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IMPACTO DA EXPOSIÇÃO GESTACIONAL A XENOBIÓTICOS NO DESENVOLVIMENTO FETAL/INFANTIL – REVISÕES SISTEMÁTICAS

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Ciências Farmacêuticas pela Universidade Federal de Alfenas. Área de concentração: Ciências Farmacêuticas.

Orientadora: Profa. Dra. Larissa Helena Lobo Torres Pacheco
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"IMPACTO DA EXPOSIÇÃO GESTACIONAL A XENOBIÓTICOS NO DESENVOLVIMENTO FETAL/INFANTIL – REVISÕES SISTEMÁTICAS"

O Presidente da banca examinadora abaixo assina a aprovação da Tese apresentada como parte dos requisitos para a obtenção do título de Doutor em Ciências Farmacêuticas pela Universidade Federal de Alfenas. Área de concentração: Ciências Farmacêuticas

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"O que sabemos é uma gota; o que ignoramos é um oceano."

(ISAAC NEWTON, data desconhecida)

RESUMO

Este estudo realizou três revisões sistemáticas independentes para avaliar os efeitos da exposição gestacional a chumbo (Pb), cafeína e triclosan (TCS) sobre desfechos neurocomportamentais na infância. As revisões foram conduzidas seguindo rigorosos protocolos metodológicos registrados no PROSPERO (CRD42022296750, CRD42023421164, CRD42024526426). Para a análise do Pb, foram incluídos 21 estudos observacionais (16 coortes prospectivas, 2 caso-controle, 1 caso-controle aninhado, 1 coorte e 1 longitudinal), identificados através de busca abrangente nas bases MEDLINE, Cochrane Library, EMBASE, Scopus, Web of Science e LILACS. Dois revisores independentes realizaram a seleção dos estudos, extração de dados e avaliação de qualidade utilizando a ferramenta Downs & Black. A certeza das evidências foi classificada como muito baixa pelo sistema GRADE, devido à heterogeneidade dos estudos e limitações metodológicas. Os resultados indicaram possíveis associações entre exposição ao Pb e déficits neurocomportamentais ou abortos espontâneos, embora sem significância estatística consistente. Notavelmente, nenhum estudo avaliou a associação com mortalidade infantil. Para a cafeína, foram incluídos 14 estudos observacionais com amostras variando de 173 a 64.189 gestantes. O consumo médio de cafeína durante a gestação variou de 0 a 1000 mg/dia, com picos no segundo trimestre. Sete estudos relataram associações positivas entre exposição pré-natal e transtornos neurocomportamentais, como TDAH e problemas de comportamento, enquanto um estudo encontrou efeitos benéficos em habilidades sociais e seis não detectaram associações significativas. A avaliação de risco de viés utilizando a ferramenta ROBINS-I classificou a maioria dos estudos como tendo viés moderado, e a certeza das evidências foi considerada muito baixa pelo sistema GRADE. No caso do TCS, a revisão sistemática incluiu 14 estudos de coorte, com tamanhos amostrais variando de 193 a 794 pares de mães e crianças. As concentrações medianas de TCS durante a gestação variaram de 0,40 a 28,2 ng/mL. Quatro estudos sugeriram associações com problemas neurocomportamentais, incluindo externalização, déficit de atenção e dificuldades de comunicação, enquanto oito estudos não encontraram efeitos significativos. A avaliação metodológica revelou limitações importantes na mensuração da exposição e controle de fatores de confusão. Os resultados das três revisões sistemáticas destacam desafios metodológicos comuns, incluindo heterogeneidade nos desenhos dos estudos,

variabilidade na avaliação da exposição e presença de múltiplos fatores de confusão não controlados. Embora alguns estudos tenham sugerido associações potenciais, a baixa certeza das evidências impede conclusões definitivas sobre os efeitos dessas exposições. Os achados reforçam a necessidade de estudos prospectivos bem desenhados, com tamanhos amostrais adequados, métodos padronizados para avaliação de exposição e controle rigoroso de fatores de confusão. Além disso, futuras pesquisas deveriam investigar os mecanismos biológicos subjacentes às potenciais associações observadas. Estas revisões contribuem para o campo da saúde ambiental perinatal ao sintetizar criticamente as evidências disponíveis e identificar lacunas importantes no conhecimento, destacando a relevância de políticas públicas preventivas e monitoramento contínuo dessas exposições durante a gestação.

Palavras-chave: chumbo; cafeína; triclosan; neurodesenvolvimento; gestação; revisão sistemática; exposição pré-natal.

ABSTRACT

This study conducted three independent systematic reviews to evaluate the effects of gestational exposure to lead (Pb), caffeine, and triclosan (TCS) on neurobehavioral outcomes. The reviews were performed following rigorous methodological protocols registered in PROSPERO (CRD42022296750, CRD42023421164, CRD42024526426). For the Pb analysis, 21 observational studies were included (16 prospective cohorts, 2 case-control, 1 nested case-control, 1 cohort, and 1 longitudinal), identified through comprehensive searches in MEDLINE, Cochrane Library, EMBASE, Scopus, Web of Science, and LILACS databases. Two independent reviewers performed study selection, data extraction, and quality assessment using the Downs & Black tool. The certainty of evidence was classified as very low according to the GRADE system, due to study heterogeneity and methodological limitations. The results indicated potential associations between Pb exposure and neurobehavioral deficits or spontaneous abortions, although without consistent statistical significance. Notably, no study evaluated the association with infant mortality. For caffeine, 14 observational studies were included with sample sizes ranging from 173 to 64,189 pregnant women. The average caffeine consumption during pregnancy varied from 0 to 1000 mg/day, peaking in the second trimester. Seven studies reported positive associations between prenatal exposure and neurobehavioral disorders, such as ADHD and behavioral problems, while one study found beneficial effects on social skills and six detected no significant associations. The risk of bias assessment using the ROBINS-I tool classified most studies as having moderate bias, and the certainty of evidence was considered very low according to GRADE. Regarding TCS, the systematic review included 14 cohort studies with sample sizes ranging from 193 to 794 mother-child pairs. Median TCS concentrations during pregnancy varied from 0.40 to 28.2 ng/mL. Four studies suggested associations with neurobehavioral problems, including externalizing behaviors, attention deficits, and communication difficulties, while eight studies found no significant effects. Methodological evaluation revealed important limitations in exposure measurement and control of confounding factors. The results of the three systematic reviews highlight common methodological challenges, including heterogeneity in study designs, variability in exposure assessment, and the presence of multiple uncontrolled confounding factors. Although some studies suggested potential associations, the low certainty of evidence precludes definitive

conclusions about the effects of these exposures. The findings underscore the need for well- designed prospective studies with adequate sample sizes, standardized exposure assessment methods, and rigorous control of confounders. Furthermore, future research should investigate the biological mechanisms underlying the potential associations observed. These reviews contribute to the field of perinatal environmental health by critically synthesizing available evidence and identifying important knowledge gaps, emphasizing the relevance of preventive public health policies and continuous monitoring of these exposures during pregnancy.

Keywords: lead; caffeine; triclosan; neurodevelopment; pregnancy; systematic review; prenatal exposure.

SUMÁRIO

1 INTRODUÇÃO GERAL

Triclosan, cafeína e chumbo são xenobióticos que têm gerado preocupações significativas em relação ao seu impacto na saúde humana, especialmente durante períodos críticos, como a gestação. Esses compostos, comumente encontrados emdiversos produtos de consumo e fontes ambientais, possuem diferentes vias de exposição e potenciais efeitos adversos no desenvolvimento fetal e infantil (Al-Saleh *et al.*, 2011; Dann; Hontela, 2011; Blanco-Castañeda *et al*., 2020).

O chumbo (Pb) é um metal pesado com efeitos tóxicos bem documentados, particularmente em fetos em desenvolvimento e crianças pequenas. A exposição ao chumbo pode ocorrer através de várias fontes, incluindo água contaminada, solo, poeira doméstica e certos produtos de consumo. A exposição ao chumbo durante a gestação é particularmente preocupante devido ao seu potencial para causar aborto espontâneo, mortalidade infantil e déficits cognitivos. O chumbo pode atravessar a barreira placentária, afetando diretamente o desenvolvimento cerebral fetal levando a problemas no neurodesenvolvimento e comportamentais a longo prazo (Olufemi *et al.,* 2022).

A cafeína é um estimulante do sistema nervoso central comumente consumido através de café, chá, refrigerantes e vários medicamentos. Embora a ingestão moderada de cafeína seja geralmente considerada segura para a população em geral, seu consumo durante a gestação tem sido examinado devido aos possíveis riscos ao desenvolvimento fetal. Estudos sugerem que a ingestão elevada de cafeína durante a gravidez pode estar associada a desfechos adversos do neurodesenvolvimento, incluindo baixo peso ao nascer, parto prematuro e atrasos no desenvolvimento. Os mecanismos precisos pelos quais a cafeína afeta o desenvolvimento fetal ainda estão em investigação, destacando a necessidade de mais pesquisas para estabelecer diretrizes claras para o consumo de cafeína durante a gravidez (Maslova *et al*., 2010).

O triclosan (TCS) é um agente antimicrobiano amplamente utilizado em diversos produtos domésticos e de cuidados pessoais, como sabonetes, pastas de dente e cosméticos. Apesar de sua eficácia no controle do crescimento bacteriano, o TCS tem sido associado a vários problemas de saúde, incluindo a disrupção da homeostase dos hormônios tireoidianos, toxicidade no neurodesenvolvimento, alteração neurocomportamental, disfunção imunológica e correlações com doenças

alérgicas e prognóstico de câncer de mama. Além disso, o TCS interfere nas vias do ácido retinoico, na microbiota intestinal e nos mecanismos estrogênicos, levantando preocupações sobre seu uso generalizado e seus potenciais efeitos a longo prazo na saúde humana (Dann; Hontela, 2011; Mustieles *et al.,* 2023).

Em resumo, a presença ubíqua de chumbo, cafeína e triclosan na vida cotidiana e seus potenciais efeitos adversos no neurodesenvolvimento e na saúde geral ressaltam a importância da avaliação científica rigorosa e da supervisão regulatória. Compreender os mecanismos e riscos associados a esses xenobióticos é crucial para desenvolver políticas de saúde pública eficazes e garantir a segurança das populações vulneráveis, especialmente de mulheres gestantes e seus descendentes em desenvolvimento.

2 REVISÃO DE LITERATURA

2.1 Desenvolvimento do SNC e Xenobióticos

O desenvolvimento do sistema nervoso central (SNC) constitui um processo dinâmico e sequencial que se inicia nas primeiras semanas de gestação e se prolonga até a adolescência, com maior intensidade nos primeiros mil dias de vida (Stiles; Jernigan, 2010). Este período de extraordinária plasticidade neural envolve uma cascata de eventos interdependentes, desde a proliferação de precursores neurais na zona ventricular até a complexa organização sináptica cortical (Silbereis *et al.*, 2016). Durante a fase embrionária (3-8 semanas), ocorre a neurogênese ativa e a migração neuronal guiada por fibras gliais radiais, estabelecendo as seis camadas corticais fundamentais. O terceiro trimestre gestacional e os primeiros anos pós-natais são marcados por explosiva sinaptogênese, com formação de aproximadamente 40.000 novas conexões sinápticas por segundo acompanhada por processos simultâneos de mielinização progressiva e poda sináptica dependente de atividade. (Stiles; Jernigan, 2010),

Esta intrincada sequência desenvolvimental apresenta janelas críticas de vulnerabilidade a agentes xenobióticos (Rice; Barone, 2000). No período embrionário, a exposição a neurotóxicos pode comprometer irreversivelmente a arquitetura básica do SNC, enquanto na fase fetal predominam alterações funcionais sutis, porém igualmente impactantes (Grandjean; Landrigan, 2014). A barreira hematoencefálica imatura, apenas parcialmente formada até o terceiro trimestre permite maior penetração de substâncias neuroativas. (Stolp *et al.*, 2013). Além disso, os sistemas de detoxificação hepática fetal apresentam atividade reduzida das enzimas do citocromo P450 prolongando a meia-vida de compostos lipofílicos como o chumbo e os disruptores endócrinos. (Hines, 2008),

Estudos de neurobiologia do desenvolvimento demonstram que xenobióticos podem interferir em múltiplos processos celulares: (1) inibição da proliferação de progenitores neurais através da disrupção do ciclo celular; (2) alteração dos mecanismos de migração neuronal mediados por moléculas de adesão; (3) comprometimento da mielinização por efeitos sobre oligodendrócitos imaturos; (4) indução de apoptose neuronal via ativação de caspases (Mason *et al.*, 2014). Particularmente preocupante é a capacidade de alguns metais pesados, como o

chumbo, de mimetizar íons cálcio, interferindo na sinalização intracelular essencial para o desenvolvimento sináptico (Mason *et al.*, 2014).

A vulnerabilidade é ainda maior considerando a dinâmica temporal específica de cada região encefálica (Tau; Peterson, 2010). O hipocampo, crucial para aprendizagem e memória, apresenta prolongado período de desenvolvimento pós-natal, mantendo-se suscetível a interferências ambientais por anos após o nascimento. Em contraste, estruturas subcorticais como o tálamo completam sua organização majoritariamente durante a vida intrauterina. Estas diferenças regionais explicam a variabilidade nos fenótipos neurocomportamentais observados após exposições pré-natais a xenobióticos (Grandjean; Landrigan, 2014).

Evidências recentes destacam que os efeitos disruptivos podem manifestar-se através de mecanismos epigenéticos com alterações persistentes nos padrões de metilação do DNA e modificações de histonas em genes críticos para o neurodesenvolvimento, como BDNF e RELN (Kundakovic; Jaric, 2017, Park, 2022). Tais modificações podem perpetuar-se transgeracionalmente, segundo Bohacek e Mansuy (2015), mesmo na ausência de exposição direta das gerações subsequentes. Esta perspectiva integrativa entre toxicologia e epigenética do desenvolvimento tem redefinido nossa compreensão sobre como exposições ambientais precoces podem programar trajetórias neurocomportamentais ao longo da vida (Kundakovic; Jaric, 2017).

2.2 Chumbo (Pb) como Disruptor Neurocomportamental

O chumbo (Pb) configura-se como um dos neurotóxicos mais estudados e preocupantes no contexto do desenvolvimento neurológico infantil (Neal & Guilarte, 2015). Como metal pesado de ampla distribuição ambiental, sua exposição durante os períodos críticos do desenvolvimento cerebral pode levar a alterações neurocomportamentais persistentes e muitas vezes irreversíveis (Mason *et al.*, 2014). Estudos de García-Lestón *et al.* (2010) demonstram que o Pb atravessa facilmente a barreira placentária e a barreira hematoencefálica ainda imatura no feto, acumulando-se preferencialmente no tecido nervoso em desenvolvimento. Os mecanismos de neurotoxicidade do Pb são multifatoriais, envolvendo principalmente a competição com íons cálcio (Ca^{2+}), essenciais para a sinalização neuronal a geração

de estresse oxidativo através da depleção de glutationa - um importante antioxidante celular - e a disrupção da função mitocondrial em neurônios e células gliais (Sanders *et al.*, 2009; Neal; Guilarte, 2015). Além disso, o Pb interfere significativamente nos processos de mielinização fundamentais para a transmissão eficiente dos impulsos nervosos (Lidsky; Schneider, 2003),

As consequências dessa exposição são bem documentadas na literatura epidemiológica. Crianças expostas ao Pb apresentam, de forma dose-dependente, redução de 2 a 5 pontos de QI para cada 10 µg/dL de aumento nos níveis sanguíneos desse metal (Lanphear *et al.*, 2005). Além dos prejuízos cognitivos globais, observa-se maior incidência de transtorno de déficit de atenção/hiperatividade (TDAH) nessa população assim como déficits específicos em funções executivas e memória de trabalho (Cecil *et al.*, 2008; Froehlich *et al.*, 2009)

- habilidades fundamentais para o aprendizado e adaptação social. Esses efeitos parecem ser particularmente pronunciados quando a exposição ocorre nos primeiros anos de vida período de máxima plasticidade neural e vulnerabilidade a agentes tóxicos. (Rice; Barone, 2000).

2.3 Cafeína e Neurodesenvolvimento

A cafeína, um alcaloide pertencente à classe das xantinas, tem seu consumo amplamente disseminado na população geral, incluindo mulheres em idade fértil e gestantes (de Paula; Farah, 2019). Durante a gravidez, a cafeína atravessa livremente a barreira placentária expondo o feto a concentrações semelhantes às maternas (Qian *et al.*, 2020). O metabolismo da cafeína no feto é particularmente lento devido à imaturidade do sistema enzimático hepático, especialmente das enzimas do citocromo P450 prolongando sua meia-vida e, consequentemente, seu tempo de ação no organismo em desenvolvimento. (Hines, 2008),

Os efeitos da exposição pré-natal à cafeína no neurodesenvolvimento têm sido objeto de intenso debate científico. Estudos prospectivos de grande porte identificaram associações entre o consumo materno de cafeína e alterações comportamentais na prole, incluindo maior incidência de hiperatividade e déficits de atenção (James, 2021). Segundo Alhowail e Aldubayan (2020), os mecanismos propostos para esses efeitos incluem a interferência da cafeína nos receptores de

adenosina, que desempenham papel crucial na modulação da atividade neuronal durante o desenvolvimento cerebral. Além disso, a cafeína pode alterar os padrões de sono fetal e o fluxo sanguíneo uteroplacentário fatores indiretos que também influenciam o desenvolvimento neural. (Christensen *et al.*, 2021).

Contudo, é importante destacar que os achados científicos sobre os efeitos da cafeína não são unânimes. O estudo de Benglundh *et al.* (2021) não encontrou associações clínicas significativas entre o consumo moderado de cafeína durante a gestação e desfechos neurocomportamentais adversos em crianças de até 8 anos. Essa divergência pode refletir diferenças metodológicas entre os estudos, variações genéticas no metabolismo da cafeína entre as populações estudadas, ou a influência de fatores de confusão não adequadamente controlados.

2.4 Triclosan e Desenvolvimento Neurológico

O triclosan, um agente antimicrobiano amplamente utilizado em produtos de higiene pessoal e itens domésticos, tem emergido como uma preocupação em saúde pública devido aos seus potenciais efeitos como disruptor endócrino e neurotóxico (Dann; Hontela, 2011). Estudos recentes demonstram que o triclosan pode atravessar a barreira placentária e ser detectado no sangue do cordão umbilical indicando que o feto em desenvolvimento está exposto a essa substância (Pycke *et al.*, 2014).

Os mecanismos pelos quais o triclosan pode interferir no desenvolvimento neurológico incluem sua ação como disruptor do eixo tireoidiano (Guilbert *et al.*, 2021). Os hormônios tireoidianos são essenciais para a migração neuronal, mielinização e sinaptogênese durante o desenvolvimento cerebral (Zoeller; Rovet, 2004). Ao interferir com a sinalização desses hormônios, o triclosan pode prejudicar esses processos fundamentais. Além disso, evidências experimentais sugerem que o triclosan pode alterar a expressão de genes envolvidos no desenvolvimento neuronal e promover processos neuroinflamatórios (Szychowski *et al.*, 2016; Ling *et al.*, 2020).

Estudos epidemiológicos que investigaram a associação entre a exposição pré-natal ao triclosan e desfechos neurocomportamentais na infância apresentam resultados mistos. Alguns relataram associações com maior incidência de comportamentos externalizantes e déficits de atenção, enquanto outros não encontraram efeitos significativos (Philippat *et al.*, 2017; Jackson-Browne *et al.*, 2019).

Essa inconsistência pode refletir diferenças nos níveis de exposição, janelas críticas de vulnerabilidade ou interações com outros fatores ambientais. Apesar das incertezas, a potencial neurotoxicidade do triclosan durante o desenvolvimento, combinada com sua ampla utilização em produtos de consumo, justifica a adoção do princípio da precaução e a realização de mais pesquisas para elucidar seus efeitos a longo prazo.

3 ARTIGO 1- Gestational lead exposure and its effects on fetal/infant development - A systematic review

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Gestational lead exposure and its effects on fetal/infant development - A systematic review

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ABSTRACT

Studies suggest that gestational exposure to lead (Pb) is related to spontaneous abortions, preterm birth, lower infant birth weight and length, and neurological dysfunctions. However, the evidence about its effects during pregnancy exposure on fetal and child development is still poor. Thus, the aim of this systematic review was to verify the association between prenatal exposure to Pb and the occurrence of neurobehavioral deficits, miscarriages, and child mortality. Observational studies with pregnant women exposed to Pb during pregnancy were included, without gender or ethnicity restrictions. The MEDLINE, Cochrane Library, EMBASE, Scopus, Web of Science, and LILACS databases were searched. The reading of titles and abstracts was conducted, followed by reading in full format and data extraction, that were performed independently by two reviewers. The included studies were evaluated by Downs and Black tool and qualitatively synthesized. Certainty of evidence was assessed by Grading of Recommendations Assessment, Development, and Evaluations (GRADE). The study protocol was registered with the Prospective Registry of Systematic Reviews (PROSPERO; CRD42022296750). Among twenty-one studies included, sixteen were classified as prospective cohort, two case-control, one nested case-control, one cohort, and one longitudinal study. No study that evaluated child mortality associated with gestational Pb exposure was found. There is a very low certainty of evidence in the association of gestational Pb exposure and neurobehavioral deficits or miscarriages. This systematic review reflects the poor evidence and the challenges of human toxicology studies, since it was not possible to associate gestational Pb exposure to neurobehavioral deficits, miscarriages, and child mortality.

1. Introduction

The exposure to xenobiotics, such as heavy metals, during gestational and perinatal periods is associated with implications for fetal and child development. Studies show that exposure to heavy metals during a critical period of development is related to spontaneous abortions, preterm birth, lower infant birth weight, length, and head circumference, and neurological dysfunctions [1,2]. Preclinical studies show deleterious effects of Pb. Rats exposed to Pb showed spatial learning deficits affecting the hippocampus and cerebellum systems. In cows, Pb contributes to induced infertility, premature parturition, and abortion [3-5].

Environmental contamination by lead (Pb) and its toxicity have been widely recorded in recent decades [6]. Pb is a ubiquitous element identified in organic/inorganic forms and its exposure occurs mainly by ingestion of contaminated food or water, and inhalation or skin contact with environment pollution or soil [7]. Exposure to Pb is related to neurological, hematological, and renal toxic effects [8]. About 800

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million children and young people aged 0–19 years old worldwide have blood Pb levels at or above 5 μg/dL, an alarming concentration of the metal in the body [9]. In 2021, the Centers for Disease Control and Prevention (CDC, United States), updated the blood Pb levels considered acceptable to 3.5 μg/dL; the previous recommendation were Pb levels of 5 ug/dl [10].

