysaccharides in Vaccines Is Not Associated with Risk of Autism*

A case–control study (U.S.; 3 managed care organizations; births 1994–1999; n=256 ASD cases, n=752 matched controls) examined whether cumulative exposure to vaccine antigens (antibody-stimulating proteins and polysaccharides) in the first 2 years of life was associated with ASD, autistic disorder, or ASD with regression. Antigen exposure was calculated from immunization registries and medical charts, and ASD diagnoses were validated by standardized in-person assessments (ADOS, ADI-R). Conditional logistic regression showed no increased risk: adjusted OR per 25-unit increase in antigen exposure was 0.999 (95% CI 0.994–1.003) at 3 months, 0.999 (95% CI 0.997–1.001) at 7 months, and 0.999 (95% CI 0.998–1.001) at 2 years. Maximum same-day antigen exposure was also unrelated to ASD outcomes.

Strength of evidence: STRONG, Risk of bias: LOW.

- Strengths include validated ASD diagnoses using gold-standard tools (ADOS, ADI-R), use of medical records rather than parental recall for vaccine exposure, prospective capture of vaccination data, and adjustment for numerous maternal and child covariates. Sensitivity analyses by time window and vaccine type confirmed findings.
- Limitations include possible recall bias for certain maternal exposures (from interviews), modest participation rates (48% of eligible cases, 32% of eligible controls), and the assumption that all antigens have equivalent immunologic weight. Diagnostic tools were standardized instruments (ADOS, ADI-R), adding rigor.

3. *Gentile et al. (2013) – Response to Measles-Mumps-Rubella Vaccine in Children with Autism Spectrum Disorders*

A hospital-based case–control study (Italy; 2010–2011; n=31 children with ASD vs. n=29 controls) measured antibody titers and seropositivity rates for measles, mumps, and rubella antigens using ELISA and chemiluminescence assays. ASD diagnoses were validated with standardized instruments (DSM-IV-TR, ADOS, ADI-R, CARS, VABS, GMDS). Results showed no differences in antibody titers or seropositivity rates between ASD cases and controls, nor between autistic disorder and other ASD subtypes.

Strength of evidence: LOW, Risk of bias: MODERATE

- Strengths include standardized ASD diagnosis using gold-standard instruments, laboratory-based antibody measurement, and comparison of both titers and seropositivity rates across subgroups.
- Limitations include small sample size (n=31 cases), hospital-based recruitment that may not represent the general population, lack of adjustment for potential confounders (e.g., time since vaccination, infection history), and limited generalizability beyond the Italian clinical setting.

Appendix 2.4: Evidence in support of a relationship between vaccination and ASD (Table 8)

1. *DeLong (2011) – A Positive Association found between Autism Prevalence and Childhood Vaccination uptake across the U.S. Population*

An ecological correlation study (U.S.; 2001–2007; state-level data from the U.S. Department of Education and CDC's National Immunization Survey) examined associations between autism prevalence at age 8 (and speech/language impairment) and the proportion of 2-year-olds completing the recommended vaccine series (4 doses of DTP or DTaP, 3 doses of polio, 1 does of measles, 3 doses of Hib, 3 doses of hepatitis B vaccines). Using fixed-effects regression across states and years, the study reported that a 1% increase in vaccination coverage was associated with a 1.7% increase in autism/speech disorder prevalence.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include use of multi-year state-level data and regression models adjusting for income, ethnicity, and year fixed effects.
- Limitations include reliance on aggregate vaccination coverage and outcomes measures (ecological fallacy), outcome misclassification (special education classifications from IDEA, not standardized diagnostic tools), potential confounding from secular changes in diagnostic practices and awareness, and assumptions that vaccination uptake is homogeneous across each state. Results are highly sensitive to analytic choices (e.g., inclusion of speech/language impairment with autism, definition of vaccination coverage).

2. *Gallagher and Goodman (2010) – Hepatitis B Vaccination of Male Neonates and Autism Diagnosis, NHIS 1997–2002*

A cross-sectional study (U.S.; NHIS 1997–2002; boys aged 3–17 years born before 1999; n=30 autism cases vs. n=7,044 controls with vaccination records) evaluated the association between neonatal hepatitis B vaccination and parental report of autism diagnosis. The study reported that boys vaccinated during the first month of life had threefold higher odds of autism (adjusted OR=3.0, 95% CI 1.1–8.1), after adjusting for race, maternal education, and household structure.

Strength of evidence: VERY LOW, Risk of bias: HIGH

- Strengths include use of a nationally representative probability sample (NHIS) and adjustment for several sociodemographic covariates.
- Limitations include small case numbers (n=30), reliance on parental report of autism diagnosis without standardized diagnostic instruments, and incomplete vaccination records that may have introduced exposure misclassification. The unexposed comparator group was small, limiting statistical power and inflating odds ratios. Results are highly sensitive to analytic assumptions (e.g., restricting to birth cohorts before 1999).