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Review article

The role of phthalate esters in autism development: A systematic review

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ABSTRACT

Background: Available evidence implicates environmental factors in the pathogenesis of autism spectrum disorders (ASD). However, the role of specific environmental chemicals such as phthalate esters that influence ASD risk remains elusive. This paper systematically reviews published evidences on association between prenatal and/or childhood exposure to phthalate and ASD.
Methods: Studies pertaining to systematic literature search from Scopus, PubMed, PsycInfo and Web of Science prior to December 2015 were identified. The authors included studies which assessed the effect of exposure to phthalates on occurrence of ASD. This comprehensive bibliographic search identified five independent studies. Each eligible paper was summarized with respect to its methods and results with particular attention to study design and exposure assessment. Because of the heterogeneity in the type of included studies, different methods of assessing exposure to phthalates and the use of different statistics for summarizing the results, meta-analysis could not be used to combine the results of included studies.
Results: The results of this systematic review have revealed the limited number of studies conducted and assessed phthalate exposure. Seven studies were regarded as relevant to the objectives of this review. Two of them did not measure phthalate exposure directly and did not result in quantitative results. Out of the five studies in which phthalate exposure was mainly measured by the examining biomarkers in biological samples, two were cohort studies (one with positive results and another one with not clear association). Among the three case control studies, two of them showed a significant relation between exposure to phthalate and ASD and the last case control study had negative results. Indeed, this case control studies showed a compromised phthalate metabolite glucuronidation pathway, as a probable explanation of mechanism of the relation between phthalate exposure and ASD.
Conclusions: This review reveals evidence showing a connection between exposure to phthalates and ASD. Nevertheless, further research is needed with appropriate attention to exposure assessment and relevant pre and post-natal cofounders.

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1. Introduction

Since the industrial revolution, synthetic chemicals have been increasingly manufactured in order to be used in almost every product with which we are in contact. From a scientific perspective, recent data have shown that nearly all the people regardless of age and sex are being exposed to hundreds of these man-made chemicals worldwide (Meeker, 2012). It has been proved that nearly two hundreds of these chemicals are neurotoxic in humans; and even worse , based on laboratory analysis, more than 1000 of such compounds can potentially be neurotoxic (Schwartzer et al., 2013). However, less than 20% of high-volume chemicals have been screened for potential neurodevelopmental toxicity during early development (Landrigan, 2010). It should be noted that human brain, at its early developing stage, is highly vulnerable and sensitive to the damages caused by environmental neurotoxicants. In fact, exposure of the brain to neurotoxicants at this stage could damage this vital organ in a way which is far worse than what it does to an adult brain (Grandjean and Landrigan, 2006; Weiss, 2000). This susceptibility roots from the fact that during the 9 months of prenatal life, the human brain develops from a strip of cells along the dorsal fetal ectoderm into a complex organ consisting billions of precisely located, highly interconnected and specialized cells. In fact, exposure to environmental chemicals, especially endocrine disruptor chemicals (EDCs), during the brain growth spurt (BGS) in prenatal period, has been suggested to be a possible causal factor for neurodevelopmental disorders (Colborn, 2004; Kim et al., 2010). In this regard, autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorder (ADHD) could be the outcomes of exposure to these chemicals (Miodovnik, 2011; Tanida et al., 2009). The BGS period usually begins during the third trimester of pregnancy and continues throughout the first two years of life (Kim et al., 2010). Although the involvement of genetic abnormalities in developing ASD is well-accepted, it is widely believed that a single genetic risk factor cannot cause ASD. In other words, the most likely cause of ASD might be genetic susceptibility besides the exposure to environmental neurotoxic compounds (Hertz-Picciotto et al., 2006). In fact, this hypothesis provides a plausible explanation for the rapid increase in the incidence of ASD over the past few decades (Hertz-Picciotto and Delwiche, 2009).