Hu et al., [11] demonstrated that moderately high levels of Pb (7.1 ± 5.1 μg/dL) in maternal blood during the first trimester of pregnancy indicated an adverse effect on neurodevelopment, observed from worse scores in the Mental Developmental Index (MDI) during the postnatal period [11]. Lamadrid-Figueroa et al. [12] observed increased incidence of miscarriage in women from Mexico City exposed to Pb during the first trimester of pregnancy in which Pb levels were 6.24 and 0.014 μg/dL in whole blood and plasma, respectively [12]. Several studies also showed that prenatal and/or postnatal exposure to Pb disturbs cognitive development in children [13,14]. A study showed that higher maternal blood Pb levels were associated to preterm birth; however, the authors did not find association between maternal or umbilical cord Pb levels and neurodevelopment deficits [15]. Yorifuji et al. [16] observed that Pb was associated to cognitive deficits when in the concomitant presence of methylmercury, while Inoue et al. [17] did not find association between prenatal Pb exposure and neurodevelopmental delay in children up to 3 years old [16,17].

Studies suggest that heavy metals such as Pb, cadmium, mercury, and arsenic can interfere with pregnancy and contribute to child mortality, a social indicator rate represented by the number of children who died before reaching one year of life for every thousand children born alive in the period of one year [18,19]. Kaur et al. [20] demonstrated that the association of cadmium and Pb increased the risk of spontaneous abortion due to environmental toxicity, being cytogenetic, immunological, and endocrinological factors [20]. Pb can replace calcium, zinc, and other divalent cations in physiological cell processes, interfering for example in trophoblasts, peripheral cells of the blastocyst that attach the zygote to the uterine wall [21]. The decrease in calcium uptake induced by Pb in the syncytiotrophoblast, a primary structure responsible for nutrient and gas exchange in the human placenta, leads to greater fetal exposure to this metal in addition to decreasing placental weight, contributing to spontaneous abortion [20]. The period of gestational exposure can exert some influence on the final result, in addition to maternal exposure that may have occurred before pregnancy. Pb can be mobilized from the mother to the fetus. An elevated bone Pb level during pregnancy can lead to more significant mobilization for the fetus compared to other mothers who were not exposed before pregnancy [22].

Although studies have suggested the deleterious effects of prenatal Pb exposure on fetal and child health, the evidence is still poor. The causality between exposure to heavy metals and neurodevelopment disturbance, spontaneous abortion, or child mortality often cannot be assumed. In this systematic review, we verify among twenty-one observational studies the effects of Pb gestational exposure on spontaneous abortion, child mortality, and cognitive deficits.

2. Methods

This systematic review was performed based on observational studies, according to the PRISMA 2020 Statement [23], being registered in the PROSPERO platform under the number CRD42022296750. The question of the study was "Does Pb exposure during a critical period of human development lead to miscarriage, infant mortality, and learning disabilities?". PRISMA was used for reporting this systematic review.

2.1. Eligibility criteria

Observational studies with pregnant women exposed to Pb during pregnancy were included. No restrictions on language, ethnicity, or publication status were imposed. Studies covering exposure exclusively in the postnatal period, exposure to other heavy metals, and co-association of heavy metals or pollutants were excluded.

2.2. Information sources

The databases selected for research were categorized as conceptual and essential (MEDLINE, Cochrane Library, and EMBASE), specialized and optional (Scopus and Web of Science) and regional (LILACS). In addition, a manual search was performed in the reference list of included studies, expert consultations and the gray literature (Biorxiv, Capes and Medrxiv). The research was performed until October 2021: Scopus and LILACS (09/28/2021), MEDLINE, Web of Science, and Cochrane Library (10/02/2021) and Embase (10/04/2021) (Supplementary material A).

2.3. Search strategy

The search strategy was planned to locate observational studies. The study question was designed according to the PECOS strategy [24]. Participants (P): Pregnant women exposed to Pb; Exposure (E): Gestational Pb exposure; Comparison (C): Pregnant women not exposed to Pb; Outcomes (O): Miscarriage, child mortality and learning disabilities; and Studies (S): Observational studies. MesH, DeCS, and Emtree descriptors were crossed with the Boolean operators "AND" (inter-category) and "OR" (intra-category).

2.4. Selection process

The retrieved studies were exported to the EndNote® (online version) and Rayyan® platforms [25], and duplicates were eliminated in both software. The selection was conducted independently by two researchers (ABS and LEWS) based on the eligibility criteria established for the present systematic review. Initially the studies were selected by reading title and abstract, followed by a full text read of those selected. Disagreements were resolved in consensus meetings with a third researcher. The agreement between researchers was analyzed by a Kappa coefficient using GraphPad QuickCalcs (Graphpad, San Diego, CA), in which a result above 0.60 was considered acceptable.

2.5. Data collection process

Two reviewers performed data extraction of the included studies independently. Data extraction provided the following information: i) General characteristics of the studies included: title, author, year, country, number of participants, study design, follow-up/evaluation time; ii) Intervention characteristics: Pb exposure indicator and index; and iii) Outcomes included: miscarriage, child mortality and/or learning disabilities. The outcome assessment instrument and the authors' conclusions were also collected with the respective results. The collected information was compared between investigators and disagreements were settled by consensus with a third investigator.

2.6. Study risk of bias assessment

The methodological quality of the studies was assessed using the Downs and Black [26] scale by two reviewers independently [26]. The scale includes 27 questions that assess internal and external validity, reporting patterns. Items can receive a score of 0 or 1, with exceptions for questions 5 and 27. This assessment indicated the quality of each study [27,28]. Scores for each study can be seen in Supplementary material B.

2.7. Synthesis methods

A qualitative synthesis of the data was performed, presenting their information in tables in a narrative way. The results were summarized

considering the methodological quality and the conclusions observed in each study. The methodological quality classification was ranked from the following scores: Excellent quality: 26–28; Good quality: 20–25; Fair: 15–19; Poor: < 15 [27,28]. Due to the heterogeneity of the included studies, no meta-analysis was performed.

2.8. Certainty assessment

The level of evidence of the data was assessed by GRADE using GRADEpro software [29]. Each outcome considered in this study was evaluated.

3. Results

3.1. Study selection

From the searches performed in the databases, 6540 registered articles were retrieved, the elimination of duplicates allowed a final result of 5238 studies. Through screening and eligibility, the selection process retrieved 33 studies, which were read in full. Twenty studies were excluded after the full reading (Supplementary Material B). The thirteen remaining studies met the inclusion criteria and were included in this review. Eight studies were added from the manual search. Therefore, twenty-one studies were included in the systematic review, as shown in the PRISMA flow diagram (Fig. 1). Agreement among researchers were considered acceptable (Kappa = 0.665).

3.2. Study characteristics

The included studies evaluated neurocognitive deficits or spontaneous abortion as outcomes. No study with the inclusion criteria established in the present systematic review that evaluated child mortality as an outcome was found. The characteristics of each of the studies are presented in the Tables 1 and 2, which were divided accordingly to the respective outcome: neurocognitive deficits or spontaneous abortion. Among the studies included, twenty-one were published between 1986 and 2013, sixteen studies were classified as prospective cohort, two case-control, one nested case-control, one cohort, and one longitudinal. Most publications come from the United States (nine studies), the remaining are divided into: Yugoslavia (three studies), Mexico (three studies), Australia (two studies), Italy (one study), China (one study), Iran (one study) and Turkey (one study).

For the neurocognitive deficit outcome (Table 1), the sample size among pregnant women ranged from 146 to 1502, and among children ranged from 146 to 2657. Ten studies did not report the sample value for pregnant women. The period of exposure evaluation varied between day 10–96 months. Related to the exposure sample indicator, nine studies used Pb in umbilical cord blood [11,30,32,33,37–41]; three studies used teeth Pb levels [32,31,40]; two studies used capillary blood [30,33]; and nine studies used venous blood samples [31,33–39,41]. In terms of serum levels of Pb in the umbilical cord, values ranged from 6.5 ± 1.8 ug/dL to 22.4 ug/dL at birth. Pb in venous blood ranged from 6.4 ± 4.1–39.9 μg/dL. Pb in dentin ranged from 2.8 ± 1.7–3.4 ± 2.4 μg/g. Pb in capillary blood ranged from 1.8 ug/dL to 17.5 ± 1.5 ug/dL.

Related to the spontaneous abortion outcome (Table 2), the number of participants (women) was from 40 to 2067. Study participants were 21 weeks pregnant or less. All studies evaluated serum Pb. One study evaluated the plasma Pb level [12]; one evaluated Pb in the umbilical cord [44]; one evaluated erythrocyte protoporphyrin [45]; and one study evaluated the levels of homocysteine [48]. The blood value of Pb levels among pregnant women ranged from 11.03 ug/dL to 22.17 ug/dL. The Pb level in the plasma average was 0.014 ± 0.013 μg/dL. The umbilical cord Pb level was 6.2 ± 0.22–10.1 ± 0.19 μg/dL.

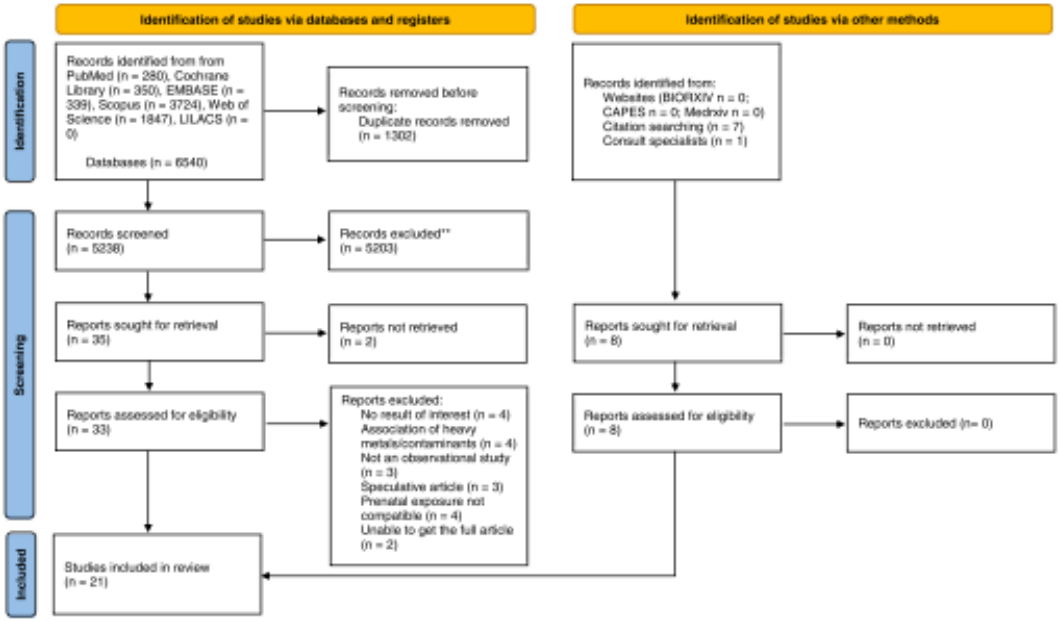

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram [23].

Table 1
General characteristics of studies included that presented neurocognitive deficits as an outcome (n = 13).

Author, Year, Country	Study design	Number of participants (women/ children) Time of exposure evaluation (months)	Exposure sample	Exposure index
[30], USA	Prospective cohort study	NR/249 6–24 months	Umbilical cord blood Pb levels (prenatal); Capillary-blood (postnatal)	Umbilical-cord blood: 6.6 ± 3.2 μg/dL Capillary-blood Pb levels at birth: Low = 1.8 ± 0.6; Medium = 6.5 ± 0.3; High = 14.6 ± 3.0 / 6 months: Low = 4.6 ± 3.9; Medium = 7.0 ± 7.8; High = 7.0 ± 8.7 / 12 months: Low = 5.8 ± 5.1; Medium = 8.5 ± 7.6; High = 8.8 ± 6.4 / 18 months: Low = 6.7 ± 5.5; Medium = 8.3 ± 5.8; High = 7.6 ± 5.8 / 24 months: Low = 5.4 ± 4.8; Medium = 7.2 ± 5.0; High = 7.7 ± 8.5
[31], USA	Prospective cohort study	NR/169 57 months	Blood Pb levels (venous blood); tooth Pb levels	Blood Pb at 57 months: 6.4 ± 4.1 μg/dL; tooth: 2.8 ± 1.7 μg/g
[32], USA	Prospective cohort study	NR/2657 96 months	Umbilical cord blood Pb levels tooth Pb levels	Blood: 6.8 ± 3.1 μg/ dL; tooth: 3.4 ± 2.4 μg/g
[33], Australia	Prospective cohort study	299/318 6–48 months	Venous blood (mothers and children ≥ 6 months); cord blood (children-at birth) Capillary bood (children-6 months all samples and ≥ 6 months half samples)	Venous blood (Maternal): 9.1 ± 1.3 μg/dL; Umbilical cord: 8.1 ± 1.4 μg/ dL; Capillary blood (6–36 months) 6 months: 15.0 ± 1.6 μg/dL; 12 months: 17.1 ± 1.4 μg/dL, 18 months: 17.5 ± 1.5 μg/dL, 24 months: 16.6 ± 1.4 μg/dL, 30 months: 16.3 ± 1.5 μg/dL, 36 months: 15.7 ± 1.4 μg/dL Venous blood (children) (12–48 months) 12 months: 13.2 ± 1.5 μg/dL; 18 months: 15.3 ± 1.5 μg/dL; 24 months: 14.2 ± 1.5 μg/dL; 36 months: 11.8 ± 1.4 μg/dL; 42 months: 10.7 ± 1.5 μg/dL; 48 months: 10.1 ± 1.4 μg/dL.
[34], USA	Prospective cohort study	NR/237 Day 10 – 48 months*	Blood Pb levels maternal (prenatal) and from children (postnatal) Venous blood samples (mostly), blood samples (heel or finger stick)	Maternal: 8.3 ± 3.7 μg/dL; neonatal: 5 ± 3.4 μg/dL; 3–12 months: 10.6 ± 5.1 μg/dL; 15–24 months: 17.1 ± 8.4 μg/dL; 27–36 months: 16.3 ± 7.7 μg/dL; 39–48 months: 14.0 ± 7.2 μg/dL;
[35], USA	Prospective cohort study	NR/259 60 months	Reported in early study	51–60 months: 11.8 ± 6.3 μg/dL
[36], USA	Prospective cohort study	NR/253 78 months	Reported in early studies	NR
[37], USA	Prospective cohort study	NR/285 6–36 months	Venous blood (children) umbilical cord Pb levels and blood Pb (maternal blood)	Blood: Maternal at delivery = 6.5 ± 1.8 μg/dL; children- 6 months = 10.05 ± 3.3 μg/dL; 2 years = 16.74 ± 6.5 μg/dL; 3 years = 16.68 ± 5.93 μg/dL / Umbilical cord = 5.99 ± 2.11 μg/dL
[38], USA	Prospective cohort study	NR/242 58 months	Venous blood (children) umbilical cord Pb levels and blood Pb (maternal blood)	Prenatal PbB - Maternal:6.5 ± 1.8 μg/dL, cord: 5.89 ± 2.10 μg/dL. Preschool PbB - 6 months: 9.99 ± 3.32 μg/dL, 24 months: 16.7 ± 6.45 μg/ dL, 36 months: 16.7 ± 6.01 μg/dL
[39], Yugoslavia	Prospective cohort study	1502/706 6–90 months	Venous blood, cord blood and blood Pb	NR
[11], Mexico	Prospective cohort study	146/146 12 and 24 months	Umbilical cord blood, blood Pb levels and plasma	Maternal: First trimester = 7.1 ± 5.1 μg/dL; Second = 6.1 ± 3.2 μ g/dL; Third = 6.9 ± 4.2 μg/dL; Delivery = 7.3 ± 4.3 μg/dL / Children: Umbilical cord = 6.2 ± 3.9 μg/dL; 12 months = 5.2 ± 3.4 μg/dL; 24 months = 4.8 ± 3.7 μg/dL; Plasma: First trimester: 0.016 ± 0.014 μg/dL, Second trimester: 0.014 ± 0.011 μg/dL, Third trimester: 0.016 ± 0.024 μg/dL
[40], USA	Prospective cohort study	NR/1923 72 months	Blood Pb levels; umbilical cord Pb levels; tooth Pb levels	Umbilical cord = 6.8 μg/dL; Tooth = 3.3 μg/g
[41], Yugoslavia	Prospective cohort study	NR/ 388 36 and 48 months	Venous blood (prenatal and postnatal), Umbilical cord, Blood Pb levels (children)	Children at birth in the exposed town: 22.4 μg/dL / 4 years= 39.9 μg/dL Children at birth in the unexposed town: 5.4 μg/dL / at 4 years old = 9.6 μg/dL

Exposure index refers to the Pb levels found in different tissues and time periods; NR: not reported.
*Blood Pb was measured in the mother at her first prenatal visit, and in the neonate at 10 days corrected for gestational age.

3.3. Neurocognitive deficit

Table 3 shows neurocognitive deficit reported in thirteen included studies. The outcomes evaluated according to the researchers were: development alterations (three studies), children behavior (six studies), cognitive function (six studies), neurobehavioral development (one study), perceptual function (one study), intellectual function (one study), and motor assessment (one study). The assessment of the outcome was made using specific scales for the children's age. Bayley Scale: 1–42 months; McCarthy scale: 2.5–8.5 years; Kaufman: 2.5–12.5 years; Wechsler: 3–7 years. Bellinger et al., [32] and Leviton et al., [40] performed a specific form for the teacher to assess the child's behavioral profile.

The Bayley Scale was used in four studies: two studies did not report the values found [11,39]; in the other two studies [30,37], the MDI found at six months of age in the high cord-blood Pb group were on average 106.1 ± 11.1 and 112.1 ± 18.6, suggesting a possible decline in performance. The McCarthy scale was used in four studies: two studies did not report the values found [39,41]; one study reported the General Cognitive Index (GCI) at 11.5 ± 14.5, in which the poor performance on the McCarthy scale at 57 months was related to high Pb levels at 24 months [31]; the other study reported 107.3 ± 14.2 at 48 months, without association with cognitive decline [33]. Kaufman was used in two studies: Mental Process Composite (MPC) values found were 80.3 ± 10.4 [34] and 87.2 ± 10.7 [35] and higher prenatal Pb exposure was not associated with developmental cognitive deficit. The Wechsler scale was used in three studies: one study did not report the unadjusted values associated to Pb levels [39]; in the other two studies the Intelligence Quotient (IQ) scale found was 86.9 ± 11.3 and 87.5 ± 16.6, not demonstrating prenatal association with Pb and cognitive decline [36, 38]. Two studies used the Child Behavior Profile Teacher Report Form: the first study demonstrated an average of behavioral problems of 47.5 ± 9.5, in which the cord blood Pb level was not associated with behavioral problems [32]; the second study had a result of 61.4 and 46.3 in the Child Behavior Checklist (CBCL 2/3) in two towns, respectively, and the blood Pb measured was associated to poor performance in test [39]. One study used the Boston teacher questionnaire with the behavioral values index at 9.5% for boys and 4% for girls, both genders showed development deficits associated with Pb levels in the umbilical cord [40]. One study used the Kent Infant Development Scale - modified

Table 2
General characteristics of studies included that presented spontaneous abortion as an outcome (n = 8).

Author, Year, Country	Study design	Number of participants (women)	Exposure sample	Exposure index
[42], Mexico	Nested case-control study	668	Blood Pb levels - maternal	Blood: 11.03 μg/dL
[43], Turkey	Case- control study	40	Blood Pb levels	SA = 18.8 μg/dL; control = 22.17 μg/dL
[12], Mexico	Prospective cohort study	207	Blood Pb levels	All woman: Blood = 6.24 ± 4.5 μg/dL; Plasma = 0.014 ± 0.013 μg/dL / Not miscarriages: Blood = 6.47 ± 4.95 μg/dL; Plasma = 0.013 ± 0.013 μg/dL / Miscarriages: Blood = 5.8 ± 3.41 μg/dL; Plasma = 0.013 ± 0.014 μg/dL.
[44], Australia	Prospective cohort study	831	Blood Pb levels; umbilical cord Pb levels	Blood < 14 weeks: PP = 10.8 ± 0.24 μg/dL; NPP = 7.7 ± 0.61 μg/dL/ 14–20 weeks: PP = 10.6 ± 0.17 μg/dL; NPP = 7.6 ± 0.19 μg/dL/ 21–29 weeks: PP = 11.0 ± 0.53 μg/dL; NPP = 10.9 ± 1.6 μg/dL/ 30–36 weeks: PP = 10.7 ± 0.19 μg/dL; NPP = 7.6 ± 0.21 μg/dL/ Delivery: PP = 11.2 ± 0.21 μg/dL; NPP = 7.5 ± 0.25 μg/dL/ Umbilical cord: PP = 10.1 ± 0.19 μg/dL; NPP = 6.2 ± 0.22 μg/dL
[45], Yugoslavia	Prospective cohort study	639	Blood Pb levels; erythrocyte protoporphyrin	Exposed town = 16 μg /dL; not exposed town = 5,2 μg/dL
[46], Italy	Cohort study	2.067	Blood Pb levels	15 μg/dL
[47], Iran	Longitudinal study	351	Blood Pb levels	Normal pregnancy = 3.83 ± 1.99 μg/dL; spontaneous abortion = 3.51 ± 1.42 μg/dL
[48], China	Case- control study	80	Blood Pb levels and homocysteine levels	Pb: Cases = 5.3 μg/dL (5.2–5.9 μg/dL); Control = 4.5 μg/dL (3.7–5 μg/dL) / Homocysteine: Cases = 262.25 μg/dL (238–328 μg/dL); Controls = 236,5 μg/dL (169–266,3 μg/dL)

Exposure index refers to the Pb levels found in different tissues and time period.
NR: not reported; PP: Port Pirie (city); NPP: non-Port Pirie.

(KID) with a value found at six months of 111.7 ± 13.8, that was not consistent with the hypothesis of an adverse effect of Pb exposure [37]. One study used the Stanford-Binet Intelligence Scale (SB) in 3-year-old children, with a mean value of 89.7 ± 15.5, not associated to prenatal Pb exposure [37]. Two studies used the Home Observation for Measurement of the Environment (HOME) assessed at 4 years with mean values of 28.6 ± 7.8 [41] and 45.5 ± 5.4 [33]. The lowest score reported by Wasserman et al. [41] was described as an association with cognitive decline, unlike Cooney et al. [33] who did not show this association. In general, ten studies demonstrated neurocognitive disorders associated with gestational Pb exposure while three studies show no association.

3.4. Spontaneous abortion

Table 4 shows spontaneous abortion reported in the eight included studies. The outcomes evaluated according to the investigators were: miscarriage (seven studies), anembryonic pregnancy (one study), early fetal death (one study), premature delivery (one study), late fetal death (one study), and congenital anomalies (one study). Considering all studies, the miscarriage rate ranged between 2.97% and 16.4%. Of the eight studies evaluated, three showed an association between gestational Pb exposure and abortion [12,42,48] and five failed to show an association between gestational Pb exposure and spontaneous abortion ([43-45]; Paredes [46,47]).

To assess the occurrence of spontaneous abortion, six of the eight studies used percentage indices: Borja-Aburto et al. [42] found a risk rate of miscarriage of 6.4%; Lamadrid-Figueroa et al. [12] found a mean of miscarriages of 0.42 (range 0–4); McMichael et al. [44] presented that 23 of 774 pregnancies had miscarriage (2.97%); Murphy et al. [45] compared the rate of miscarriage, finding 16.4% in Pb exposed pregnant women and 14% unexposed pregnant women; Paredes Alpaca et al. [46] showed that the miscarriage rate was 5.2%; and Vigeh et al. [47] demonstrated that the miscarriage rate was 4.3%. Faikoglu et al. [43] evaluated the distribution of serum levels of Pb in the control and aborted groups and found no significant correlation between pregnant women who have been exposed to Pb and unexposed pregnant women. Yin et al. [48] demonstrated the mean value of the level of Pb, folate, vitamin B12, and homocysteine between anembryonic and normal pregnancies. The authors suggest that Pb exposure may increase the risk of anembryonic pregnancies since the mean Pb levels were higher in anembryonic pregnancies than in the control group (5.3 versus 4.5 μg/dL).

3.5. Risk of bias in studies

The analysis of the methodological quality of the included studies shows that two studies are of excellent methodological quality, obtaining a score greater than 26 points, fifteen had good quality, three had fair quality and one had poor quality (Table 5; Supplementary material C). Inclusion/exclusion criteria differed between studies. Among the studies that reported neurocognitive deficits, gestational age was reported as an exclusion criterion for twelve studies and birth weight as an inclusion criterion reported by four studies. According to the World Health Organization (WHO) preterm infants are born at less than 37 weeks gestational age and low birth weight infants are born with a birth weight below 2.5 kg of gestational age, which leads to consequences in child development [49,50]. Among the six studies that reported miscarriage, a pregnancy loss less than the 20th week was considered as abortion. This range is defined as predictor of miscarriage for several studies [51,52].

3.6. Certainty of evidence

Both outcomes evaluated in this systematic review had overall certainty of the evidence classified as very low (Supplementary material D).

4. Discussion

This systematic review showed poor evidence on the effects of gestational exposure to Pb on neurocognitive deficits and miscarriage, and the absence of evidence on child mortality. The conclusions of the included studies were divergent for both neurocognitive disorders and miscarriage, and in addition, the quality of evidence was very low. No studies were found on the effects of gestational exposure to Pb on child mortality.

Regarding studies that evaluated neurocognitive disorders, the difference in conclusions can be partially explained due to methodological variations, age of the cognitive function tests, the number of participants, and the statistical modeling techniques. The lack of standardization of maternal blood collection performed in the first/second

Table 3
Neurocognitive deficits reported in the included studies. Evaluation instrument, effect measures, and conclusions.

Author, Year	Outcome	Outcome evaluation instrument	Outcome measurement	Conclusions
[30]	Development alterations	Bayley Scales of Infant Development (BSID-I)	MDI at 6 months: Low = 109.2 ± 12.9; Medium = −108.6 ± 12.0; High = 106.1 ± 11.1 / at 12 months: Low = 113.1 ± 12.5; Medium = 115.4 ± 12.9; High = 108.7 ± 12.8/ at 18 months: Low = 113.4 ± 15.5; Medium = 116.6 ± 16.7; High = 109.5 ± 17.5 / at 24 months: Low = 115.9 ± 17.2; Medium = 119.9 ± 14.4; High: 110.6 ± 16.5	Prenatal Pb exposure was associated with neurocognitive deficits at 2 years
[31]	Development alterations	McCarthy Scales of Children's Abilities	GCI = 11.5 ± 14.5; Verbal = 59.7 ± 10; Perceptual- Performance = 57.6 ± 8.7; Quantitative = 57.8 ± 8; Memory = 56.4 ± 9; Engine = 50.6 ± 8.5	Prenatal Pb exposure was associated with neurocognitive deficits from 2 to 5 years
[32]	Behavior alterations	Teacher Report Form of the Child Behavior Profile	Total problem behaviors = 47.5 ± 9.5; Internalizing = 50.3 ± 8.1; Externalizing = 49.1 ± 8; Anxious = 56.6 ± 3.6; Social withdrawal = 57.2 ± 4.9; Depressed = 56.7 ± 4.4; Unpopular = 57.5 ± 4.1; Self -destructive = 58 ± 3.7; Obsessive-compulsive = 57.2 ± 4.7; Inattentive = 56.6 ± 4; Nervous-overactive = 56.8 ± 4.3; Aggressive = 56.4 ± 3.9.	Prenatal Pb exposure was unrelated with neurocognitive deficits at 8 years
[33]	Neurobehavioral development	McCarthy Scales of Children's Abilities Home Observation for Measurement of the Environment	Mental (GCI) 36 months: Mean-105.2 ± 12.5; 48 months: Mean-107.3 ± 14.2; Engine 36 months: Mean-50.4 ± 7.9; 48 months: Mean-48.3 ± 9.2; HOME score: Assessed at 4 years old= 45.5 ± 5.4	Prenatal Pb exposure was unrelated to neurobehavioral deficits
[34]	Children behavior	Kaufman Assessment Battery for Children	MPC = 80.3 ± 10.4; SEQ = 83.1 ± 10.4; SIM = 81.7 ± 11.6; NONVB = 83.5 ± 10.4; ACHIV = 79.4 ± 6.8; Vocabulary = 83.8 ± 8.8; Faces/ places = 80.3 ± 6.3; Arithmetic = 82.1 ± 9.7; Riddles = 81.7 ± 8.1.	Prenatal Pb exposure was unrelated with neurocognitive deficits at 4 years
[35]	Children behavior	Kaufman Assessment Battery for Children; Screening Test for Auditory Processing Disorders	MPC = 87.2 ± 10.7; SIM = 88.9 ± 11.8; SEQ = 88.4 ± 10.6; NONVB = 88.5 ± 10.4; ACHIV = 84.0 ± 8.5; SCAN: FWS= 25.3 ± 5.2; AFGS= 25.6 ± 5.0	Prenatal Pb exposure was associated with cognitive developmental deficits
[36]	Children behavior	Wechsler Intelligence Scale for Children (WPPSI-R)	Full Scale IQ = 86.9 ± 11.3; Performance IQ = 88.5 ± 12.6; Verbal IQ = 87.4 ± 11.5	Prenatal Pb exposure was associated with cognitive developmental deficits
[37]	Children development - motor and cognitive	Bayley Scales of Infant Development (BSID-I); Kent Infant Development Scale - modified (KID); Stanford-Binet Intelligence Scale (SB)	6 months: MDI = 112.1 ± 18.6; PDI = 111.8 ± 14.6; KID = 111.7 ± 13.8 / 1 year: MDI = 111.5 ± 15.1 / 2 year: MDI = 101.8 ± 17.5 / 3 year: SD IQ = 89.7 ± 15.5	Prenatal Pb exposure was associated to development at six months
[38]	Cognitive function	Wechsler Preschool and Primary Scale of Intelligence (WPPSI)	IQ = 87.5 ± 16.6	Prenatal Pb exposure was associated to development
[39]	Cognitive function and behavior	Mental Development Index from Bayley Scales of Infant Development (BSID-I); General cognitive Index from McCarthy Scales of Children's Abilities; Wechsler Intelligence Scale for Children (WISC-III); Child Behavior Checklist (CBCL 2/3)	CBCL 2/3 = 61.4 and 46.3	Prenatal Pb exposure was associated with neurodevelopment deficits
[11]	Cognitive function and behavior	Bayley Scales of Infant Development (Spanish version)- BSID-II	MDI= 91.5 ± 11.6	Prenatal Pb exposure was associated with neurodevelopment deficits
[40]	Cognitive and perceptual function	Boston teacher questionnaire	Behavior: boys = 9.5%; girls = 4% / Hyperactive: boys = 6.1%; girls = 1.5% / Reading: boys = 11.6%; girls = 7.7% / Arithmetic: boys = 4.5%; girls = 10.3% / Directions: boys = 5.1%; girls = 4.7% / Daydream: boys = 18.5%; girls = 11.5% / Tasks: boys = 5.5%; girls = 5.4%.	Prenatal Pb exposure was associated with developmental deficits
[41]	Intellectual function	McCarthy Scales of Children's Abilities; Home Observation for Measurement of the Environment – adapted-R	HOME score: Assessed at 4 years old= 28.6 ± 7.8 (n = 319); Not assessed at 4 years old = 30.1 ± 7.2 (n = 179) / MSCA: not clear	Prenatal Pb exposure was associated with neurodevelopment deficits

NR: not reported; MDI: Mental development index; GCI: General cognitive index; MPC: Mental process composite; SEQ: Sequential processing scale; SIM: Simultaneous processing scale; NONVB: Nonverbal scale; ACHIV: Achievement scale; IQ: Intelligence Quotient; HOME: Home Observation for Measurement of the Environment; MSCA: McCarthy Scales of Children's Abilities; PDI: Psychomotor Index; KID: Kent Infant Development Scale.

trimester by Dietrich et al. [34–36] or at delivery as performed by Cooney et al. [33], can interfere and affect the results and conclusions of the studies. Plasma Pb concentration has been suggested as the toxicologically active fraction of Pb in blood that would be able to cross the placental barrier [53]; however, most studies did not differentiate between Pb in blood and plasma. Another factor that contributes to inconclusive results is that Pb can be mobilized from the bone of the mother to the fetus, but the knowledge of whether the mobilization would be greater in the first, second or third trimester of pregnancy is not consolidated [11].

The studies included in this review evaluated children under nine years of age exposed to Pb during the gestational period. Intellectual and cognitive tests are routinely given to children in clinical settings as part of their general psychological assessment. The scales used to measure the cognitive index varied according to the age of the study population. Prospective studies conducted by Bellinger et al. [30,31], Dietrich et al.

Table 4
Spontaneous abortion reported in the included studies. Evaluation instrument, effect measures, and conclusions.

Author, Year	Outcome	Outcome evaluation instrument	Outcome measurement	Conclusions
[42]	Spontaneous abortion	Spontaneous abortions index	Risk of spontaneous abortion = 6.4%	Prenatal Pb exposure was associated with spontaneous abortion
[43]	Spontaneous abortion	NA	NA	It was not possible to associate Pb exposure and spontaneous abortion
[12]	Spontaneous abortion	Miscarriages index	Mean number of miscarriages = 0.42	Prenatal Pb exposure was associated with spontaneous abortion
[44]	Spontaneous abortion, premature delivery, late fetal death, congenital anomalies	Number of spontaneous abortions before the 20 weeks; rate of pre-term delivery; Number of late fatal death; rate of premature rupture of membranes; rate of congenital anomalies	Spontaneous abortion = 23 of 774 pregnancies / Pre-term delivery: PP = 5.3%; NPP = 2.9% / Late fatal death from 740 pregnancies: PP = 11; NPP = 1 / Premature rupture of membranes: 3.1% of 672 deliveries / Congenital anomalies = 5.4%	Prenatal Pb exposure was unrelated to spontaneous abortion
[45]	Spontaneous abortion and early fetal death	Rate of spontaneous abortion	Exposed = 16.4%; note exposed = 14.0%	Prenatal Pb exposure was unrelated to spontaneous abortion
[46]	Spontaneous abortion	Miscarriage rate	5.2%	Prenatal Pb exposure was unrelated to spontaneous abortion
[47]	Spontaneous abortion	Rate of spontaneous abortion after the first trimester	4.3%	Prenatal Pb exposure was unrelated to spontaneous abortion
[48]	Anembryonic pregnancy	NA	NA	There is a possible association between Pb exposure and miscarriage

NA: not apply.

[35,36], Ernhart et al. [37,38], Factor-Litvak et al. [39], Hu et al. [11], Leviton et al. [40] and Wasserman et al. [41] found evidence that prenatal Pb exposure was associated with cognitive development deficits. Bellinger et al. [30] discussed the difficulty of distinguishing the neurodevelopmental impacts of prenatal from postnatal Pb exposure in an epidemiological study. The authors evaluated three groups according to the Pb levels in the umbilical cord and observed an inverse correlation between Pb levels and score on cognitive tests. Even the lowest levels of Pb were associated with worsening of cognitive function in children evaluated from 6 to 24 months, a critical period of development [11,30]. Bellinger et al. [32], showed a modest association between dentin Pb levels and behavioral changes, but did not perform adjustment measures, which may have compromised the result, as the interpretation that reflects the outcome may be uncertain and/or speculative; another question is that this value of Pb in teeth is more sensitive to the postnatal period, not being the focus of our evaluation.

The Pb levels in the umbilical cord seem to be related to the MDI test scores. Bellinger et al. [30], Ernhart et al. [37], and Hu et al. [11] obtained umbilical cord Pb levels of 6.6 μg/dL (3.4–9.8), 5.99 μg/dL (3.9–8.1), and 6.2 μg/dL (2.3–10.1), respectively; and the MDI test scores at 24 months were similar: 119.9 ± 14.4 (BSID-I), 101.8 ± 17.5 (BSID-I), and 91.5 ± 11.6 (BSID-II), respectively. P é rez-López et al. [54] compared the descriptive data of the means and standard deviations obtained with each scale and subscale between these two Bayley tests, reaching the conclusion that the original version overestimates the abilities of full-term and pre-term newborn children,

Table 5
Bias analysis in observational studies (Downs, S.H., Black, N., 1998) and parameters of exclusion/inclusion criteria for Neurocognitive deficits/Spontaneous abortion.

Studies	Punctuation	Classification	Gestational age exclusion criteria (weeks)	Birth weight exclusion criteria (g)	Pregnancy loss (weeks)
Observational studies					
[30]	26	Excellent	< 34	NR	-
[31]	23	Good	< 34	NR	-
[32]	23	Good	< 34	NR	-
[42]	24	Good	-	-	< 20
[33]	26	Excellent	< 37	< 2500	-
[34]	25	Good	< 34	> 1500	-
[35]	20	Good	< 34	> 1500	-
[36]	20	Good	< 34	> 1500	-
[37]	20	Good	< 37	NR	-
[38]	22	Good	< 37	NR	-
[39]	21	Good	< 28 and > 44	NR	-
[43]	16	Fair	-	-	< 20
[11]	23	Good	< 37	NR	-
[12]	23	Good	-	-	< 20
[40]	19	Fair	< 37	NR	-
[44]	20	Good	-	-	< 20
[45]	14	Poor	-	-	NR
[46]	18	Fair	-	-	NR
[47]	21	Good	-	-	< 20
[41]	22	Good	NR	NR	-
[48]	22	Good	-	-	< 20

Classification. Excellent: 26–28, Good: 20–25, Fair: 15–19, Poor: < 15. NR: not reported

compared to the outcome they would obtain using a more recent version of these scales. The use of assessment instruments more sensitive to the processes of change and development of children is suggested [54].

Wasserman et al. [41] observed that cord levels greater than 10 μg had deleterious effects at 36 and 48 months. Ernhart et al. [38] and Dietrich et al. [35] evaluated children of 58 and 60 months of age. The hypothesis evaluated by Ernhart et al. [38] that low level Pb exposure in fetal and early preschool years affects intelligence made from the gestational and postnatal analysis was not supported after adjusting for sex, race, birth order, birth weight, gestational age in birth, parental education, maternal vocabulary, maternal authoritarianism, maternal alcohol use, maternal use of alcohol in pregnancy, maternal use of tobacco in pregnancy, maternal use of marijuana in pregnancy, maternal use of other illicit drugs, quality of home environment, medical problems, and psychosocial problems. Dietrich et al. [35] observed a similar outcome, however only postnatally, and when adjusted for the effect of cognitive decline, was not significant.

Leviton et al. [40] evaluated prenatal exposure to Pb in the umbilical cord and postnatally in dentin and found that levels below 15 ug/dL in the cord at 72 months were able to affect brain function, including reduced intelligence, behavioral problems, and school performance. The authors used the Boston Teacher Questionnaire to measure the outcome at the age described, which is able to assess cognitive and perceptual function, but depends on the teacher's perception of the child. This is unlike the Wechsler Scale, which is used not only as an intelligence test, but also as a clinical test. Dietrich et al. [36] and Factor-Litvak et al. [39] observed that the blood Pb levels, used to assess gestational exposure, were predictors of IQ deficit at older ages such as 78 and 90 months. Factor-Litvak et al. [39] reported that increasing serum Pb from 10 μg to 20 μg can reduce cognitive test scores during the postnatal period.

The studies conducted by Borja-Aburto et al. [42], Lamadrid-Figueroa et al. [12], and Yin et al. [48] showed a possible relationship between Pb exposure and spontaneous abortion. All these studies evaluated pregnant women during the first trimester. Another point that makes the connection between abortion and Pb difficult in these studies is to have full methodological certainty that other metals such as nickel and cadmium cannot be interfering with female reproductive health [55]. Lamadrid-Figueroa et al. [12] evaluated abortion based on the history of previous pregnancies. However, it is important to note that the time between the Pb measurement and the outcome make it difficult to establish a causal association. During the third trimester occur fastest fetal growth and the greatest mobilization of Pb from maternal bone with consequent increase of the serum Pb levels [56]. Since fetuses are directly exposed to the maternal Pb fraction via umbilical cord transfer, higher exposure to Pb during the third trimester may coincide with adverse effects on fetal development [57]. The study by Yin et al. [48] also showed an association between exposure to Pb and the occurrence of miscarriage and had as exclusion criteria pregnant women who used mineral supplements. Deficiency of calcium, as well as iron and zinc, can increase the retention of ingested Pb by increasing its gastrointestinal absorption [58].

The results collected from Vigeh et al. [47], Paredes Alpaca et al. [46], McMichael et al. [44], and Faikoğlu et al. [43] showed that there was no increased risk of miscarriage with a gestational age of less than 20 weeks, with Pb concentrations ranging from 10.8 to 22.1 μg/dL for the abortion and non-abortion groups. Lamichhane et al. [59] suggest that gestational Pb exposure effects are related to GSTM1/GSTT1 polymorphism, which interferes with the levels of Glutathione S-transferases (GST) that is involved in the detoxification process. Prenatal Pb exposure may contribute to lower birth weight and head circumference in boys of GSTM1 null mothers [59].

This systematic review has limitations since the included studies showed high heterogeneity. Furthermore, we found a very low quality of evidence synthesis, which thwarts adequate conclusions about the effects of gestational Pb exposure.

5. Conclusions

In humans, toxicological studies represent a challenge, as several uncontrolled factors may interfere with the results, such as co-exposure to different heavy metals and other xenobiotics, in addition to socioeconomic and environmental conditions. Scientific evidence on the impact of gestational exposure to Pb is important to prioritize future public health policies. The present study shows that the evidence on the effects of gestational exposure to Pb on child development is inconclusive, and more toxicological studies are needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2023.108342.

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4 ARTIGO 2 - Prenatal caffeine consumption and neurobehavioral disorders - A systematic review

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Review

Prenatal caffeine consumption and neurobehavioral disorders - A systematic review

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ABSTRACT

Studies have suggested associations between gestational exposure to caffeine and adverse outcomes, however the evidence is still limited. Therefore, a systematic review was conducted to investigate the association between prenatal caffeine exposure and neurobehavioral disorders. The MEDLINE (PubMed), EMBASE, Scopus, Web of Science, and LILACS databases were searched. Observational studies involving women with documented caffeine consumption during pregnancy were eligible for inclusion. The outcomes evaluated were behavioral and intellectual development, Attention Deficit Hyperactivity Disorder, and related behaviors. The data were analyzed by qualitative synthesis. The ROBINS-I tool was employed to assess the risk of bias, and the certainty of evidence was evaluated using GRADE (PROSPERO: CRD42023421164). The search yielded fourteen studies that met the inclusion/exclusion criteria. The sample size among pregnant women ranged from 173 to 64,189, and among children ranged from 88 to 49,190. Maternal caffeine consumption during pregnancy ranged from 0 to 1000 mg/day, with the highest levels observed during mid-pregnancy. Seven studies indicated a potential association between prenatal caffeine exposure and neurobehavioral/neurodevelopment deficits, one study showed that prenatal caffeine exposure improved peer problems, and six studies did not show a significant effect of prenatal caffeine consumption on neurobehavioral disorders. The included studies were classified as moderate for the risk of bias and with very low certainty of evidence. Thus, the evidence is insufficient to confirm with certainty that the prenatal caffeine exposure leads to neurobehavioral disorders. Studies heterogenicity, as well as their variable quality and the presence of several confounding factors, generate uncertainty.

1. Introduction

Prenatal caffeine consumption has been a subject of growing interest and concern due to its potential impact on neurodevelopment. Caffeine, chemically known as 1,3,7-trimethylxanthine, is an organic heterocyclic compound based on the purine base xanthine. It is a psychoactive substance commonly consumed and found in various sources of food and beverage, such as coffee, tea, chocolate, and energy drinks [1,2]. In 1980, the United States Food and Drug Administration (FDA) responded to research indicating teratogenic effects induced by caffeine in rodents by issuing a warning that advised pregnant women to limit or completely avoid its consumption [3].

Although organizations such as the Food Standards Agency (FSA) and The American College of Obstetricians and Gynecologists (ACOG) deem a daily caffeine intake of up to 200 mg safe for pregnant women, a unanimous safe threshold for maternal caffeine consumption during pregnancy is lacking [4–6]. Galéra et al., [7] found that children born to mothers who consumed ≥ 200 mg/day of caffeine during pregnancy exhibited an increased probability of presenting borderline or lower intelligence quotient (IQ) levels compared to those consuming

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100 mg/day, with decreased scores on cognitive Wechsler Preschool tests in children at age five. These findings were supported by Zhang et al., [8], who showed that higher maternal caffeine consumption of two or more cups (≥ 200 mg) was significantly associated with an increased risk of displaying oppositional defiant behaviors, accompanied by a higher total score for externalizing problems, compared to the absence of caffeine exposure.

In contrast, other studies have found no significant associations between prenatal caffeine consumption and neurobehavioral disorders or social behavior, even at similar doses of caffeine. Loomans et al., [9] did not identify any effects of intrauterine exposure to caffeine ranging from 86 to 425 mg/day on problem behavior in school-aged children (5 years). The authors used the Strengths and Difficulties Questionnaire (SDQ), a psychosocial assessment instrument developed to identify strengths and behavioral difficulties in children and adolescents. Miyake et al., [10] found that increased maternal caffeine intake during pregnancy, particularly from Japanese and Chinese tea consumption, was linked to a decreased probability of peer problems in Japanese children.