ASDs are comprised of a broad spectrum of heterogeneous, neurodevelopmental disorders (Ashwood et al., 2006). Previously, disorders which were considered as part of the autism spectrum were divided into the following discrete categories: Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS), as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The DSM-V, published in May 2013, combined the previous categorical disorders into a single category of “Autism Spectrum Disorder,” with varying degrees of severity depending on the amount of support required by an individual. Because no medical or biological marker exists for ASD, the diagnosis is mostly based on behaviors (APA, 2013). Thus ASD, similar to the one first described in 1943 by Kanner, is a complex developmental disability with social, cognitive, and communicative deficits (Kanner, 1943). The symptoms of autism usually appear before a child reaches the age of three and last throughout the life (CDC, 2012). From a social point of view, some children with ASD have difficulty in understanding the fact that others think differently from the way they do, and in coordinating attention with a social partner. Regarding the cognitive deficit, some children have weak central coherence and executive dysfunction and these continue into adulthood in some individuals (Mendes, 2013).

In a scientific statement published by the Endocrine Society in 2009, it was argued that endocrine disruptors indeed pose a “significant concern for public health”(Diamanti-Kandarakis et al., 2009). Recently, due to proven adverse effects on human health, concerns over a class of chemicals namely, phthalates has also emerged (Myers, 2012). Phthalates with a di-ester structure are additive polymers applied as plasticizers to produce high volumes of synthetic chemicals (Miodovnik et al., 2014a). In fact, these chemicals are being used to provide flexibility, durability, and solubility and can be found in a wide range of products used in daily life (Lyche et al., 2009); many of these products do not require labeling of phthalates as an ingredient (Dodson et al., 2012). Currently, over a dozen forms of phthalates are in commerce among which di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), butylbenzyl phthalate (BBP or BBzP), diisoheptyl phthalate (DiHP), di-n-butyl phthalate (DBP or DnBP) and diethyl phthalate (DEP) are the most commonly produced forms (Miodovnik et al., 2014a). Concerns over human exposure to phthalates root from the fact that these compounds do not form a covalent bound with the polymer matrix. In other words, phthalates may leach or outgas into their surroundings. Humans are exposed to phthalates via ingestion, inhalation and dermal exposure during their whole lifetime including intrauterine development. (Heudorf et al., 2007; Zare Jeddi et al., 2015). In fact, it is not surprising that metabolites of some phthalates can be detected in saliva, urine, amniotic fluid and breast milk (Fromme et al., 2007; Koch et al., 2006; Koch et al., 2011; Völkel et al., 2014). Since, the potential consequences of human exposure to phthalates have raised

concerns among the general population, these compounds have been studied in terms of their effects on susceptible subjects, including pregnant women, infants and children (Jurewicz and Hanke, 2011).

Ubiquitous environmental contaminants (phthalates in particular) can be potential risk factors for the pathogenesis of ASDs while interfering with neurological development (Schug et al., 2015; Ventrice et al., 2013). Carbone et al. (2013) have conducted a study that shows DEHP has anti-androgenic effects, and this can be related to anxiogenic-like effects in rats. This finding shows that the endocrine effects of phthalates can lead to other neurological/psychiatric diseases (Carbone et al., 2013). Furthermore, EDCs can interfere with the thyroid's hormonal functions and in turn result in neurodevelopmental outcomes (De Cock et al., 2012). Regarding this, it can also be implied that exposure to exogenous agents, particularly during critical prenatal or early post-natal windows of development, might interfere with the expression of genetic susceptibility (Hertz-Picciotto and Delwiche, 2009; McDonald and Paul, 2010).

So far, among the environmental neurotoxicant factors, a number of epidemiological studies have been conducted in order to evaluate the possible association between exposure to phthalate and the risk of developing autism in human subjects. However, to the best of our knowledge, there is no thorough study in which the issue at hand is fully discussed and studied. There are Review articles such as those conducted by Kalkbrenner et al. (2014), Rossignol et al. (2014) and De Cock et al. (2012) and other related reviews investigating the relationship between environmental contaminants, including phthalates and other endocrine disruptors and autism (De Cock et al., 2012; Kalkbrenner et al., 2014; Matelski and Van de Water, 2016; Rossignol et al., 2014; Sealey et al., 2016). However, they did not fully discuss the existing data on phthalates and risks of ASD. Moreover, in these reviews, the limitations and strengths of related studies were not taken into account.. Thus, the present study aimed to systematically review the previous studies on exposure of humans to phthalates which had resulted in autism in order to help producing more reliable evidence for future studies.

2. Material and method

2.1. Search strategy and selection criteria

The present systematic review was conducted by means of following the preferred reporting items for systematic reviews and meta-analyses guidelines. To identify pertinent articles which have been published up to December 2015, specific terms relating to exposure in combination with outcome-related keywords were used to search the literatures in multiple international databases, including Pub Med (www.ncbi.nlm.nih.gov), ISI Web of Science (www.isiknowledge.com), Scopus (www.scopus.com), PsycInfo (www.ebscohost.com/academic/psycinfo) databases, and Google Scholar. The authors formulated the search strategy by employing a combination of the following concepts: “Autism spectrum disorder”, “phthalate”, and all of their possible variations and synonyms and the use of Boolean operators, such as “OR” to explode and “AND” to combine. The final terms used in the search strategy were:

((("Autism spectrum disorder*") OR (autism) OR ("autistic disorder*") OR ("Pervasive Development* Disorder*") OR (PDD) OR (ASD)) AND ((*phthalate*) OR ("*phthalic acid*") OR (plasticizer*) OR ("endocrine disrupter*") OR ("*phthal* ester*) OR (plastic*) OR ("polyvinyl chloride*"(OR ("polyethylene terephthalate*") OR (PET) OR (PVC))).

Moreover, to ensure that relevant papers were not missed, the reference lists of retrieved articles were screened for additional relevant studies. The researchers have searched and located the papers in which those most relevant studies have been cited (forward citation). Also, grey literature was searched on the World Health Organization (http://www.who.int/en), FDA (http://www.fda.gov/) and Health Canada (http://www.hc-sc.gc.ca/ahc-asc/pubs/index-eng.php) websites in order to identify relevant missed articles. The authors did not impose any restrictions on the time of publication or language, study design and publication status.

2.2. Study selection and eligibility criteria

Having removed duplicates, two authors independently screened titles and abstracts to ensure that articles met the inclusion criteria and irrelevant papers were excluded. Where uncertainty arose regarding the eligibility of an article from its abstract, the authors retrieved the full-text version of the article and evaluated it against the inclusion criteria. Also, discrepancies were resolved through consultation and consensus building. Finally, the full text of identified papers was deeply explored in order to be sure that only relevant papers were selected to be included in the review for quality assessment and data extraction.

2.3. Inclusion criteria

In our review, we included studies which met the following criteria: (a) original articles (b) all observational (i.e., cohort, case-control and cross-sectional) studies; (c) studies with assessment of pre- or post-natal exposure to phthalate esters (PEs) through a biomarker of exposure; (d) publications were only included if the outcome measured and reported in those studies was related to autism and not autistic-like disorders or other health outcomes; (e) Studies conducted on human subjects.

2.4. Exclusion criteria

Review articles, hypothesis papers, conference papers and letters to the editor which did not present unique or new data were excluded from this study. Publications of animal models were also excluded. The researchers also excluded articles that their outcomes were related to autistic-like but not ASD or other behavioral disorders.

Fig. 1 shows the process of selecting relevant papers for our systematic review based on the PRISMA flow diagram (Moher et al., 2009).

2.5. Quality assessment

The methodology of each eligible paper was assessed using a checklist based on Strengthening and Reporting of Observational Studies in Epidemiology (STROBE) statement to assess the methodological quality of the observational studies (Daneshparvar et al., 2016; Olmos et al., 2008; Ricci-Cabello et al., 2010; Scales et al., 2008). (Von Elm et al., 2008). This tool was initially developed to assess clarity in reporting research results of the observational studies. The STROBE tool uses a systematic approach to appraise three broad areas namely, study validity, an evaluation of methodological quality and presentation of results, and assessment of external validity.

Of 22 items listed in the checklist, 9 items that were related to the methods section were selected; in other words, these selected items can be used to assess the different aspects of methodology in an observational study (Appendix A). It should be mentioned that the authors equipped each question in the modified checklist with "Yes" or "No" answers and scored them with 1 and 0, respectively; therefore, the final score was in the range of 0-9. After performing the assessment, the methodological quality was