The absence of agreement among studies and the challenge in establishing a safe limit for caffeine intake during pregnancy highlight the importance of evaluating the available evidence in this field. The aim of this systematic review of observational studies was to verify the causal association between prenatal caffeine exposure and neurobehavioral disorders such as neuromotor development, IQ, attention-deficit hyperactivity disorder (ADHD), and related behaviors.

2. Methods

This systematic review was performed based on observational studies, according to the PRISMA 2020 Statement [11], being registered in the PROSPERO platform under the number CRD42023421164. The question of the study was "Does caffeine consumption during the gestational period lead to neurobehavioral disorders?".

2.1. Eligibility criteria

Observational studies involving women with documented caffeine consumption tracking method (self-reported, serum metabolite concentration or others) during pregnancy were included in this review, without limitations on language, ethnicity, date, or publication status. Studies meeting the following criteria were excluded from the analysis: 1) Nature of the exposure and the conclusions were not focused on caffeine, 2) Not the outcome of interest, and 3) studies available only in summary format or lacking detailed data. Studies that focused solely on postnatal caffeine consumption or that examined caffeine consumption in the context of other substances without adjusting for confounding variables were excluded from the analysis.

2.2. Information sources

The databases selected for research were categorized as conceptual and essential (MEDLINE and EMBASE), specialized and optional (Scopus and Web of Science), and regional (LILACS). A manual search was performed in the reference list of included studies, and expert consultations were conducted through email and ResearchGate to gather additional relevant information. Additionally, the gray literature was explored, including preprint servers such as Biorxiv and Medrxiv, as well as CAPES, to ensure comprehensive coverage of available research. The research was conducted in April 2023 (Supplementary material A).

2.3. Search strategy

The search strategy was planned to locate observational studies. The study question was designed according to the PECOS strategy [12]. Participants (P): Women with documented caffeine consumption during pregnancy; Exposure (E): Prenatal caffeine exposure; Comparison (C): Women who abstain from caffeine consumption during pregnancy; Outcome (O): Neurobehavioral disorders; and Studies (S): Observational studies. MesH, DeCS, and Emtree descriptors were crossed with the Boolean operators "AND" (inter-category) and "OR" (intra-category), according to the specificity of each database selected for the search.

2.4. Selection process

The retrieved studies were exported to the EndNote® (online version) and Rayyan® platforms [13], and duplicates were eliminated in both software. The selection was conducted independently by two researchers (ABS and LEWS) in Rayyan based on the eligibility criteria established for the present systematic review. Initially the studies were selected by reading title and abstract, followed by a full text read of those selected. Disagreements were resolved in consensus meetings with a third researcher. The agreement between researchers was analyzed by a Kappa coefficient using GraphPad QuickCalcs (Graphpad, San Diego, CA), in which a result above 0.60 was considered acceptable.

2.5. Data collection process

Two reviewers performed data extraction of the included studies independently. Data extraction provided the following information: i) General characteristics of the studies included: author, year and country, study design, study database, recruitment period/location, and number of participants; ii) Exposure characteristics: Exposure and interview period, caffeine consumption tracking method, caffeine sources, amount of caffeine/source, caffeine intake, and experimental groups; and iii) Outcome characteristics: outcomes evaluated, period of evaluation, instruments used and their scores, main results, and conclusions. The collected information was compared between investigators and disagreements were settled by consensus with a third investigator.

2.6. Study risk of bias assessment

The assessment of study bias was conducted using the ROBINS-I (Risk Of Bias In Non-randomized Studies of Interventions) assessment tool [14]. The ROBINS-I tool consists of specific questions, which are divided among seven main domains, and can be used to assess the risk of bias in observational studies. According to the signaling questions for each domain, studies will receive categorical responses that can vary between NA (Nor Applicable) / Y (Yes) / PY (Probably Yes) / PN (Probably No) / N (No) / NI (No Information) following the response options, along with a risk of bias judgement for the end of each section [15,16].

2.7. Synthesis methods

A qualitative synthesis of the data was performed, presenting their information in tables in a narrative way. The results were summarized considering the risk of bias and the conclusions observed in each study. The performance of quantitative synthesis (meta-analysis) was contingent upon the presence of methodological homogeneity among the studies, considering study type, exposure characteristics, and clinical outcomes obtained.

2.8. Certainty assessment

The level of evidence of the data was assessed by GRADE (Grading of Recommendations Assessment, Development, and Evaluations) using GRADEpro software [17]. The analysis was carried out for the outcome neurobehavioral disorders.

3. Results

3.1. Study selection

From the searches performed in the databases, 2873 registered articles were retrieved, the elimination of duplicates allowed a final result of 2538 studies. Through screening, the selection process retrieved 20 studies, which were read in full. Eight were excluded after the full reading. The twelve remaining studies met the inclusion criteria and were included in this review. Two studies were included through manual search. Therefore, fourteen studies were included, as shown in Supplementary Material C. Agreement among researchers were considered acceptable (Kappa = 0.629).

3.2. Study characteristics

The included studies were published between 1984 and 2021; thirteen were classified as cohort studies, and one case-control. The year of recruitment ranged from 1959 to 2015. Most publications come from the United States (five studies), with the remaining divided among: Denmark (two studies), Norway (two studies), Brazil, England, France, Japan, and the Netherlands. The sample size among pregnant women ranged from 173 to 64,189, and among children ranged from 88 to 49,190. The 14 included studies that met the inclusion and exclusion criteria used the following data reservoirs: Adolescent Brain and Cognitive Development Study (ABCD) [8,9,18]; Father and Child Cohort Study (MoBa) and the Medical Birth Registry of Norway (MBRN) [19,20]; Seattle Longitudinal Study on Alcohol and Pregnancy [21]; Aarhus Birth Cohort [22]; EDEN (*Etude des Déterminants pré- et postnatals précoces du développement et de la santé de l'Enfant*) [7]; Danish National Birth Cohort (DNBC) [23]; Pelotas (Brazil) Birth Cohort Study [24]; Kyushu Okinawa Maternal and Child Health Study (KOMCHS) [10]; Early Autism Risk Longitudinal Investigation (EARLI) [25]; Health Outcomes and Measures of the Environment (HOME) Study [25]; and Longitudinal study on prenatal exposure to an environmental toxin, polychlorinated biphenyls (PCBs) [26]. The case-control study was conducted as part of the Collaborative Perinatal Project at multiple sites across the United States [27] (Table 1).

3.3. Dietary caffeine consumption

Table 2 shows the characteristics of the caffeine consumption in the included studies. The period of caffeine consumption evaluated varied across studies: before pregnancy [21]; early pregnancy – in the first weeks [25], from the fifth week [10], and not accurate data [18,21]; mid-pregnancy – between the 15th and 22nd weeks of gestation [9,19–23,25]; late pregnancy – between the 30th and 40th week of gestation [7,10,18,19,23,25]; or post-pregnancy period – after delivery [24,26] or one month later [23]. Two studies did not report the specific timing of caffeine exposure or maternal interviews [8,27].

Table 1
Characteristics of the included studies (n= 14).

Author, Year Country	Study design	Study database	Recruitment period / Location	Number of participants (women / children)
(Jacobson et al., 1984), USA	Cohort study	Longitudinal study on prenatal exposure to an environmental toxin; PCBs	NR	173 / 173
(Barr et al., 1991), USA	Cohort study	Seattle Longitudinal Study on Alcohol and Pregnancy	1974–1975 / Two Seattle health care facilities	1529 / 500
(Linnet et al., 2009), Denmark	Prospective cohort study	Aarhus Birth Cohort	1990–1998 / Aarhus University Hospital	24068 / 88
(Bekkhus et al., 2010), Norway	Prospective population-based cohort study	MoBa and MBRN	1999–2008 / NR	25343 / 25343
(Loomans et al., 2012), Netherlands	Community based cohort study	ABCD®	2003–2004 / NR	8202 / 3439
(Klebanoff & Keim, 2015), England	Case control study	Collaborative Perinatal Project	1959–1965 / twelve academic medical centers in the United States	2197 / 2197
(Del-Ponte et al., 2016), Brazil	Cohort study	Pelotas Birth Cohort Study	2004–2015 / five hospitals in the city of Pelotas	3485 / 3485
(Galéra et al., 2016), France	Population-based cohort study	EDEN	2003–2005 / Department of Obstetrics and Gynecology in two French university hospitals (Nancy and Poitiers)	1083 / 1083
(Mikkelsen et al., 2017), Denmark	Cohort study	DNBC	1996–2002 / NR	47491 / 47491
(Miyake et al., 2019), Japan	Cohort study	KOMCHS	2007–2008 / 423 obstetric hospitals in seven prefectures on Kyushu Island in southern Japan	1199 / 1199
(Berglundh et al., 2021), Norway	Prospective population-based cohort study	MoBa and MBRN	1999–2008 / NR	64189 / 15819–49190
(Christensen et al., 2021), USA	Cohort study	ABCD® from the NDA	Upon completing 9 or 10 years / Through school and community settings	NR / 9157
(Patti et al., 2021), USA	Cohort study	EARLI; HOME	EARLI: 2009–2012 / four sites in the US: Pennsylvania (Drexel/ Children's Hospital of Philadelphia), Maryland (Johns Hopkins/ Kennedy Krieger Institute), and Northern California (UC Davis and Northern California Kaiser Permanente); HOME: between 2003 and 2006 / nine prenatal clinics affiliated with three delivery hospitals in the greater Cincinnati, Ohio area	EARLI:120 / 120; HOME: 269 / 269
(Zhang et al., 2022), USA	Cohort study	ABCD®	NR / 22 sites across the United States	9978 / 9978

ABCD: Adolescent Brain and Cognitive Development; DHQ: Diet history questionnaire; DNBC: Danish National Birth Cohort; EARLI: Early Autism Risk Longitudinal Investigation; EDEN: Etude des Déterminants pré- et postnatals précoces du développement et de la santé de l'Enfant; FFQ: Food Frequency Questionnaire; HOME: Health Outcomes and Measures of the Environment; KOMCHS: Kyushu Okinawa Maternal and Child Health Study; MBRN: Medical birth registry of Norway; MoBa: Norwegian Mother, Father and Child Cohort Study; NDA: National Institute of Mental Health Data Archive; NR: not reported; PCBs: polychlorinated biphenyls; QFV: quantity-frequency-variability.

Table 2
Characteristics of the caffeine consumption in the included studies.

Author, Year, Country	Exposure period/ Interview period	Caffeine consumption tracking method	Caffeine sources	Amount of caffeine per source	Caffeine intake (mg/ day) Mean ± SDM	Experimental groups (N)
(Jacobson et al., 1984), USA	Before and during pregnancy / Post pregnancy (2–3 days after delivery)	Self-reported (retrospectively parental reports about frequency)	Coffee, tea, and cola	Cup of coffee is equivalent to about 225 mL – 66 mg; instant – 66 mg; percolated –74 mg, drip or filter –112 mg.	Before pregnancy 192.12 (NR); During pregnancy: 121.44 (NR).	Four groups: 0 mg/d (NR); 30–168 mg/ d (NR); 169–504 mg/ d (NR); >505 mg/ d (NR)
(Barr et al., 1991), USA	Before, Early pregnancy, Mid-pregnancy / 5th month	Self-reported (QFV)	Coffee, tea, cola, chocolate candy, and chocolate milk	Tea: cup of non-herbal tea – 27 mg; Coffee: cup of dripped – 112 mg; cup of percolated – 74 mg; cup of dripped; instant coffee – 66 mg; Chocolate: glass of chocolate milk – 10 mg; chocolate bar (113 g) – 20 mg Caffeinated soft drinks – 31 mg.	Early pregnancy 193 ± 234 Mid pregnancy: 152 ± 195	Three groups: 0–295 mg/ d (1162); 296–443 mg/ d (124); ≥444 mg/ d (113)
(Linnet et al., 2009), Denmark	NR / Mid-pregnancy (16th week of gestation)	Self-reported (parental reports frequency on daily consumption)	Coffee, tea, chocolate, and cola	Cup of coffee - 100 mg of caffeine; Cup of tea or chocolate - 50 mg; Bottle of cola (25 cl-250 mL) - 50 mg.	NR	Four groups: <100 mg/ d (10886); 100–399 mg/ d (8254); 400–999 mg/ d (4340); ≥1000 mg/ d (588)
(Bekkhus et al., 2010), Norway	NR / Mid-pregnancy (17th week) to late pregnancy (30th week)	Self-reported (mailed questionnaire)	Coffee, Coca Cola, Pepsi Cola regular; and Coca Cola, Pepsi Cola diet	One mug = two cups; one small bottle = four cups; one large bottle, 1.5 L = 12 cups. boiled / percolated / filtered – 85 mg/cup; Instant/ espresso coffee – 60 mg/cup; Tea – 50 mg/cup; Regular and diet soft drinks - 30 mg/cup.	**Mid pregnancy: 33.7 Late pregnancy: 11.3	* Four groups: No intake of caffeine (NR); 33–85 (NR); 99–251 (NR); ≥ 255 (NR)
(Loomans et al., 2012), Netherlands	One week before interview / Mid-pregnancy (16th week)	Self-reported (parental reports frequency on amount consumed in the last week)	Coffee, tea, and cola (caffeinated, decaffeinated, both, or herbal tea)	One regular coffee = 85 mg; Decaffeinated coffee = 3 mg; Both regular and decaffeinated coffee = 44 mg; Regular tea = 45 mg; Regular cola = 35 mg; Decaffeinated cola = 0 mg; Regular and decaffeinated cola = 17 mg; No cola, coffee, tea, only herbal tea = 0 mg.	NR	Four groups: 0–85 mg/ d (963); 86–255 mg/ d (1614); 256–425 mg/ d (719); >425 mg/ d (143)
(Klebanoff & Keim, 2015), England	NR	Serum paraxanthine concentration	NR	NR	NR	Four groups: 25th: 97 μg/L / 187 μg/L (561/ 505); 50th: 383 μg/L / 522 μg/L (571/ 473); 75th: 870 μg/L / 941 μg/L (521/ 552); 90th: 1405 μg/L / 1526 μg/L (543/535)
(Del-Ponte et al., 2016), Brazil	NR / Post pregnancy (at birth)	Self-reported (parental reports frequency on daily consumption)	Coffee and yerba mate	Filtered coffee: strong coffee = 0.25 mg/mL; medium coffee = 0.20 mg/mL; weak coffee = 0.11 mg/mL; Yerba mate = 17 mg/100 mL; Instant coffee (3 mg of caffeine per g of powder): coffee spoon - 2.6 g; level coffee spoon - 2.3 g; full small coffee spoon - 2.5 g; level	NR	Three groups: <100 mg/ d (1534); 100–299 mg/ d (902) ≥300 mg/ d (698)

(continued on next page)

Table 2 (*continued*)

Author, Year, Country	Exposure period/ Interview period	Caffeine consumption tracking method	Caffeine sources	Amount of caffeine per source	Caffeine intake (mg/ day) Mean ± SDM	Experimental groups (N)
				small coffee spoon - 1.5 g; full dessert spoon - 7.5 g; and level dessert spoon - 7.0 g.		
(Galéra et al., 2016), France	Before pregnancy (52 weeks before) and late pregnancy (28–40 week) / At enrollment and after delivery	Self-reported (FFQ)	Regular coffee, tea, and caffeinated soda/soft drink	Cup of coffee - 100 mg; cup of tea - 37 mg; glass of caffeinated soda/soft drink - 37 mg	NR	Three groups: 0–100 (634); 100–200 (316); ≥200 (133).
(Mikkelsen et al., 2017), Denmark	NR / Mid-pregnancy (15th week) to late pregnancy (30th week) and post pregnancy (6th and 18th month)	Self-reported (parental reports frequency on daily consumption)	Coffee, tea	Mug = two cups; less than one cup/day = 0.5 cup/day; One cup of coffee – 100 mg; One cup of tea – 50 mg.	NR	Four groups (cups per day coffee/tea): 0 (26590/ 16762); 0.5–3 (15220/ 21848); 4–7 (4396/ 6831); ≥8 (1285/ 2050)
(Miyake et al., 2019), Japan	NR / Early to late pregnancy (5th to 39th week)	Self-reported (DHQ)	Chinese tea (such as green tea and oolong tea), coffee, black tea, cola and sports drinks, hot chocolate, diet cola and non-energy-containing soft drinks, and confectionaries (mainly chocolate)	NR	*During pregnancy: 227 (NR)	Four groups: Q1: 80.8 (299); Q2: 183.3 (300); Q3: 279.2 (300); Q4: 434 (300)
(Berglundh et al., 2021), Norway	Early to Mid-pregnancy / Mid-pregnancy (22nd week)	Self-reported (FFQ)	Coffee, tea, caffeinated beverages and chocolate	NR	***Before pregnancy: 172; Mid pregnancy: 56; Late pregnancy: 80; Total cohort: 56. IQR caffeine intake [18 months/3 yr/ 5 yr/8 yr]: Re. 56/55/63/57 Non Re. 56/59/60/55	Five groups: 0–22 mg (NR); > 22–56 mg/ d (NR); > 56–200 mg/ d (NR); > 200–300 mg/ d (NR); > 300 mg/ d (NR)
(Christensen et al., 2021), USA	NR / Early to late pregnancy	Self-reported (parental reports frequency on day/ week/no consumption)	NR	NR	Highly uncertain	NR
(Patti et al., 2021), USA	Early to mid-pregnancy (first 20 weeks), and mid to late pregnancy (≥ 21 weeks to birth) / Mid to late pregnancy	Self-reported (specific questionnaire about frequency and quantities)	Coffee, tea, soda, and chocolate	8 fluid ounces of hot coffee/ tea/chocolate: 95 mg/29 mg/ 5 mg (Ref). 12 fluid ounces was assigned 33 mg of caffeine per serving. Chocolate candy - 43 mg of caffeine based on a 1.5 ounce size candy bar. 'Cups' or 'bottles – it was assumed that serving size referred to the standard 8 fluid ounce cup or 12 fluid ounce bottle.	EARLI: 20 (NR); Early pregnancy to mid pregnancy: 28 (NR); Mid pregnancy to late pregnancy: 7 (NR); HOME:18 (NR); Early pregnancy to mid pregnancy:17 (NR); Mid pregnancy to late pregnancy: 15 (NR).	NR
(Zhang et al., 2022), USA	NR	Self-reported (Developmental History Questionnaire)	Coffee, espresso, tea with caffeine, soda with caffeine, and energy drinks	Coffee = 8 oz (240 mL); espresso = 1 shot; tea = 8 oz (240 mL), soda = 12 oz (355 mL); energy drink = 5 oz (148 mL) or 2 oz (59 mL) for 5-hr energy drink. Two cups of coffee- 200 mg	NR	Three groups: 0 mg/d (4170); 0–200 mg/ d (1829); >300 mg/ d (221)

BMI: Body mass index; CAF: mean mg caffeine per day from all sources during mid pregnancy; CAFP: for the period prior to pregnancy recognition, or early pregnancy; Cl: centiliter; D: day; EARLI: Early Autism Risk Longitudinal Investigation; HOME: Health Outcomes and Measures of the Environment; IQR: inter quartile range; NR: Not reported; OZ: ounce; Q: Quartile; Re: Responders; Ref: Referent; SDM: Standard deviation mean; *Caffeine consumption ranges; ** Sum of Squares for the variability of total caffeine consumption at 17 and 30 weeks of pregnancy; ***Values corresponding to the median

The majority of studies assessed maternal caffeine intake through self-reporting, by a Food Frequency Questionnaire (FFQ) [7,20], Quantity Frequency Variability (QFV) Questionnaire [21], or Diet History Questionnaire (DHQ) [10]. Additionally, four studies employed specific questionnaires to assess daily caffeine consumption [18,22–24], while two others focused on weekly consumption [9,18]. Two studies examined both frequency and/or quantity of caffeine intake without specifying time periods [25,26]. One study evaluated serum levels of paraxanthine to assess caffeine exposure [27]. It is important to note that these studies primarily focused on dietary sources of caffeine, and

other potential sources of caffeine such as medications were not evaluated.

Two studies did not clearly report the sources of caffeine [18,27]. Among the highlighted sources are coffee (regular and espresso), tea (yerba mate, herbal tea, Chinese tea, and black tea), cola (Coca-Cola, Pepsi-Cola regular, Pepsi-Cola diet type, and diet cola), chocolate (candy, milk, and hot chocolate), and various caffeinated beverages (soda, soft drink, sports drink, and non-energy-containing soft drinks). When assessing daily and weekly dietary caffeine intake and their respective quantities, there were variations in milligrams across studies that solely considered a standard cup of coffee without accounting for the preparation method, type of coffee, or its strength. The measurements ranged approximately from 66 to 100 mg.

Maternal caffeine consumption during pregnancy exhibited variations across different stages. Pre-pregnancy: two studies reported daily consumption ranging from 0 to over 505 mg, with a mean of 192.12 mg/day and a median of 172 mg/day [20,26]. Early pregnancy: daily consumption ranged from 0 to over 444 mg, with mean ranging from 17 to 193 mg/day [21,25]. Mid-pregnancy: daily consumption varying from 0 to over 1000 mg, with mean ranging from 7 to over 152 mg/day [21,23,25]. Late pregnancy: daily consumption between 0 and over 800 mg, with a median varying from 80 to potentially exceeding 800 mg/day [20,23]. Throughout pregnancy: daily consumption varying from 0 to over 505 mg, with a mean ranging from 18 to potentially exceeding 505 mg/day [7,9,19,20,22,24,26].

3.4. Prenatal caffeine and neurobehavioral disorders

Table 3 shows the outcome characteristics and the main results of the included studies. The main parameters assessed in the included studies were IQ, cognition, memory efficiency and working memory, anxiety, depression, ADHD and other attention tests, internalizing and oppositional behavior, externalizing and internalizing behavior, neuromotor functioning, mental/intellectual, and language and motor development. Specific measures related to neurodevelopment in autism spectrum disorder (ASD) were analyzed in one study [25]. Some studies evaluated anthropometric measures, such as birth size, length, gestational age, and birth complications [21,26]. Two studies examined physical health outcomes and brain structure [8,18]. The evaluation period for the outcomes and their respective frequencies spanned from the early postnatal period, with a minimum interval of one day [21] to an extended period of up to 11 years [8,23,24].

The studies included in this systematic review employed a range of assessment tools. Two separate studies applied the Wechsler Preschool and Primary Scale of Intelligence (WPPSI), the first study found no significant relationship between variables [21], while the second study, using the WPPSI-III, revealed a negative association between continuous caffeine consumption and IQ scores, with the higher caffeine exposure associated with lower IQ scores [7]. Two studies employed the Wechsler Intelligence Scale for Children (WISC), both finding no significant associations between caffeine exposure and childhood IQ [21,27]. The first study focused on IQ scores, while the second examined associations between maternal serum paraxanthine concentrations at different pregnancy points and child IQ and behavior at 7 years. Two studies used the National Institutes of Health (NIH) Toolbox tests to assess cognitive aspects, measuring processing speed, working memory, visual-spatial processing, learning, memory, and fluid intelligence [8,18]. Three studies utilized the SDQ, assessing the impact of maternal caffeine consumption on child psychopathology and behavior [9,10,23]. In one study, caffeine consumption was divided into groups, but no significant associations were observed. In another study, caffeine consumption showed significance in relation to specific psychopathological domains, and the third study found that higher consumption during the first trimester was associated with adverse cognitive effects in children. Four studies employed the Child Behavior Checklist (CBCL) to measure psychopathological and behavioral aspects [8,18–20]. Some significant associations were found, such as caffeine consumption linked to internalizing and externalizing behavior problems. Two independent studies investigated the relationship between prenatal caffeine intake and ADHD, with one failing to demonstrate a significant association and the other revealing a potential link between high caffeine intake and ADHD [22,24]. One study examined the association between caffeine intake during pregnancy and Social Responsiveness Scale (SRS) scores in children, suggesting that prenatal caffeine consumption may be associated with higher SRS scores [25]. Lastly, Jacobson et al., [26] assessed neonatal behavior using the Neonatal Behavioral Assessment Scale (NBAS). Their findings suggested an inverse relationship between caffeine intake during pregnancy and newborn neuromuscular maturity (Table 3). Table 4 shows the main confounding factors of the included studies in this systematic review.

3.5. Risk of bias in studies

The risk of bias analysis of the included studies, along with categorical responses and judgments for each study, indicating a moderate overall bias across all studies, can be found in Supplementary Material B. Given the presence of numerous confounders that can affect cognitive outcomes, the studies were classified as moderate to confounding bias in the ROBINS-I assessment. Considering this and the methodological heterogeneity, it was decided not to perform a quantitative synthesis (meta-analysis).

3.6. Certainty of evidence

The outcome evaluated in this systematic review had overall certainty of the evidence classified as very low (Supplementary material D).

4. Discussion

This systematic review revealed limited evidence regarding the effects of gestational exposure to caffeine on neurobehavioral disorders. Among the fourteen studies included in this systematic review, seven indicated a potential association between prenatal caffeine exposure and neurobehavioral/neurodevelopment deficits. Prenatal exposure to caffeine was associated to poorer neuromuscular development and reflex functioning, lower IQ scores, anxiety and attention disorders, hyperactivity, and behavior problems. The studies also related that prenatal exposure to caffeine affected brain structure and increased modestly behaviors related to ASD [7,8,18–20,23,25,26]. However, one study showed that prenatal caffeine reduced the risk of peer problems, including being solitary, having good friends, being popular, being picked on or bullied, and being best with adults, in children [10] and six studies did not show a significant effect of prenatal caffeine consumption [9,20–22,24,27]. Also, the studies presented moderate risk of bias and the quality of the evidence was rated as very low.

In this review, the included studies that observed negative effects associated with prenatal exposure to caffeine showed a mean caffeine consumption that ranged from 7 to ≥800 mg/day [7,8,18,23,25,26]. Among studies that did not observe a significant effect of prenatal exposure to caffeine, only two reported caffeine consumption, which varied between 56 and 193 mg/day. Miyake et al. [10], who observed an improvement in children's behavior, reported consumption of 227 mg/day [10]. With the data obtained, it is not possible to correlate the dose of caffeine ingested during pregnancy and the observed outcomes.

Considering children aged 5 years, Galéra et al. [7], linked children of mothers consuming 200 mg/day or more of caffeine to borderline IQ scores [7], while the Berglund et al. [20] research found internalization issues for caffeine intake between 22 and 56 mg and internalization/externalization problems between 22 and 200 mg [20]. The Miyake et al. [10] study associated SDQ scores for peer problems with caffeine intake ranging from 183.4 mg to 434.2 mg (Q2 to Q4) [10].

Table 3
Neurobehavioral disorders evaluated in the included studies. Period of evaluation, instruments used and measurement of the outcomes. **Values in bold**: statistical difference for caffeine effect.

Author, Year, Country	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main results of prenatal caffeine consumption	Conclusions
(Jacobson et al., 1984), USA	Birth size; Gestational age; Behavior	1d; 2d; 3d; 4d	NBAS	1d, 3d and 4d: Orientation: **4.22** Range of state: **4.87** General Irritability: **5.09** Neuromuscular maturity: **5.53** Reflexes: **6.18** 2d: Head circumference: 3.54 Gestational age (EDC): **5.69**	Caffeine was linked to shorter and poorer neuromuscular development and reflex functioning.	Intrauterine exposure to caffeine predicted behavioral effects that were independent of physical or gestational age deficits.
(Barr et al., 1991), USA	Height; Weight; HC; IQ and attention tests; Pregnancy complications; Newborn measures; Physical size; Mental/ intellectual development; Neuromotor functioning	1 d; 2 d; 8 m; 4 y; 7 y	NBAS; ST; Bayley Scales; FSIQ; FGMB; TPT; WPPSI; WISC-R; CPT	*1 d: Orientation: 0.01; Reactivity: 0.22; Habituation: **7.37**; Tremulousness:0.01; Arousal: 0.38; Activity/Muscle tone: 1.13; 2 d: Sucking pressure: 1.08; Latency to first suck: 0.83; 8 m: MDI: -; PDI: -; 4 y: IQ: 0.22, Fine motor Steadiness Errors: 3.18; Grooved Pegboard - pegs dropped: 2.97; 7 y: IQ: 0.17; Vigilance: Errors of omission (AX): 0.01; Errors of commission (AX): 0.03	Breech presentation associated to CAFD.	There was no long-term impact of prenatal caffeine exposure.
(Linnet et al., 2009), Denmark	HYPD; HYPCD; attention-deficit disorder without hyperactivity	NR	Inattention; hyperactive/ impulsive scales;	<100 mg/d: 1; 100–399 mg/d: 0.9; 400–999 mg/d: 1.3; ≥1000 mg/d: 2.3	No significant effect	The prenatal consumption of high doses of caffeine does not increase the risk of hyperkinetic disorder and childhood ADHD.
(Bekkhus et al., 2010), Norway	Infant inattention; Overactive behavior	1.5 y	CBCL	**Inattention and overactivity: Inattention/overactivity: Mid-pregnancy: **0.001** Overactivity: Late pregnancy: **0.000**	Small effect of high caffeine intake on inattention/ overactivity for caffeine exposure during mid-pregnancy and effect of overactivity for caffeine exposure during mid to late pregnancy	Soft drinks consumption during mid-pregnancy can lead to inattention / overactivity in children aged 1.5 y
(Loomans et al., 2012), Netherlands	Prosocial behavior; Behavior; EP; CP; Hyperactivity-inattention; PP	5 y	SDQ	[Overall problem behavior / Hyperactivity-inattention / Emotional symptoms / CP / PP / Prosocial behavior] 0–85 mg/d: - (reference) 86–255 mg/d: [1.05/0.94/ 0.74/0.96/0.85/0.85] 25–425 mg/d: [0.86/0.87/ 0.93/0.67/0.64/1.09] 425 mg/d: [1.04/1.08/ 1.02/1.16/0.65/0.45]	No significant effect	There was no long-term impact of prenatal caffeine exposure
(Klebanoff & Keim, 2015), England	Hyperactive; Internalizing; Oppositional behavior; EB; IB	4 y; 7 y	SBIS; WISC	4 y: SB Full-Scale IQ / Hyperactive behavior / IB/ OB: 0.71/**0.02**/0.96/0.16; WISC Full-Scale IQ / EB / IB: 0.21/0.51/0.49; 7 y: SB Full-Scale IQ / Hyperactive behavior/ IB / OB: 0.32/0.77/NA/0.9; WISC Full-Scale IQ / EB / IB: NA/NA/0.37	Association between maternal paraxanthine levels in early pregnancy and hyperactive at age 4. Also, correlation analysis showed association between paraxanthine levels in early pregnancy and internalizing behavior at age 4; and association between paraxanthine levels in mid-	Despite the data obtained, there was no clinical relevance to the association between serum paraxanthine levels and childhood IQ or problem behaviors

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Table 3 (*continued*)

Author, Year, Country	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main results of prenatal caffeine consumption	Conclusions
					pregnancy and child's IQ at age 7.	
(Del-Ponte et al., 2016), Brazil	ADHD	3 m; 1 y; 2 y; 4 y; 6 y; 11 y.	DAWBA	[1st trimester / 2nd trimester / 3rd trimester / Entire pregnancy] <100 mg/d: [1/1/1/1] 100–299 mg/d: [1.04/1.03/0.96/1.12] ≥300 mg/d: [1.27/0.95/1.05/0.90]	No significant effect	There was no significant association between prenatal caffeine consumption and ADHD in children at the age of 11 y.
(Galéra et al., 2016), France	Verbal IQ; Performance IQ; Full-scale IQ	4 m; 8 m; 1 y; 2 y; 3 y; 5.5 y	WPPSI-III	Full IQ / Verbal IQ / Performance IQ for: Continuous CI: −0.94/-0.63/-1.12; Verbal IQ: Ref/.66/1.43; Performance IQ: Ref/1.48/1.93 Per experimental group (0–100/100–200/ ≥ 200): CI Full IQ: Ref/-0.99/-2.60; CI Verbal IQ: Ref/-0.40/-0.99; CI Performance IQ: Ref/-1.75/-3.64; Borderline Intellectual Functioning and Below - Full IQ: Ref/1.24/2.30	According to the authors, prenatal caffeine exposure had a negative association with children's IQ at age 5.	The authors suggest an association between prenatal caffeine consumption and adverse effects on cognitive development in the offspring.
(Mikkelsen et al., 2017), Denmark	Emotional behavioral; Conduct-oppositional disorders; Hyperactivity inattention disorders; Anxiety-depressive disorders	11 y	SDQ	Per experimental group (0/0.5–3/4–7/≥8): First/third trimester (FT/TT) Anxiety-depressive disorder Coffee FT: Ref/0.93/1.06/1.21; TT: Ref/0.91/1.02/1.09 Tea FT: Ref/0.96/0.96/1.28; TT: Ref/**0.85**/**0.85**/**0.88** Conduct-oppositional disorder Coffee FT: Ref/0.93/1.13/1.22; TT: Ref/0.87/0.97/1.16. Tea FT: 0.92/0.96/1.21; TT: Ref/0.97/0.92/0.89 Hyperactivity-inattention disorder	Prenatal coffee consumption (≥8 cups) in the first trimester can lead to hyperactivity-inattention behavior at 11 y, while prenatal tea consumption (≥8 cups) in the third trimester was associated to anxiety-depressive, hyperactivity-inattention and any psychiatric disorder.	Excessive maternal caffeine consumption from both coffee and tea was related to behavioral disorders at age 11.
				FT: Ref/0.93/1.06/1.21; TT: Ref/0.91/1.02/1.09 Tea FT: Ref/0.96/0.96/1.28; TT: Ref/**0.85**/**0.85**/**0.88** Conduct-oppositional disorder Coffee FT: Ref/0.93/1.13/1.22; TT: Ref/0.87/0.97/1.16. Tea FT: 0.92/0.96/1.21; TT: Ref/0.97/0.92/0.89 Hyperactivity-inattention disorder Coffee FT: Ref/**0.97**/**1.09**/**1.47**; TT: Ref/0.94/0.96/1.21 Tea FT: Ref/0.93/0.97/1.21; TT: Ref/**0.90**/**0.79**/**0.84** Any psychiatric disorder Coffee FT: Ref/0.93/1.04/1.23; TT: Ref/0.90/0.96/1.08 Tea FT: Ref/0.95/0.96/1.24; TT: Ref/**0.92**/**0.89**/**0.93**	depressive, hyperactivity-inattention and any psychiatric disorder.	

				T1: Ref/**0.92**/**0.89**/**0.93**		
(Miyake et al., 2019), Japan	EP; CP; HP; PP	5 y	SDQ (Japanese version)	EP / CP/ HP / PP: Q1: Ref Q2:0.89/1.26/1.04/**0.61** Q3:1.22/0.97/0.99/**0.52** Q4: 0.77/0.95/0.84/**0.51**	Prenatal caffeine consumption, mainly from Japanese and Chinese tea, was related to lower scores of peer problems, independent of other factors.	Prenatal caffeine intake may have a protective effect against peer problems in children.
(Berglundh et al., 2021), Norway	Fine and gross motor development; LD; Behavior; Temperament.	0.5 y; 1.5 y; 3 y; 5 y; 8 y	ICQ; CBCL; EAS; ITSEA; SMFQ; SCARED; RS-DBD; ASQ;	Full model NE/HA/SH/LS: **0.010**/**0.022**/0.008/**0.020** Re / Non Re LS: 3.96/3.96; NE: 2.73/2.74; SH: 2.04/2.03; HA: 4.03/4.04;	Prenatal caffeine consumption was associated with alterations in child behavior and development. It was related to negative emotionality, high activity and low sociability in the offspring. Caffeine intake higher than 56 mg/d was	The authors suggest that low to moderate prenatal caffeine consumption in pregnancy does not result in clinical significant long-term effects on the neurodevelopment of children up to 8 years of age.

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Table 3 (*continued*)

Author, Year, Country	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main results of prenatal caffeine consumption	Conclusions
			CDI; CCC-2	IB: 1.25/1.26; EB: 1.49/1.49; FM: 1.13/1.13; GM: 1.10/1.10; LD: 1.40/1.39. Per experimental group (TCI;0–22 mg; > 22–56 mg/d; > 56–200 mg/d; > 200–300 mg/d; > 300 mg/d): IB 1.5 y: 1.001/Ref/0.956/**0.909**/0.976/1.017 3 y: 0.976/Ref/1.012/1.003/0.932/0.977 5 y: 0.962/Ref/**0.854**/0.898/0.837/0.833 EB 1.5 y: 0.995/Ref/1.005/1.009/1.097/0.921 3 y: 1.030/Ref/0.991/0.975/1.059/1.166 5 y: 1.007/Ref/**0.869**/**0.870**/0.917/1.033 Conduct disorder 8 y: 1.027/Ref/0.917/0.862/1.127/0.948 ADHD-symptoms 8 y: 1.025/Ref/0.967/0.873/1.045/1.125 Oppositional defiant 8 y: 1.015/Ref/0.958/**0.837**/0.958/1.194 Depression symptoms 8 y: 1.042/Ref/0.917/0.932/1.065/1.281 Scared/anxiety 8 y: 0.975/Ref/1.016/0.955/0.987/0.956 – Gross motor 1.5 y: **1.055**/Ref/1.058/**1.180**/**1.174**/**1.299** 3 y: 0.941/Ref/1.062/0.962/1.005/0.891 5 y: 0.978/Ref/0.953/0.953/0.952/0.988 8 y: 0.968/Ref/1.174/1.229/1.048/0.726 Fine motor 1.5 y: 0.990/Ref/0.945/1.006/**0.880**/0.945 3 y: 0.963/Ref/0.925/0.953/0.972/0.817, 5 y: 0.996/Ref/0.897/1.005/1.087/0.763, 8 y: - Language 1.5 y: **1.043**/Ref/0.978/**1.102**/1.085/1.107, 3 y: 1.004/Ref/0.947/1.033/0.997/1.158, 5 y: 0.960/Ref/**0.848**/0.901/0.821/0.888, 8 y: 0.986/Ref/1.017/0.981/**0.754**/0.911	associated to delayed motor and language development at 1.5 years. While children appear to regain their motor development, the same is not true for language.	

(Christensen et al., 2021), USA	Fiber tracts interaction; TE; WM; Psychopathology measures	During 10 y	MRI; CBCL; NIH CB	**ROI / P(>χ2): IFOF-LH/ 0.0034 CST-LH / **0.0436** CST-LH WM P(>χ2) for GCE/FA/Interaction: 0.2393/**6.2400e-4**/ 0.1233; CST-LH TE P(>χ2) for GCE/FA/Interaction:	Prenatal caffeine consumption led to decreased fractional anisotropy in the left corticospinal tract and the inferior fronto-occipital fasciculus of the left hemisphere, which was associated to impairment in the working memory, task	Prenatal caffeine exposure can affect neurodevelopment, which can be associated to its effects on microstructure of fiber tracts, such as IFOF-LH and CST-LH.

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Table 3 (*continued*)

Author. Year. Country	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main results of prenatal caffeine consumption	Conclusions
				0.3120/**2.3404e-4**/ **0.0245**; CST-LH Externalizing P (>χ2) for GCE/FA/ Interaction: **2.3452e-7**/ **0.00746**/0.3136; CST-LH Internalizing P (>χ2) for GCE/FA/ Interaction: **3.2935e-8**/ **0.0176**/0.1133; CST-LH Somatization P (>χ2) for GCE/FA/ Interaction: **0.0063**/ **0.0176**/0.2098; CST-LH Neurodevelopment P(>χ2) for GCE/FA/Interaction: **5.7835e-6**/**1.4758e-5**/ 0.2627; IFOF-LH WM P(>χ2) for GCE/FA/Interaction: 0.2393/**9.6555e-5**/ 0.1881; IFOF-LH TE P(>χ2) for GCE/FA/Interaction: 0.3120/**7.5649e-5**/ 0.1045; IFOF-LH Externalizing P (>χ2) for GCE/FA/ Interaction: **2.3452e-7**/ 0.14414/0.3216; IFOF-LH Internalizing P (>χ2) for GCE/FA/ Interaction: **3.2935e-8**/ 0.08295/0.1551; IFOF-LH Somatization P (>χ2) for GCE/FA/ Interaction: **0.0063**/ 0.2215/0.1869; IFOF-LH Neurodevelopment P(>χ2) for GCE/FA/Interaction: **5.7835e-6**/**3.8778e-5**/ 0.1637	efficiency, and neurodevelopment.	

(Patti et al., 2021), USA	ASD-related behaviors; Interpersonal behaviors; Communication; Repetitive behavior; Stereotypical behavior	3 y; 8 y	SRS; ADOS; MSEL; KBIT	Gestational Period/ IQR adjusted value: EARLI: Mean/ 2.0; <20w/1.0. >20w/1.8. HOME: Mean/ 0.6; <20w/ 0.4. >20w/ 0.3	Prenatal caffeine intake has a positive clinically association with social responsiveness scale in children aged 3–8 years in both studies, although the results were more evident in the EARLI study.	The authors suggest that higher levels of caffeine intake during pregnancy was associated with modest increases in behaviors related to ASD in children aged 3–8 years
(Zhang et al., 2022), USA	Birth outcomes; Physical health; Behavior problems; Cognition; Substance use; Brain structure in children	9 y to 11 y	CBCL; NIH CB; LMT; RAVLT; SST; SID	[At least once a day/less than once a day but more than once a week/less than once a week/no exposure] Behavior problems by frequency caffeine consumption: Internalizing: 47.8/48.0/48.0/47.9; Externalizing:**45.7**/**45.1**/**45.0**/**44.7**; Depressive:53.4/53.4/53.2/53.6; Anxiety: 53.4/53.2/53.3/53.6; Somatic: **55.5**/**55.6**/**55.5**/**55.0**; ADHD: 53.4/53.2/53.1/53.1; Opposite: **53.6**/**53.2**/**53.2**/**53.1**; Conduct: **53.4**/**52.8**/**52.9**/**52.9**. Cognition	Prenatal caffeine exposure, even at lower doses, was associated to increased externalizing problems. Girls showed more somatic complaints and conduct problems, while boys had higher total scores for externalizing problems. The consumption of more than three cups of coffee daily was associated to higher rates of oppositional defiant and conduct problems in male individuals.	Prenatal caffeine exposure was associated with behavior problems and affected brain structure in offspring.

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Table 3 (*continued*)

Author, Year, Country	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main results of prenatal caffeine consumption	Conclusions
				RP:0.01/0.02/0.04/0.06; LM: −0.09/-0.12/-0.05/-0.06; EF: 0.18/0.16/0.20/0.24. Cortical thickness CP1: NA CP2: NA CP3:**0.119**/**0.099** (D > No/Less than W) CP4:**0.106**/**0.084** (No > D/W) CP5:**0.098** (D > No) Cortical thickness (ROIs): L_Cuneus: All |b| > **0.013** (D/W>No/Less than W) R_Lateral occipital: All |b| > **0.012** (D>other groups) R_Cuneus: **0.018**/**0.017** (D>No/Less than W) L_Lateral occipital: **0.013**/**0.014** (D>No/Less than W) L_Isthmus cingulate: **0.024** (D>No) L_Superior frontal: **0.015** (No>W) Sulcal depth (ROIs): L_Cuneus: All |b| > **0.007** (D>other groups) R_Cuneus: - R_Pericalcarine: **0.009** (No>D) L_Lingual: All |b| > **0.004** (D>other groups) L_inferiorparietal: **0.005** (Less than W>No) L_Supramarginal: **0.004**/**0.004** (No>D/Less than W)		

ADHD: Attention Deficit Hyperactivity Disorder; ADOS: Autism Diagnostic Observation Schedule; ASD: Autism Spectrum Disorder; ASQ: The Ages and Stages Questionnaire; CAFD: mean mg caffeine per day from all sources during mid-pregnancy; CPT: Continuous Performance Test; CBCL: Child Behavior Checklist; CB: Cognition Battery Test; CCC-2: The Children's Communication Checklist-2; CDI: The Child Development Inventory; CI: Caffeine Intake; CP: Conduct Problems; CST-LH: Corticospinal Tract of the Left Hemisphere; DAWBA: Development and Well-Being Assessment; DSM: Diagnostic and Statistical Manual of Mental Disorders; D: Day; EARLI: Early Autism Risk Longitudinal Investigation; EAS: Activity and Shyness Temperament Questionnaire; EB: Externalizing Behavior; EDC: Estimated Date of Confinement; EF: Executive Function; FA: Fractional anisotropy; FGMB: Fine and Gross Motor Battery; FM: Fine Motor; FSIQ: Full Scale IQ score; GCE: Gestational caffeine exposure; HA: High Activity; HC: Head Circumference; HOME: Health Outcomes and Measures of the Environment; HP: Hyperactivity Problems; HYPCD: Hyperkinetic Conduct Disorder; HYPD: Hyperkinetic Disorder; IB: Internalizing Behavior; ICQ: The Infant Characteristics Questionnaire; IQ: Intelligence Quotient; IQR: Interquartile Range; ITSEA: Infant–Toddler Social and Emotional Assessment; KBIT: Kaufman Brief Intelligence Test; LD: Language Development; LM: Learning Memory; LRTs: likelihood ratio tests; LS: Low Sociability; M: Months; MDI: Mental Development Index; MSEL: Mullen Scales of Early Learning; MRI: Magnetic Resonance Imaging; NA: not applicable; NBAS: Neonatal Behavioral Assessment Scale; NE: Negative Emotionality; NIH: National Institutes of Health; OB: Oppositional Behavior; PDI: Psychomotor Developmental Index; PP: Peer Problems; RAVLT: Rey Auditory Verbal Learning Matrix Reasoning Task; Ref: reference value; RP: Reward Processing; RS-DBD: Parent/Teacher Rating Scales for Disruptive Behaviors; SCARED: Screen for Child Anxiety-Related Disorders; SBIS: Stanford-Binet Intelligence Scale; SDM: Standard Deviation Mean; SDQ: Strengths and Difficulties Questionnaire; SID: Structural Imaging Data; SMFQ: Short Moods and Feeling Questionnaire; SRS: Social Responsiveness Scale; SST: Stop-Signal Task; ST: Sucking Test; T: Task; TCI: Total Caffeine Intake; TE: Task Efficiency; W: Week; WISC-R: Wechsler Intelligence Scale for Children-Revised; WM: Working Memory; WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence Third Edition; Y: Years; *All values in the cell represent Partial F(df) for caffeine consumption with respect to cognitive tests **p-values.

However, the Loomans et al. [9] study did not observe a significant relationship between caffeine intake and cognitive outcomes.

It is important to note that except Klebanoff & Keim [27], the studies included used self-reporting to assess caffeine consumption ranges, which can impact the accuracy and validity of the obtained results due the possibility of memory bias. Participants may have difficulty in accurately recalling past events, especially specific details. This can lead to underestimation or overestimation of the reported values [28,29]. Furthermore, self-reporting can be susceptible to social desirability bias, where participants tend to report their behaviors in alignment with social or cultural expectations. Another challenge is the lack of objectivity in interpreting questionnaires, as participants may interpret questions differently or respond ambiguously, resulting in inconsistent and imprecise data [28,29].

Volqvartz et al. [30] revealed that up to 60% of pregnant Danish women who reported not consuming caffeine had detectable caffeine levels in their blood samples [30]. Beyond susceptibility to social desirability bias, this data suggests that many women might be unaware of the sources of caffeine, including chocolate and energy drinks, which were not thoroughly investigated in this study [30]. Other sources of caffeine are only superficially examined in the included studies. Only one study mentioned medications [21] or energy drinks [8], while chocolate fountains were mentioned by five studies [10,20–22,25]. These limitations in evaluating caffeine sources can influence the results and underscore the significance of addressing all potential sources of caffeine exposure during pregnancy [31].

Table 4
Confounders factors of the included studies (n= 14).

Author, Year, Country	Confounders factoes
(Jacobson et al., 1984), USA	Demographics characteristics: Baby's sex, Gravidity, Maternal age, Maternal education, Parity, Prenatal care, Socio-economic status. Nutrition: Weight gain based on amount milk and cheese consumed. Drug use: Acetaminophen, Alcohol intake, Antacids, Aspirin, Hydroxizine, Mepivacaine, PCB exposure, Smoking.
(Barr et al., 1991), USA	Demographics characteristics: Gravidity, Marital status, Maternal age, Maternal education, Number of children in the household, Parity, Race, Socio-economic status. Nutrition: Adequate diet, Weight gain. Drug use: Acetaminophen, Alcohol intake, Antibiotic, Aspirin, Smoking.
(Linnet et al., 2009), Denmark	Demographics characteristics: Baby's sex, Employment status, Maternal age, Marital status, Number of siblings' psychiatric hospitalizations or contacts as outpatients, Parental years of schooling after basic school. Drug use: Alcohol intake, Smoking.
(Bekkhus et al., 2010), Norway	Demographics characteristics: Age, Baby's sex, Birth weight, Head circumference, Marital status, Maternal education, Maternal mood symptoms. Drug use: Alcohol intake, Smoking.
(Loomans et al., 2012), Netherlands	Demographics characteristics: Baby's sex, Education, Ethnicity, Family size, Maternal age, Marital status, Prenatal maternal anxiety. Drug use: Alcohol intake, Smoking.
(Klebanoff & Keim, 2015), England	Demographics characteristics: Baby's sex, Duration of gestation at blood draw, Maternal age, Maternal education, Race, Pre-pregnancy weight, Weight. Drug use: Smoking.
(Del-Ponte et al., 2016), Brazil	Demographics characteristics: Baby's sex, Birth weight, Child gestational age at birth, Marital status, Maternal age, Maternal and paternal education, National Economic Index (IEN), Number of antenatal care consultations, Maternal mood symptoms. Nutrition: Maternal nutritional state before pregnancy. Drug use: Alcohol intake, Smoking.
(Galéra et al., 2016), France	Demographics characteristics: Baby's sex, Birth weight, Breastfeeding duration, Gestational age, Gestational diabetes, Household income, Marital status, Maternal depression, Maternal vomiting, Parity, Stimulation of the child at home. Drug use: Alcohol intake, Smoking.

Klebanoff & Keim. [27] evaluated the levels of paraxanthine, a caffeine metabolite, in maternal urine. This method provides a valuable means to differentiate between women with different levels of caffeine consumption [32]. However, paraxanthine has a longer half-life in blood than caffeine and concentrations vary throughout the day [33]. In a comprehensive study, Grosso et al. [34] discuss that maternal self-reported intake remains the most optimal and valid assessment of prenatal caffeine exposure, as biomarkers fail to accurately capture caffeine exposure throughout the entirety of pregnancy.

The variability in caffeine content among different sources can have a substantial impact on the accuracy of estimating caffeine intake in research studies. If one study uses a higher estimate for caffeine content in coffee, it may indicate higher caffeine consumption among the participants compared to another study that uses a lower estimate. These differences in measurements may lead to inconsistent findings and affect the accuracy of dose-response relationships in studies examining the effects of prenatal caffeine consumption on neurodevelopmental outcomes. To improve research reliability, standardized and validated methods should be employed for measuring caffeine content in beverages, ensuring consistent and precise data for meaningful conclusions [25,35].

The included studies exhibited high results heterogeneity, primarily due to different methodological approaches, as well as adaptations of the tools employed for some of them. For instance, in the ADHD assessment, Linnet et al. [22] used the DSM-IV, focusing on symptomatic criteria essential for diagnosis, while Del-Ponte et al. [24] employed DAWBA, considering a spectrum of mental and behavioral conditions. Another example is the NBAS, initially designed for identifying individual differences among healthy Caucasian infants. A comparison of cluster scores between Japanese and American-Caucasian newborns revealed significant differences in outcomes related to habituation and orientation, emphasizing the need to modify assessment tools according to specific cultural contexts [36–38].

Studies have shown that women who consume caffeine are more likely to smoke and drink alcohol compared to those who do not consume caffeine regularly [39,40]. Caffeine, tobacco, and alcohol are all psychoactive substances that can act on the central nervous system, and it is possible that women who consume one of these substances are also more likely to consume the others due to reinforcing and addictive effects. In addition, alcohol and tobacco consumption during pregnancy

(Mikkelsen et al., 2017), Denmark	Demographics characteristics: Baby's sex, Birth year, BMI, Birth weight, Gestational age, Marital status, Maternal age, Parity, Socio-economic status Drug use: Smoking
(Miyake et al., 2019), Japan	Demographics characteristics: Baby's sex, Birth weight, Breastfeeding duration, Gestation at baseline, Household income, Maternal age, Maternal and paternal education, Maternal depressive symptoms, Number of children at baseline, Region of residence at baseline. Drug use: Alcohol intake, Smoking.
(Berglundh et al., 2021), Norway	Demographics characteristics: Baby's sex, Body mass index (BMI), Household income, Marital status, Maternal age, Maternal depression symptoms, Maternal education, Nausea during pregnancy. Nutrition: Dietary fibre, Energy intake. Drug use: Alcohol intake, Smoking.
(Christensen et al., 2021), USA	Demographics characteristics: Age, Baby's sex, Household income, Maternal education, Premature birth, Race/Ethnicity. Data collection: Collection site. Drug use: Smoking.
(Patti et al., 2021), USA	Demographics characteristics: Household income, Maternal race/ethnicity, Maternal age, Maternal education, Parity. Drug use: Smoking.
(Zhang et al., 2022), USA	Demographics characteristics: Age, Baby's sex, Household income, Highest household education, Marital status, Parents' psychopathology, Parents' age at child's birth, Race/ethnicity, Sibling status. Drug use: Alcohol intake, Smoking.

can also be an important confounding factor, since both substances are known to impact fetal development and may be related to both caffeine consumption and children's cognitive outcomes [41,42]. By incorporating these confounders into analysis models, researchers can obtain more accurate results. The study by Klebanoff & Keim [27] demonstrated no significant interaction between paraxanthine values, sex, or smoking habits concerning behavioral outcomes. Patti et al. [25] identified a positive association between caffeine consumption and SRS scores after adjusting cotinine concentrations and smoking status. Although Christensen et al. [18] observed a low prevalence of individuals reporting smoking in their sample, and Mikkelsen et al. [23] suggested an association between consuming more than 10 cigarettes per day and ingesting at least 8 cups of coffee per day, they did not independently assess smoking habits in their analyses.

In fact, assessing the effects of caffeine consumption during pregnancy on cognitive outcomes in children is a complex issue due to the presence of potential confounders [7,43]. For instance, the diagnosis of neurobehavioral disorders in children is not always accurate, given its multifactorial and complex nature, and, in a significant percentage of cases, it is not clearly elucidated because of the confounding factors. Bekkhus et al. [19] reported inattention and overactivity associated to prenatal exposure to caffeine in children aged 1.5 years [19], an age at which, through normal development, behaviors related to hyperactivity and inattention are expected.

The lack of an assessment of the quality of the maternal diet, environmental exposures, and lifestyle factors represents another potential

source of bias. Inadequate nutrition during pregnancy and early life can impact various aspects of offspring growth, metabolism, brain development, and cognitive function [44,45]. The presence of food additives, as highlighted by McCann et al. [46], may have adverse effects, potentially contributing to an increase in hyperactivity. Living in areas with high pesticide or harmful chemical exposure, as well as lifestyle factors like stress, were not comprehensively addressed in the included studies and could also be associated with neurobehavioral disorders.

This systematic review has limitations since the included studies showed high heterogeneity. In observational studies, researchers have no control over assigning participants into different groups, such as exposed or not exposed to caffeine during gestation. In addition, there are several potential confounding factors, such as maternal age, education, socioeconomic status, lifestyle habits, and general health. Although researchers attempt to account for these confounders through study design and statistical analyses, it is difficult to comprehensively address all of them. Furthermore, we found a very low quality of evidence synthesis, which thwarts adequate conclusions about the effects of gestational caffeine exposure.

5. Conclusions

There is no evidence that prenatal exposure to caffeine leads to neurobehavioral impairment in children. While some studies suggest an association between prenatal caffeine exposure and various deficits in neurobehavioral development, their heterogenicity, including their variable quality, as well as the presence of several confounding factors, generate uncertainty.

This systematic review highlights the complexity of assessing the effects of gestational caffeine exposure on neurobehavioral disorders. Furthermore, the challenges in controlling confounding factors and the lack of standardization in measuring caffeine consumption across sources underscore the need for more rigorous and well-controlled future research to accurately assess the impacts of caffeine during pregnancy. Therefore, new studies with more consistent methodological approaches need to be carried out to better understand the complex relationships between caffeine and neurobehavioral outcomes in children.

Author contributions

Aleksander Brandão Santana: Conceptualized and developed the research concept, conducted data collection, interpreted the data, drafted the manuscript, and provided final approval of the version to be published. Lídia Emmanuela Wiazowski Spelta: Evaluated the research concept, contributed to data collection, critically reviewed the manuscript, made substantial intellectual contributions, and provided final approval of the version to be published. Joselin Valeska Martinez Sobalvarro: Evaluated the research concept, interpreted the data, critically reviewed the manuscript, and provided final approval of the version to be published. Raphael Caio Tamborelli Garcia: Evaluated the research concept, critically reviewed the manuscript, and provided final approval of the version to be published. Tiago Marques dos Reis: Evaluated the research concept, critically reviewed the manuscript, and provided final approval of the version to be published. Larissa Helena Torres: Conceptualized and developed the research concept, supervised the project, critically reviewed and corrected the manuscript, and provided final approval of the version to be published.

CRediT authorship contribution statement

Raphael Caio Tamborelli Garcia: Writing – review & editing, Supervision, Conceptualization. **Joselin Valeska Martinez-Sobalvarro:** Writing – review & editing, Methodology, Investigation. **Larissa Helena Torres:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization. **Tiago Marques dos Reis:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Lídia Emmanuela Wiazowski Spelta:** Writing – review &

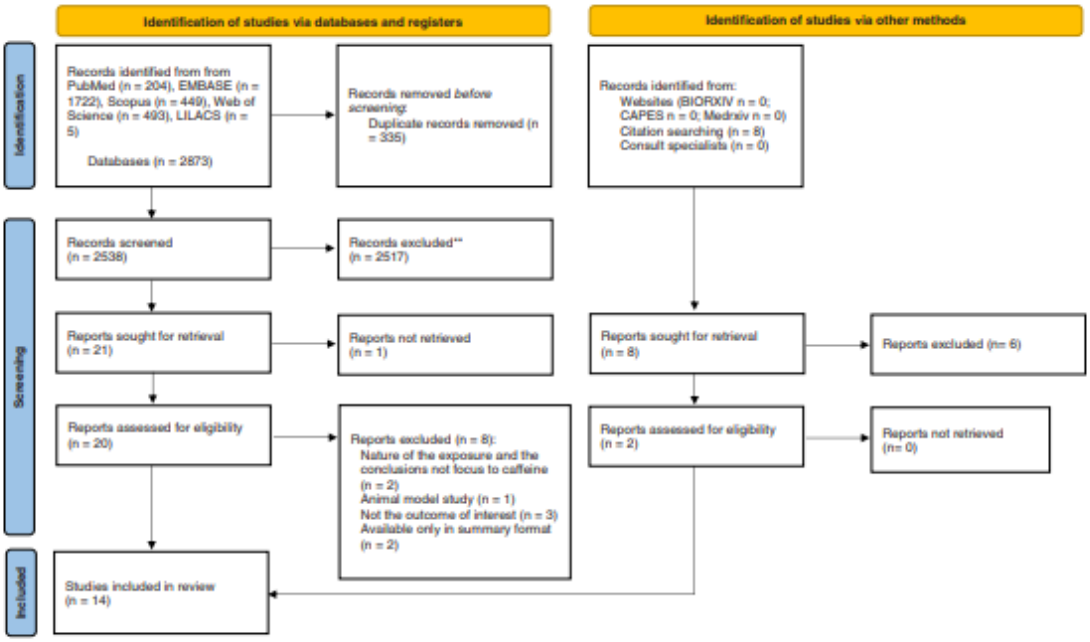

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Page et al., 2020).

editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Aleksander Brandão Santana:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Larissa Helena Torres reports financial support was provided by Coordination of Superior Level Staff Improvement - Brazil (CAPES, Finance Code 001). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2024.108563.

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5 ARTIGO 3 - Gestational triclosan exposure and its effects on child neurodevelopment – A systematic review

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Review

Gestational triclosan exposure and its effects on childneurodevelopment – A systematic review

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ABSTRACT

Triclosan (TCS) is a lipophilic antimicrobial agent present in commercial and healthcare products. Despite its beneficial properties, TCS disrupts thyroid hormone homeostasis and may be linked to metabolic disorders, cardiotoxicity, and increased cancer risk. Evidence on prenatal TCS exposure and adverse neurobehavioral outcomes is limited. This systematic review aimed to verify whether prenatal exposure to TCS is associated with neurobehavioral impairments. Observational studies with pregnant women exposed to TCS during pregnancy were included. The MEDLINE, EMBASE, Scopus, Web of Science, and LILACS databases were searched for studies up to February 27, 2024. Titles and abstracts were first screened, followed by full-text readings by two independent reviewers. Data extraction was performed independently, with conflicts resolved by consensus with a third reviewer. The included studies were assessed using an adapted Downs and Black tool and qualitatively synthesized. Certainty of evidence was assessed by GRADE. The study protocol was registered with PROSPERO (CRD42024526426). Among 17 studies, 14 cohort studies met the inclusion criteria. The sample size ranged from 193 to 794 pairs of pregnant women and children. Exposure to TCS throughout pregnancy resulted in median concentrations from 0.40 ng/mL to 28.2 ng/mL. Four studies suggested a potential association between prenatal TCS exposure and neurodevelopmental deficits, such as externalizing problems, attention issues, hyperactivity, somatization, emotional symptoms, social awareness, and communication; in contrast, eight studies found no significant effect. The studies had low certainty of evidence. Considering the heterogeneity and confounding factors, further investigation is required to confirm that prenatal TCS exposure leads to neurobehavioral disorders.

1. Introduction

Triclosan (TCS) is an aromatic and non-persistent chemical that is metabolized relatively rapidly and excreted in urine. Known for its broad-spectrum antibacterial properties, it was developed in the 1960s and has been extensively used across North America, Europe, and Asia [1,2]. TCS is found in various household products such as soaps, toothpaste, and cosmetics. It has also been detected in contaminated food, including seafood, raising concerns about its pervasive presence in the environment and food chain [3]. TCS is recognized as a known toxicant with a range of harmful effects, including potential thyroid function damage, neurodevelopmental and neurobehavioral toxicity, immune dysfunction, cytotoxicity, and correlations with allergic diseases and breast cancer prognosis [4]. TCS also interferes with retinoic acid pathways, gut microbiota, and presents estrogenic mechanisms, suggesting that it may cause endocrine disrupting effects [5].

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Research on animals has suggested that TCS may exhibit neurobehavioral toxic effects, including neurotoxicity observed in mouse brain tissue, disturbances in locomotor activity and motor coordination, and the induction of anxiety-like behaviors [6]. Regarding observational studies, Jackson-Browne et al. [7] showed that higher gestational TCS levels were associated to increased behavioral problem scores such as externalizing and attention problems, hyperactivity, and somatization among boys at eight years. Mustieles et al. [5] suggested that infant TCS exposure was associated with worse childhood social behavior in a cohort at three years of age. In contrast, Braun et al. [8] found no evidence that prenatal exposure to TCS could affect spatial learning skills and spatial memory in eight-year-old children, and Guo et al. [9] found no significant association of prenatal urinary TCS levels with motor scores. Instead, they observed an increase in development quotient (DQ) scores in the motor area of children.

Despite these issues, the use of TCS is not highly regulated, unlike some other organochlorine compounds. This lack of stringent regulation has led to growing apprehension about its potential impact on human health and ecosystems, calling for more rigorous monitoring and evaluation of its safety [1]. Causality between these xenobiotic and neurodevelopmental disturbances cannot be conclusively assumed. Thus, this systematic review aimed to verify whether prenatal exposure to TCS compared to no exposure can cause neurobehavioral impairments.

2. Methods

This systematic review was conducted following the PRISMA 2020 guidelines [10] and is registered on the PROSPERO platform with the number CRD42024526426. The study focused on observational studies to answer the question: "Does exposure to TCS during pregnancy lead to neurobehavioral disorders in children?".

2.1. Eligibility criteria

This review included observational studies involving pregnant women who were exposed to TCS during pregnancy. No restrictions based on language, ethnicity, or publication status were applied. Studies were excluded from the analysis if they failed to meet the following criteria:1) clear separation between TCS exposure and other xenobiotics, 2) absence of neurobehavioral assessments related to TCS use, and 3) absence of assessment of TCS exposure levels during pregnancy.

2.2. Information sources

For this research, the databases were divided into primary sources (such as MEDLINE, and EMBASE), secondary sources (including Scopus and Web of Science), and regional databases (LILACS). Additionally, a manual search was conducted in the reference lists of the included studies, expert consultations, and preprint servers such as Biorxiv and Medrxiv. Furthermore, the CAPES database was consulted to ensure a comprehensive coverage of available research. The research was carried out on February 27, 2024 (Supplementary material A).

2.3. Search strategy

The search strategy was developed to target observational studies pertinent to the research query. Following the PECOS framework [11], the study question was organized into five components: 1- Population (P): Newborns and children; 2- Exposure: TCS exposure during pregnancy; 3- Comparison (C): Lowest or no exposure during pregnancy; 4- Outcome (O): Postnatal neurobehavioral disorders; and 5- Studies (S): observational studies. Mesh, DeCS, and Emtree descriptors were integrated using Boolean operators "AND" (between categories) and "OR" (within categories).

2.4. Selection process

Upon retrieval, the studies were imported into both the EndNote® (online version) and Rayyan® platforms [12], where duplicate entries were meticulously eliminated. The selection process was performed independently by two researchers (ABS and LEWS) using Rayyan, adhering strictly to the predetermined eligibility criteria for this systematic review. Initially the studies were selected by reading the title and abstract, followed by a full text read of those selected. Any disparities were resolved through consensus meetings involving a third researcher. The concordance between researchers was assessed using the Kappa coefficient via GraphPad QuickCalcs (Graphpad, San Diego, CA), with a value exceeding 0.60 deemed satisfactory. Disagreements were resolved by consensus with a third reviewer.

2.5. Data collection process

Two reviewers independently conducted data extraction from the included studies. The extracted data included: i) General study characteristics, such as author, year, country, study design, database used, recruitment period, and participant count; ii) exposure characteristics, such as the assessment period and sample, analytical methods, and quantity of TCS; iii) outcome characteristics comprised neurocognitive outcome subcategories, evaluation period, instruments employed with their respective scores, key findings, and conclusions. A list of confounding factors considered was also extracted. Any discrepancies in collected information were resolved through consensus among investigators, with a third party involved if necessary.

2.6. Study risk of bias assessment

The methodological quality of the studies was evaluated independently by two reviewers using an adapted version of the Downs and Black scale [13,14]. This scale comprises 13 questions that evaluate internal and external validity as well as reporting patterns. Each item can be scored as either 0 or 1. The assessment aimed to gauge the quality of each study [15,16]. Disagreements were resolved by consensus with a third reviewer (JVMS).

2.7. Synthesis methods

A qualitative synthesis of the data was conducted, presenting the information in tables narratively. The results were summarized, considering the methodological quality and conclusions of each study. The methodological quality was estimated and classified based on the following score ranges shown in brackets: excellent quality (11–13), good quality (9–10), fair (7–8), and poor (≤6) [14–16]. Due to the substantial methodological variability and inconsistencies in outcome measures and exposure assessments among the included studies, conducting a meta-analysis was not feasible. These discrepancies would compromise the validity and reliability of any pooled estimates, making it challenging to draw accurate and meaningful conclusions from a meta-analysis in this context.

2.8. Certainty assessment

The data level of evidence was evaluated using the GRADE (Grading of Recommendations Assessment, Development, and Evaluations) approach via GRADEpro software [17]. This analysis specifically focused on the outcome of neurobehavioral disorders.

3. Results

3.1. Study selection

Following the database searches, a total of 348 articles were initially

identified. After removing duplicates, 295 unique studies remained. Through screening and full-text assessment, 17 studies were selected, of which four were subsequently excluded (Supplementary Material B). The remaining 13 studies met the inclusion criteria and were included in the review. Identification of studies via gray literature screened 38 studies. Of these, three were read in full; one met the established criteria and was included, while the other two were excluded for not meeting the criteria (Fig. 1). Inter-rater agreement among researchers was considered almost perfect, with a Kappa value of 0.822.

3.2. Study characteristics

Table 1 shows the characteristics of the included studies. The cohort studies included in this review were published between 2017 and 2023 and recruited participants between 2003 and 2015. Most of the publications originated from France [5,22–24,27] and the United States of America (USA) [7,8,19,21], followed by China [9,20,26], and Canada [18,25]. The 14 included studies used various database sources, including the Health Outcomes and Measures of the Environment (HOME) dataset [7,8,19,21] (recruitment from nine prenatal care clinics in Cincinnati between 2003 and 2006); the Sheyang Mini Birth Cohort Study (SMBCS) [9,26] (recruitment from Sheyang Maternal and Child Care Center between 2009 and 2010); the Maternal-Infant Research on Environmental Chemicals (MIREC) dataset [18,25] (recruitment from obstetric and prenatal clinics of ten cities between 2008 and 2011); the Etude des Déterminants pré et post natals du développement et de la santé de l'Enfant (EDEN) dataset [22,27] (recruitment from obstetric departments of Nancy and Poitiers university hospitals between 2003 and 2006); the French Assessment of Air Pollution Exposure during Pregnancy and Effect on Health (SEPAGES) dataset [5,23] (recruitment from eight obstetrical ultrasonography practices in the Grenoble metropolitan area between 2014 and 2017); the Human Early-Life Exposome project [24] (HELIX, recruitment from six established population-based cohorts across Europe between 2003 and 2009); and the longitudinal prenatal Wuhan cohort [20] (LPWC, recruitment from Wuhan Women and Children Medical Care Center between 2014 and 2015). The sample sizes of pregnant women and child pairs ranged from 193 to 794.

3.3. TCS exposure

Table 2 presents the characteristics of TCS exposure in the included studies. The period of TCS exposure evaluation varied across studies, with samples taken during different weeks or trimester of pregnancy and at delivery. Most studies assessed TCS exposure during mid-pregnancy (14–26 weeks) [5,7,8,18–23,27], while a few studies focused on early pregnancy (0–13 weeks) [18,20,25] and late pregnancy (27 weeks to delivery) [5,20,22,23,27]. Five studies assessed TCS exposure on the day of birth (post-pregnancy) [7,9,21,26].

All included studies used urine samples for detecting TCS levels by GC-MS/MS (4), LC-MS/MS (5), and HPLC-MS/MS (5). Creatinine was used as a normalizing factor for urine dilution. The TCS levels were described during the first (T1; 0–13 weeks), second (T2; 14–26 weeks), and third (T3; 27 weeks to delivery) trimesters and at delivery. The 50th percentile values for T1ranged from 0.63 ng/mL to 9.29 ng/mL, based on three studies [18,20,25]. For the T2, the median values ranged from 0.40 ng/mL to 28.2 ng/mL. During the T3, the median values ranged from 0.45 ng/mL to 28.2 ng/mL, based on five studies [5,20,22,23,27]. At delivery, the median values were 0.87 ng/mL and 1.02 ng/mL, based on two studies [9,26].

3.4. Neurocognitive deficit

Table 3 displays the neurobehavioral disorders evaluated in the included studies. The outcomes evaluated spanned several domains, including cognitive abilities [8,18–22], executive function [18,19,22,23], attention and behavior disorders [7,9,18,22–24], emotional and social function [5,7,9,18,22–24], cognition and social communication

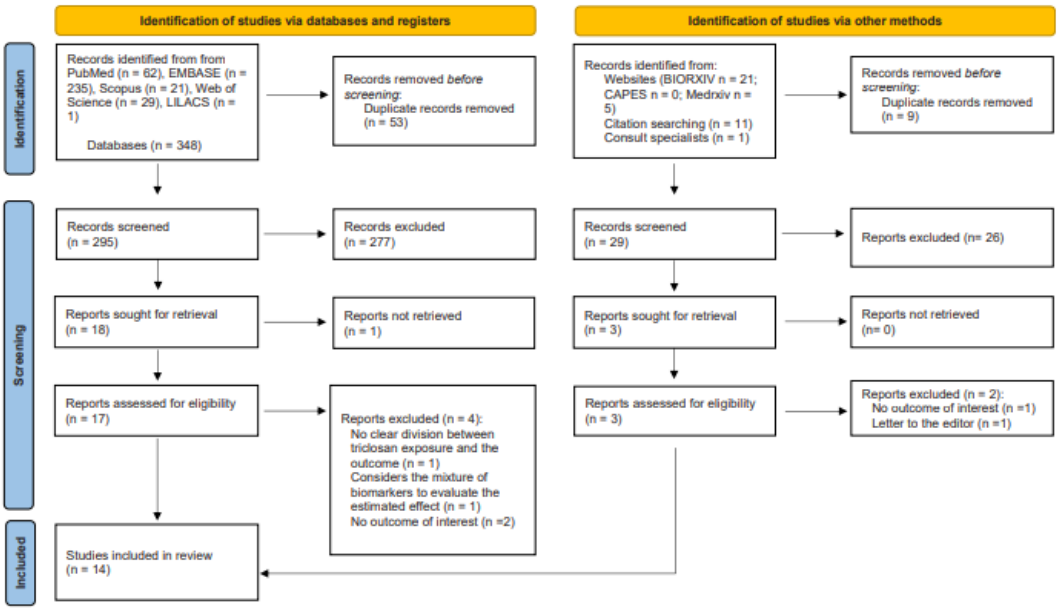

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

Table 1
Characteristics of the included studies (n = 14).

Author, Year, Country	Study design	Study database	Recruitment period / Location	Number of participants (women / children)
Alampi et al., 2021, Canada	Cohort study	MIREC	2008–2011 / Obstetric and prenatal clinics of ten cities	478 / 478
Braun et al., 2017, USA	Cohort study	HOME	2003–2006 / nine prenatal care clinics in Cincinnati	198 / 198
Etzel et al., 2018, Canada	Cohort study	MIREC	2008–2011/ Obstetric and prenatal clinics of ten cities	794 / 794
Guilbert et al., 2021, France	Cohort study	SEPAGES	2014–2017 / eight obstetrical ultrasonography practices in the Grenoble metropolitan area	416 / 416
Guo et al., 2020, China	Cohort study	SMBCS	2009–2010 / Sheyang Maternal and Child Care Center	386 / 386
Guo et al., 2020, China	Cohort study	SMBCS	2009–2010 / Sheyang Maternal and Child Care Center	377 / 377
Jackson-Browne et al., 2018, USA	Cohort study	HOME	2003–2006/ nine prenatal care clinics in Cincinnati	198 / 198
Jackson-Browne et al., 2019, USA	Cohort study	HOME	2003–2006 / nine prenatal care clinics in Cincinnati	202 / 202
Jackson-Browne et al., 2020, USA	Cohort study	HOME	2003–2006 / nine prenatal care clinics in Cincinnati	193 / 193
Jedynak et al., 2021, France	Cohort study	HELIX (BiB/ EDEN/ INMA/ KANC/ MoBa/ RHEA)	2003–2009 / Public hospitals, maternity wards, health centers and clinics	708/708
Jiang et al., 2019, China	Cohort study	LPWC	2014–2015 / Wuhan Women and Children Medical Care Center	478 / 478
Mustieles et al., 2023, France	Cohort study	SEPAGES	2014–2017 / eight obstetrical ultrasonography practices in the Grenoble metropolitan area	406 / 406
Nakiwala et al., 2018, France	Cohort study	EDEN	2003–2006/Obstetric departments of Nancy and Poitiers university hospitals	452/452
Philippat et al., 2017, France	Cohort study	EDEN	2003–2006/Obstetric departments of Nancy and Poitiers University hospitals	546/546

EDEN: *Etude des Déterminants pré- et postnatalsprécoces du développement et de la santé de l'Enfant*; HELIX: Human Early Life Exposome; HOME: Health Outcomes and Measures of the Environment; LPWC: Longitudinal prenatal Wuhan cohort; MIREC: Maternal-Infant Research on Environmental Chemicals; SEPAGES: French Assessment of Air Pollution exposure during Pregnancy and Effect on Health; SMBCS: Sheyang Mini Birth Cohort Study.

Table 2
Characteristics of the triclosan (TCS) exposure in the included studies (n = 14).

Author, Year	Exposure assessment	Exposure sample/ n°of samples per subject	TCS analysis method/ Biomarker	TCS concentration
Alampi et al., 2021	T1(6–13w)	Urine / one	GC-MS/MS; -	TCS (ng/mL) PE 25/MD/75/95: 2.62/9.29/ 97.2/569. IQR:94,58
Braun et al., 2017	T2(16w e 26w)	Urine / two	LC- MS/MS; CC, COT	*TCS (ug/g CC) PE 5/25/MD/ 75/95: 2.6/8.1/18/44/ 307. IQR:35.9
Etzel et al., 2018	T1-T2 (AVR 12w)	Urine / one	GC-MS/MS; CC	8.8 ng/mL (MD). IQR: 104.5 ng/mL
Guilbert et al., 2021	T2-T3 (16–18w; 32–35w)	Urine / forty-two	HPLC- MS/ MS; -	TCS (ng/mL) PE 33/MD/66: 1.92 ng/ mL (GM) 0.61/1.03/1.84
Guo et al., 2020	ATD	Urine / one	GC-MS/MS; CC	UDJ: 0.54 ng/ mL (MD). IQR: 1.53 ng/mL ADJ: 0.87 ng/ mL (MD). IQR: 2.44 ng/mL
Guo et al., 2020	ATD	Urine / one	GC-MS/MS; CC	UDJ:0.65 ng/ mL (MD). IQR: 1.99 ng/mL ADJ: 1.02 ng/ mL (MD). IQR: 4.42 ng/mL
Jackson-Browne et al., 2018	T2 (16w and 26 w)	Urine/ two	LC- MS/MS; CC	T2: 23 ng/mL (MD). IQR: 65.5 ng/mL T3: 16 ng/mL (MD). IQR: 36.4 ng/mL
Jackson-Browne et al., 2019	T2and PD (16w & 26w)	Urine / two	LC- MS/MS; CC	17 ng/ mL (GM). GSD: 3.6 T2:18 ng/mL (MD). IQR: 65.5 ng/mL T3:13 ng/mL (MD). IQR: 36.2 ng/mL
Jackson-Browne et al., 2020	T2and PD (16w & 26w)	Urine / two	LC- MS/MS; CC, COT	17 ng/ mL (GM) T2:17 ng/mL (MD). IQR: 59.5 ng/mL T3:12 ng/mL (MD). IQR: 34.4 ng/mL
Jedynak et al., 2021	NR	Urine / one	LC- MS/MS; CC	*TCS (ug/g CC) 15.9 (MD). IQR: 99.2
Jiang et al., 2019	T1, T2and T3 (T1(13.0 ± 1.2 w), T2(23.6 ± 3.4 w) and T3 (36.1 ± 3.3 w))	Urine / three	HPLC- MS/ MS; CC	TCS (ng/mL) T1/T2/T3MD (PE 25/75): T1:0.63(0.15/ 2.08). IQR:1.93 T2:0.40(0.08/ 1.64). IQR:1.56 T3:0.45(0.07/ 1.72). IQR:1.65 AVR (T1,T2, T3):0.43(0.12/ 1.38). IQR:1.26
Mustieles et al., 2023	T2-T3 (16–18w; 31–34w)	Urine / forty-two	HPLC- MS/ MS; -	TCS (ng/mL) PE 25/MD/75 on T2/T3: T2:0.45/0.92/ 2.29. IQR:1.84

(continued on next page)

Table 2 (*continued*)

Author, Year	Exposure assessment	Exposure sample/ n°of samples per subject	TCS analysis method/ Biomarker	TCS concentration
Nakiwala et al., 2018	T2-T3 (22–29w)	Urine / one	HPLC- MS/ MS; CC	T3:0.44/0.84/ 2.08. IQR:1.64 °TCS (ng/mL): PE 5/MD/95 < 2.3/25.6/691
Philippat et al., 2017	T2-T3 (22–29w)	Urine / one	HPLC- MS/ MS; CC	°TCS (ng/mL): PE 33/MD/66 7.09/28.2/99.7 GM:23.7

° Value corrected by level of creatinine. ADJ: Adjusted; ATD: At delivery day (within 24 hours after delivery); AVR: Average; CC: Creatinine concentration; COT: Cotinine concentration; PD(within 48 hours after delivery): Post delivery; T1: First trimester (0–13 weeks); GC: Gas chromatography; GM: Geometric mean; GSD: Geometric standard deviation; HPLC: High Performance liquid chromatography; IQR: Interquartile range; LC: Liquid chromatography; MD: Median; MS: Mass Spectrometry; NR: Not report; PE: Percentiles; T2: Second trimester (14–26 weeks); TCS: Triclosan; T3: Third trimester (27 weeks to delivery); UDJ: Unadjusted; W: week.

[5,18,25], motor function [20,26], language development [20,23,26], and social development [20,23,36]. Additionally, two studies evaluated general measures of difficulties [9] and adaptive function [26] with different tests. The instruments used were heterogeneous. The most frequent cognitive test employed was SRS-2 [5,18,25] and SDQ (Strengths and Difficulties Questionnaire) [9,24,26], followed by WPPSI-III (Wechsler Preschool and Primary Scale of Intelligence - Third Edition) [18,22] and BASC-2 (Behavior Assessment System for Children - Second Edition) [7,18]. NEPSY-II (Neuropsychological Assessment - Second Edition) [18], BRIEF-P (Behavior Rating Inventory of Executive Function - Preschool Version) [18], WISC-IV (Wechsler Intelligence Scale for Children - Fourth Edition) [19], GDS (Griffiths Development Scales) [26], BSID (Bayley Scales of Infant and Toddler Development) [20], WRAT-4 (Wide Range Achievement Test - Fourth Edition) [21], CBCL (Child Behavior Checklist) [23] and VMWM (Virtual Morris Water Maze) [8] were used in one study each. The most frequently evaluated age was three years [5,18,24–26], followed by eight [7,8,19,21], four [18,25], five years [22,27] and seven [24].

Four studies suggested a potential association between prenatal TCS exposure and neurobehavioral or neurodevelopmental deficits [5,7,23,27], such as externalizing problems, attention issues, hyperactivity, somatization, emotional symptoms, social awareness, and communication. Guo et al. [26] and Jackson-Browne et al.,[7] suggest that boys are more vulnerable to behavioral disturbance induced by TCS. Notably, all four studies that reported adverse associations [5,7,23,27] were classified as having high methodological quality, strengthening the evidence of potential adverse effects. However, nine studies found no significant effect of prenatal TCS exposure on neurobehavioral domains [8,9,18–22,24,25]. Table 4 shows the main confounding factors of the included studies in this systematic review.

3.5. Risk of bias in studies

The analysis of the methodological quality of the included studies shows that four studies [9,18,24,26] are of excellent methodological quality, obtaining a score greater than 11 points. Ten studies [5,7,8,19–23,25,27] had good quality, and no study had fair or poor quality (Supplementary material C). The use of the SRS-2 questionnaire might overestimate autistic behaviors in children with lower IQ scores or behavioral disorders, impacting the accuracy of reported outcomes. Participants in the MIREC study, who were wealthier and more likely to be white, may not adequately represent the broader Canadian population, affecting generalizability. The small sample size in the HOME cohorts can introduce biases, reducing statistical power. Gestational exposure measurements were taken at different periods, potentially missing critical developmental stages effects. The assessment of multiple toxicants could confound results, and the short half-life of TCS is particularly important in this context. As TCS is a non-persistent chemical, its short half-life generally results in attenuation bias and a tendency toward null results. This implies that the effects of early-life exposure to TCS are more likely to be underestimated rather than overestimated, impacting the interpretation of outcomes. Differences in outcome assessment tools and selection bias, influenced by household income, potentially introduce bias due to socioeconomic disparities. Maternal education disparities between retained and lost-to-follow-up subjects are noteworthy. Exposure misclassification and attenuation bias resulting from single spot urine samples for exposure characterization are additional concerns.

3.6. Certainty of evidence

The outcome evaluated in this systematic review had overall certainty of the evidence classified as low (Supplementary material D).

4. Discussion

This systematic review found limited evidence on the effects of prenatal exposure to TCS on neurobehavioral outcomes. Out of the 14 studies included, only four suggested a possible link between prenatal TCS exposure and neurobehavioral or neurodevelopmental impairments [5,7,23,27]. In general, the certainty of evidence was low, however, it is important to note that the four studies that associated prenatal TCS exposure to neurobehavioral or neurodevelopmental impairments were classified as high methodological quality. The difference in the conclusion of the studies is due to the methods employed, such as the age at which cognitive function tests were administered, the sample size, temporal urinary sample collection, and statistical analysis. Also, given the existing toxicological support from animal studies, such as those conducted in rodents that demonstrate neurodevelopmental effects [28–30], and the adverse outcomes reported in the human literature reviewed [31–33], more studies are needed to achieve a more definitive conclusion on the impact of prenatal TCS exposure on neurobehavioral development.

Legislative measures vary across countries regarding permissible limits for TCS usage [34,35]. In Canada, TCS is allowed in concentrations of up to 0.03 % in mouthwash and 0.3 % in other cosmetic products [36]. Similarly, in China, the maximum permitted TCS concentration in soaps, deodorants, and oral care products is 0.3 % [37]. The European Commission allows TCS concentrations up to 0.3 % in personal care products and 0.2 % in mouthwashes [38,39]. Japan has set a stricter limit, reducing the allowable TCS concentration in cosmetic products to 0.1 %. In contrast, the USA Food and Drug Administration banned TCS in over-the-counter antiseptic wash products in 2016 [37]. This difference in regulation among countries is not directly reflected in the studies included. The Chinese cohorts [9,20,26] have lower TCS levels than the USA cohorts [7,8,19,21]. The TCS levels in the Canadian MIREC cohort were lower than the USA HOME and France EDEN cohorts, but higher than the levels found in the France SEPAGES cohort. These differences may occur due to consumption habits, the enforcement of regulations, the prevalence of products containing TCS in the market, the methodology used to measure TCS exposure in the cohorts, and the variation in recruitment locations, even among cohorts within the same country.

Other studies also show differences in TCS exposure between countries. Liao and Kannan [40] showed that dermal intake of TCS among USA women was approximately four times higher than Chinese women, with a daily dermal intake difference of 67.8 ng/day versus 15.9 ng/day. This disparity was primarily attributed to the use of body lotions and face creams. Field measurements from a Swiss wastewater

Table 3
Neurobehavioral disorders evaluated in the included studies. Period of evaluation, instruments used, results and conclusion (n = 14).

Author, Year	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main result	Conclusions
Alampi et al., 2021	**Emotional and Social Function and Cognition and Social Communication** ASD behaviors	3y–4y	SRS–2	β(95 %CI) SRS Distribution: β (−0.2, 0.2) SRS Full sample (ng/mL) PLD/PQD: 0.930/0.996	TCS showed no significant changes on SRS scores.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Braun et al., 2017	**Cognitive abilities** spatial learning skills and spatial memory	8 y	VMWM	latency to complete the VMWM: p = 0.46 distance to complete the VMWM: p = 0.28 probe trial time: p = 0.49	TCS did not affect VMWM performance.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Etzel et al., 2018	**Cognitive abilities** (QI)- FSIQ/PIQ/VIQ; **Executive function** working memory, plan/organize subscales, **Attention and Behavior Disorders** ADHD, **Emotional and Social Function** ASD (affect recognition), attention, atypicality, aggression, anxiety, depression, hyperactivity, somatization, and withdrawal; **Cognition and Social Communication** social cognition, social communication, social awareness, social motivation and restricted interests and repetitive behaviors	3y- 4 y	NEPSY-II WPPSI-III BRIEF-P BASC–2 SRS–2	BASC–2 Summary scales β/95 %CI: BSI: −0.3/−0.7, 0.2 EB: −0.5/ −1.1,0.0 IB: −0.2/ −0.9,0.4 BASC–2 Clinical scales β/95 %CI: HI: −0.6/−1.2,−0.1 AG: −0.3/ −0.9,0.3 AN: 0.2/ −0.5,0.9 DE: −0.3/−0.9,0.3 SOM: −0.5/−1.1,0.1 AT: 0.0/ −0.5,0.6 WITH: 0.1/ 0.8,0.5 ATT: 0.1/−0.2,0.3 BRIEF-P β/95 %CI: WM: −0.3/−1.0,0.4 P/O: −0.4/−1.1,0.3 WPPSI-III Summary scales β/95 %CI: FSIQ: −0.1/ −1.2,1.1 VIQ: 0.6/−0.5,1.8 PIQ: −0.8/−2.1,0.6 WPPSI-III Subtests β/95 %CI: VOC: 0.1/ −0.1,0.4 BD: −0.1/ −0.3,0.2 IN: 0.1/−0.1,0.3 OBD: −0.2/−0.4,0.1 OBD: −0.2/−0.4,0.1 PC: 0.2/0.0,0.5 SRS–2 β/95 %CI: TD: −0.2/−0.7,0.3 SA:−0.2/0.9,0.4 SC: 0.1/−0.4,0.6 SCOM: −0.4/−0.9,0.1 SM: −0.1/−0.7,0.5 RES: −0.1/−0.6,0.5 DSM Social: −0.2/−0.7,0.3 DSM RES: −0.1/−0.6,0.5 NEPSY β/95 %CI: Affect Recognition: 0.0/−0.2,0.2	A 10-fold increase in TCS was associated with a reduction in externalizing and hyperactive behaviors, suggesting a potential positive effect.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Guilbert et al., 2021	**Emotional and Social Function** emotional symptoms, problems with peers, **Social development** Social Interest and Engagement, Response to Social Stimuli **Language and Communication** Social Interaction and Non-Verbal Communication **Attention and Behavior Disorders** Conduct problems, Hyperactivity and inattention **Executive function** Planning and organization skills	2 y	CBCL	EB CBCL β^{bc} (95 %CI): 0.27 (−0.10, 0.63) IB CBCL β^{bc} (95 %CI): 0.02 (−0.25, 0.29)	TCS were associated with increased externalizing scores in girls.	Neurobehavioral aspects were linked to urinary TCS concentrations measured during pregnancy.
Guo et al., 2020	**Emotional and Social Function** emotional symptoms, prosocial behavior, peer problems **Attention and Behavior Disorders** conduct problems, hyperactivity/inattention,	10 y	SDQ	OR/95 %CI for ES/CP/HI/PP/PB/TD ES:0.89 (0.57,1.38) CP:0.95(0.67,1.35) HI:0.97(0.77,1.21) PP:0.93(0.77,1.23)	No association was observed between urinary TCS concentrations and SDQ scores.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.

(continued on next page)

Table 3 (*continued*)

Author, Year	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main result	Conclusions
	General Measure of Difficulties total difficulties			PB: 1.20(0.94,1.53) TD:1.20(0.90,1.60)		
Guo et al., 2020	**Motor Function** lift head, roll over, sit, crawl, stand, walk and manipulate objects **Adaptative function** Self-care, Communication, Social Skills, Functional skills **Language and Communication** Expressive Language Development, Receptive Language Development, Social Interaction and Non-Verbal Communication, Pragmatic Development **Social Interaction and Relationships** Initiating Social Contact, Participating in Social Activities, Developing Relationships, Conflict Resolution, and Mediation Skills	3 y	GDS	C/Q 1,2,3,4 β (95 %CI) for MA/ADA/LA/SOCA/AA C: 0.28 (0.03, 0.54)/ 0.11 (−0.18, 0.40) / 0.29 (−0.07, 0.66) / 0.10 (−0.17, 0.36) / 0.29 (−0.03, 0.62) Q1:Ref Q2: 0.49 (−0.44, 1.42) / 0.67 (−0.37, 1.71) / 0.43 (−0.91, 1.77) / 0.34 (−0.63, 1.31) / 0.70 (−0.48, 1.89) Q3: 0.25 (−0.68, 1.18) / 0.33 (−0.71, 1.36) / 0.43 (−0.90, 1.76) / 0.39 (−0.57, 1.35) / 0.31 (−0.87, 1.49) Q4: 0.91 (−0.03, 1.86) / 1.00 (−0.05, 2.04) / 1.38 (0.03, 2.74) / 0.85 (−0.12, 1.83) / 0.78 (−0.41, 1.98)	Postnatal urinary TCS concentrations were inversely associated with DQ scores in the social area among 3-year-old boys.	Neurobehavioral outcomes were associated with higher motor scores in relation to prenatal urinary TCS concentrations.
Jackson-Browne et al., 2018	**Cognitive abilities** global cognitive abilities; verbal abilities; perceptual reasoning and organization skills; **Executive function** speed of mental and motor processing; working memory	8 y	WISC-IV	FSIQ/VC/PR/PS/WM at 16 W/26 W: FSIQ: −0.5(−3.2,2.2)/ −0.4(−3.1,2.4) VC: 0.3(−2.4,3.0)/2.1 (−0.3,4.5) PR: −0.1(−2.8,2.5)/−0.6 (−3.5,2.4) PS: 0.7(−2.3,3.6)/−1.1 (−4.3,2.1) WM: −2.5(−5.5,0.4)/ −2.5(−5.4,0.3)	At 8 years of age, this cohort showed significantly lower cognitive test scores linked to urinary TCS concentrations measured at delivery.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Jackson-Browne et al., 2019	**Emotional and Social Function** atypicality, depression, anxiety, somatization, withdrawal **Attention and Behavior Disorders** aggression, attention problems, hyperactivity, and conduct problems	8 y	BASC−2	BSI/EB/IB/AG/AN/ATT/AT/CP/DE/HI/SOM/WITH BSI: 1.6 (−0.8, 4.1) EB: 1.9 (−0.7, 4.5) IB: 0.4 (−2.0, 2.9) AG: 1.1 (−1.7, 3.9) AN: 0.2 (−2.6, 3.0) ATT: 2.5 (−0.5, 5.6) AT: 1.7 (−1.4, 4.7) CP: 1.2 (−1.8, 4.2) DE: 0.7 (−1.6, 3.0) HI: 2.8 (0.3, 5.3) SOM: 0.1 (2.8, 3.0) WITH: −1.4 (−4.4, 1.6)	In boys, TCS exposure was linked to increased scores in behavioral symptom index, externalizing problems, attention issues, hyperactivity, and somatization.	Neurobehavioral aspects were linked to urinary TCS concentrations measured during pregnancy with elevated behavior problem scores in boys, but not in girls.
Jackson-Browne et al., 2020	**Cognitive abilities** word reading, sentence comprehension, math	8 y	WRAT−4	β(95 %CI) RC/Math at 16/26w: RC: −1.2 (−3.8, 1.4)/ 1.4 (−1.3, 4.2) Math: −0.9 (−3.5, 1.7)/ 0.7 (−1.8, 3.1)	The timing of exposure modified the associations between TCS and reading composite scores, but not math scores. A tenfold increase in TCS concentrations at delivery was associated with lower reading composite scores. Additionally, weaker and less precise inverse associations were observed between math scores and TCS concentrations at delivery and at age 1 year.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Jedynak et al., 2021	**Emotional and Social Function:** Emotional symptoms and peer problems, prosocial behavior **Attention and Behavior Disorders:** Conduct problems and hyperactivity/inattention.	3–7 y	SDQ	IRR EB(95 %CI):1.03 (0.93; 1.12) IRR IB(95 %CI): 1.08 (0.96; 1.21)	No association was observed between urinary TCS and SDQ scores.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.

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Table 3 (*continued*)

Author, Year	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main result	Conclusions
Jiang et al., 2019	**Cognitive abilities** Visual Perception and Discrimination, Problem Solving, Exploration and Interest in the Environment, Memory, Imitation, Selective Attention, **Language development** Verbal Comprehension, Verbal Expression, Grammatical Development, Pragmatic Development, Pre-Language Development **Social development** Social Interest and Engagement, Response to Social Stimuli, Interaction with Examiner, Gaming Behavior, **Motor function** gross motor skills (crawling, sitting, walking); fine motor skills (isolation of fingers, grasping)	2 y	BSID-CR	β(95 %CI) MDI: −0.56 (−1.35,0.24) β(95 %CI) PDI: −0.47 (−1.09,0.15)	No significant associations were observed between the average prenatal TCS exposure and BSID results.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Mustieles et al., 2023	**Emotional and Social Function** Social Awareness, Social Cognition, Social Communication, Social Motivation, and Restricted Interests and Repetitive Behaviors (RRBs); ASDs **Cognition and Social Communication** ASDs, Social Communication and Interaction (SCI)	3 y	SRS−2	β(95 %CI) in second trimester for TS/SA/SC/ SCOM/SM/RRB: TS:0.21(−0.22,0.65)/ SA:0.07(−0.02,0.16)/ SC:0.00(−0.11,0.11)/ SCOM:0.06(−0.11,0.22)/ SM:0.09(−0.04,0.21)/ RRB:0.00(−0.10,0.10) β(95 %CI) in third trimester for TS/SA/SC/ SCOM/SM/RRB: TS: −0.36(−0.81,0.10)/ SA:−0.03 (−0.12,0.07)/ SC:−0.09(−0.21,0.02)/ SCOM:−0.06 (−0.23,0.11)/SM:−0.08 (−0.21,0.05)/RRB:−0.10 (−0.20,0.01)	Doubling urinary concentrations of TCS was linked to higher total SRS scores.	Neurobehavioral aspects were linked to urinary TCS concentrations measured during pregnancy with worse childhood social status
Nakiwala et al., 2018	**Cognitive abilities** Verbal IQ; information, vocabulary, word reasoning, **Executive function** block design, matrix reasoning, picture concepts subtest and coding	5–6 y	WPPSI-III	VIQ β /95 %CI/ PIQ β/ 95 %CI 0.101 / [0.008;0.194]/ 0.007/ [−0.111;0.125]	TCS did not exhibit evidence of an inverse association with verbal and performance IQ following in-utero exposure.	Neurobehavioral aspects were not linked to urinary TCS concentrations measured during pregnancy.
Philippat et al., 2017	**Attention and Behavior Disorders** conduct problems, hyperactivity–inattention, prosocial behavior, externalizing behavior **Emotional and Social Function** internalizing behavior peer relationship problems, emotional symptoms	3 y 5 y	SDQ	EDEN 3 y IRR/95 %CI for ES/CP/PRP/HI/PB/EB/ IB ES:1.02/(1.00,1.04)/ CB:1.01/(1.00,1.03)/ PRP:1.00/(0.98,1.03)/ HI:1.01/(1.00,1.03)/ PB:1.01/(0.99,1.03)/ EB:1.01/(1.00,1.03)/ IB:1.01/(0.99,1.03) EDEN 5 y IRR/95 %CI for ES/CP/PRP/HI/PB ES:1.01/(0.99,1.03)/ CP:1.00/(0.98,1.02)/ PRP: 1.01/(0.98,1.04)/ HI:1.00/(0.98,1.01)/ PB:0.99/(0.97,1.02)/ EB:1.00/(0.98,1.02)/ IB:1.01/(0.99,1.03) SDQ 3 y IRR/95 %CI for ES/CP/PRP/HI/PB/EB/ IB ES:1.13/(0.98,1.30)/ CP:1.18/(1.01;1.38)/ PRP:1.10/(0.95,1.28)/ HI:1.03/(0.90,1.18)/ PB:1.13/(0.98,1.30)/ EB:1.08/(0.95,1.23)/ IB:1.09/(0.95,1.24) SDQ 5 y IRR/95 %CI for	TCS tended to be associated with emotional symptom subscales at both 3 and 5 y, SDQ scores at 3 y with IRRs closer to 1 than those observed for BPA	Neurobehavioral aspects were linked to urinary TCS concentrations measured during pregnancy associated negatively with emotional symptom subscales

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Table 3 (*continued*)

Author, Year	Outcomes evaluated (Child)	Period of evaluation	Instruments	Measurement of results (Scores)	Main result	Conclusions
				ES/CP/PRP/HI/PB/EB/IB ES:1.15/(0.99,1.32)/ CP:1.10/(0.94,1.29)/ PRP:1.17/(0.99,1.39)/ HI:1.01/(0.88,1.16)/ PB:0.99/(0.85,1.16)/ EB:1.00/(0.88,1.15)/ IB:1.11/(0.97,1.28)		

AA: Average area; ADA: Adaptative Area; ADHD: Attention-deficit hyperactivity disorder; AG: Aggression; AN: Anxiety; ASD: Autism spectrum disorder; AT: Atypicality; ATT: Attention; β: beta; BASC-2: Behavior Assessment System for Children, Second Edition; BD: Block Design; BPA: Bisphenol-A; BP-3: Benzophenone-3; BRIEF-P: Behavior Rating Inventory of Executive Function - Preschool Version; BSID-CR: Bayley Scales of Infant Development Chinese Revision; BSI: Behavioral Symptom index; BWQS: Bayesian Weighted Quantile Sum; CI: Confidence intervals; CP: Conduct problems; C/Q: Continuous/Quartiles; DE: Depression; DQ: Developmental quotient; EB: Externalizing behavior; EDEN: Etude des Déterminants pré et post natals du développement et de la santé de l'Enfant; ES: Emotional symptoms; FDR: False discovery rate; FSIQ: Full Scale IQ; GDS: Gesell Developmental Schedules; HI: Hyperactivity/inattention; IB: Internalizing behavior; IN: Information; IQ: Intelligence quotient; IRR: Incidence rate ratio; LA: Language area; MA: Motor area; MBzP: Monobenzyl phthalate; MDI: Mental developmental index; MEP: mono-ethyl phthalate; MnBP: Mono-n-butyl phthalate; MP: Methyl paraben; NEPSY-II: Neuropsychological Assessment, Second Edition; OBD: Object Design; OR: Odds ratio; PB: Prosocial behavior; PC: Picture Completion; PD: Psychomotor development index; PIQ: Performance IQ; PLD: p linear deviation; P/O: Plan/Organize; PQD: p quadratic deviation; PP: Peer problems; PR: Perceptual Reasoning; PRP: Peer relationship problems; PS: Processing Speed; RC: Reading Composite; Ref: Reference quartile; RES: Restricted; RRB: Restricted Interests and Repetitive Behaviors; SA: Social awareness; SC: Social cognition; SCOM: Social communication; SDQ: Strengths and Difficulties Questionnaire; SM: Social motivation; SOM: Somatization; SRS-2: Social Responsiveness Scale Second Edition; TCS: Triclosan; TD: Total difficulties; TS: Total score; VC: Verbal Comprehension; VIQ: Verbal IQ; VMWM: Virtual Morris Water Maze; VOC: Vocabulary; W: Week; WISC-IV: Wechsler Intelligence Scale for Children, 4th edition; WITH: Withdrawal; WM: Working memory; WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence, Third Edition; WRAT-4: Wide Range Achievement Test-4; ΣDEHP: 2-ethylhexyl phthalate; 4-OH-BP: 4-hydroxybenzophenone

Table 4
Confounders factors of the included studies (n = 14).

Author, Year	Parental and Child Demographics	Socio-economic Status	Environmental Factors	Health Conditions	Drug Use/exposure or supplement consumption
Alampi et al., 2021	Child's sex, Maternal ethnicity, Maternal age, Mother's education Parity, Marital status	Household income	Caregiver environment score, City of residence	-	Folic acid supplementation
Braun et al., 2017	Child's sex, Maternal and child age, Mother's education, Marital status, Race, Parity	Household income, Employment	-	Maternal depressive symptoms	Vitamin supplementation
Etzel et al., 2018	Parity, Maternal race, Maternal age, Mother's education, breastfeeding duration	Household income, Employment	-	Parental stress, Maternal depressive symptoms	Alcohol, smoking
Guilbert et al., 2021	Parity, Child's sex, Mother's education, Breastfeeding duration	Mother's employment situation	-	BMI, Anxiety, Maternal depression	Vitamin supplementation, Smoking
Guo et al., 2020	Maternal age, Mother's education,	Family annual income	-	BMI, Depression of the caregiver	Passive smoking
Guo et al., 2020	Child's sex, Maternal and child age, Mother's education	Family annual income	Time spent playing outdoors	BMI	Passive smoking
Jackson-Browne et al., 2018	Child's sex, Child's race/ethnicity, Mother's education, Marital status,	Family income	Caregiver environment at 1 year of age	FSIQ	Passive smoking
Jackson-Browne et al., 2019	Child's sex, Child's race/ethnicity, Mother's education, Marital status	Household income	-	Maternal depression, ADHD behaviors	Passive smoking
Jackson-Browne et al., 2020	Child's sex, Race/ethnicity, Mother's education, Marital status	Household income	-	Maternal and children's IQ	-
Jedynak et al., 2021	Parity, Child's sex, Mother's education, Maternal age, Child age at SDQ, Cohort	Mother's employment situation	Season of conception	BMI	Active smoking
Jiang et al., 2019	Child's sex, Maternal age, Mother's education	-	-	BMI	Passive smoking
Mustieles et al., 2023	Parity, Child's sex, Maternal and child age, Mother's education,	Family environment	Caregiver environment until 1 year of age	BMI, Anxiety, Maternal depression	Active smoking, passive smoking
Nakiwala et al., 2018	Maternal and child's age, Parity, Parental education, Breastfeeding duration	Monthly household income	Recruitment center,	Maternal psychological difficulties, Maternal depression, Anxiety, BMI, Child cognitive stimulation	Smoking
Philippat et al., 2017	Parity, Maternal and child's age, Parental education, Breastfeeding duration	Household income	Recruitment center	Maternal psychological difficulties, Maternal depression, Anxiety, BMI	Smoking

ADHD: attention deficit hyperactivity disorder; BMI: Body mass index; FSIQ: Full-scale IQ; IQ: Intelligence quotient

treatment plant highlight notable differences in TCS levels in influent (incoming wastewater) and effluent (treated water) between the USA and Canada. In the USA, influent TCS levels range from 2.70 to 26.80 μg/L, while they are much lower in Canada, ranging from 0.01 to 4.01 μg/L. Similarly, effluent TCS levels in the USA range from 0.03 to 2.7 μg/L, compared to 0.01–0.324 μg/L in Canada [1].

The median TCS levels during pregnancy reported in the studies included ranged from 0.40 ng/mL to 28.2 ng/mL in urine. It is important to note that the limited number of spot urine samples for TCS exposure assessment can lead to inaccuracies due to the short half-life of TCS, which only reflects recent exposure [41]. Although continuous biomonitoring is necessary for a full characterization of TCS exposure, which involves multiple urine samples analysis from the same individual at several times, this procedure has economic constraints. Future studies should implement serial urine sampling with larger and more diverse cohorts to reduce misclassification and enhance generalizability [21, 22]. Notably, Mustieles et al. [5] avoided this issue by asking pregnant women to collect three urine samples per day (morning, midday, and evening) for seven consecutive days, twice during pregnancy, providing a more accurate assessment of TCS exposure. The study by Smith et al. [42] suggests that a single urine sample can be reasonably adequate for evaluating phenolic compounds, even with one sample.

Additionally, a significant limitation of this work is its exclusive focus on prenatal exposure to TCS. While this approach provides valuable insights into the impacts of exposure during critical developmental windows, it does not capture the broader effects of early-life exposure, including preconception and postnatal periods. During the literature search, we identified studies suggesting that postnatal exposure may also play a significant role in neurodevelopment. For instance, Jackson-Browne et al. (2019) reported that exposure at delivery was associated with poorer neurodevelopmental outcomes, particularly in boys [7]. Similarly, Mustieles et al. observed that exposure during infancy (1 year) was linked to adverse neurodevelopmental effects [27]. Additional studies also emphasize the role of early-life exposure to environmental toxicants in general, indicating that the postnatal period constitutes a critical window for neurodevelopmental vulnerabilities. For example, Sotelo-Orozco et al. highlighted that childhood exposure to endocrine-disrupting chemicals, including TCS, could interfere with cognitive and behavioral development [43]. Moreover, Kim et al. demonstrated that postnatal exposure to endocrine-disrupting compounds, including phthalates, bisphenol A, triclosan, parabens, and per- and polyfluoroalkyl substances, independently and synergistically affect maternal postpartum depression and infant neurodevelopment [44]. These findings collectively underscore the importance of considering early-life exposure comprehensively, encompassing preconception, prenatal, and postnatal periods. Future research should aim to address these broader exposure windows to achieve a more holistic understanding of the neurodevelopmental risks associated with TCS exposure.

Related to neurobehavioral disorders, five of the included studies have identified associations between TCS exposure and neurobehavioral outcomes [5,7,23,26,27]. Guo et al. [26] found associations with DQ scores in motor skills, while Jackson-Browne et al. [7] reported increased attention, hyperactivity, and somatization scores in boys associated with higher TCS levels. Mustieles et al. [5] also observed worse social behavior linked to increased TCS exposure, and Philippat et al. [27] found associations with conduct problems. However, the majority of studies did not find significant associations. Alampi et al. [25] and Etzel et al. [18] reported limited variation in SRS scores, possibly due to sample limitations and non-representative cohorts. Etzel et al. [18] did not find significant early-life effects, and Philippat et al. [27] did not support the hypothesis that TCS exposure impacts neurobehavioral development at later ages. The lack of significant results in studies such as Braun et al. [8] further supports the notion that TCS exposure might not have a direct impact on assessed neurobehavioral parameters.

Variations in findings could stem from differences in study methodologies, including the measurement tools and concentration levels of TCS. Notably, studies used diverse assessment tools, such as the Revised Chinese Version of the Gesell Developmental Scale in Guo et al. [26] and the Bayley Scales of Infant Development in Jiang et al. [20], which may influence results. The use of different scales and concentration thresholds could contribute to the observed discrepancies across studies. Additionally, the small sample sizes in some studies, such as those by Jackson-Browne et al. [7,19,21] and Braun et al. [8], could limit the generalizability and reproducibility of their findings.

Pre-clinical studies have shown that xenobiotics can interfere with brain development [45,46], while systematic reviews focused on xenobiotics have highlighted neurodevelopmental aspects, though they often lack definitive conclusions due to the inherent uncertainties in the data [47,48]. This systematic review on gestational TCS exposure highlights significant challenges due to study heterogeneity and the multitude of confounding factors. The potential interference of TCS with various biological pathways such as anti-thyroid activity, retinoic acid pathways, alterations in gut microbiota, and hormonal signaling pathways complicates the interpretation of findings related to neurobehavioral outcomes [5]. Despite employing diverse study designs and statistical approaches, the synthesis of evidence reveals a generally low quality, limiting definitive conclusions regarding its effects. Addressing these complexities comprehensively remains crucial for advancing our understanding of the impact of TCS during gestation.

5. Conclusions

The evidence regarding prenatal exposure to TCS and its potential impact on neurobehavioral development in children remains inconclusive. While certain studies indicate a possible link between prenatal TCS exposure and deficits in neurobehavioral outcomes, such as emotional symptoms, childhood social behaviors, and increased behavior problems, the diverse quality and heterogeneous nature of these studies, along with the presence of numerous confounding factors, contribute to low certainty of evidence in drawing definitive conclusions, highlighting the necessity of continuous monitoring and investigation.

CRediT authorship contribution statement

Torres Larissa Helena Lobo: Writing – review & editing, Supervision, Methodology, Conceptualization. **Santana Aleksander Brandão:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Spelta Lídia Emmanuela Wiazowski:** Writing – original draft, Investigation, Formal analysis, Data curation. **Martinez-Sobalvarro Joselin Valeska:** Writing – original draft, Investigation, Formal analysis, Data curation. **Garcia Raphael Caio Tamborelli:** Writing – review & editing, Supervision, Methodology, Conceptualization. **dos Reis Tiago Marques:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Larissa Helena Lobo Torres reports financial support was provided by Coordination of Superior Level Staff Improvement - Brazil (CAPES, Finance Code 001). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2025.108849.

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6 CONSIDERAÇÕES FINAIS

Com base nas revisões sistemáticas realizadas sobre chumbo, cafeína e triclosan as evidências atuais permanecem inconclusivas quanto ao seu potencial para causar alterações neurocomportamentais, mortalidade infantil ou aborto espontâneo. Embora essas substâncias estejam amplamente presentes em produtos do cotidiano e em fontes ambientais, as análises de qualidade e certeza das evidências não forneceram clareza suficiente sobre seus efeitos adversos.

Novos estudos devem ter como objetivo minimizar o risco de viés por meio de análises de regressão que incluam mais fatores de confusão e amostras populacionais maiores. Esses são exemplos dos requisitos necessários para melhorar a qualidade dos estudos existentes.

Esses achados ressaltam a necessidade de estudos mais rigorosos e abrangentes para entender plenamente os impactos desses xenobióticos na saúde. Garantir a segurança das populações vulneráveis, especialmente mulheres gestantes e seus descendentes em desenvolvimento, requer uma avaliação científica contínua e uma supervisão regulatória mais rigorosa.

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