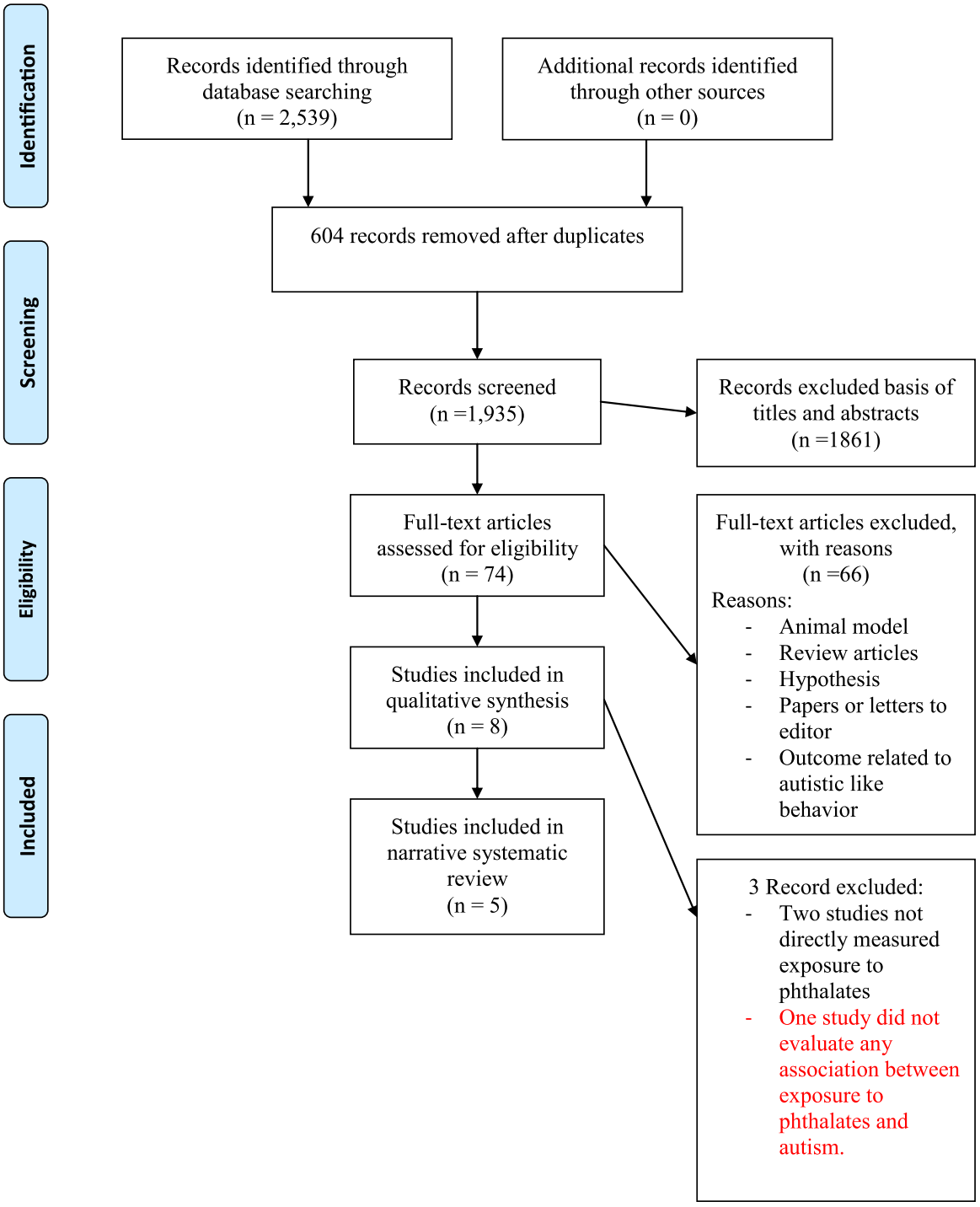

Fig. 1. PRISMA flow chart of publications examining estimated phthalate esters relation to autism spectrum disorder (ASD).

classified according to the following procedure: articles which had their final scores in the range of 0–3, 4–6, and 7–9 were respectively considered with low, medium and high methodological qualities.

2.6. Data extraction and abstraction

In addition, a pre-designed standard data collection form was used to systematically extract the data from each selected study. The required data to be extracted from each article was the general characteristics of the study (i. e. first author's name, year of publication, study location, study design, type of study and study period), as well as the characteristics of the study population (i. e. age and sex of studied participants, the sample size, type of exposure, exposure measurements, outcome scales, and effects studied). Two reviewers (MZJ) and (LJ) extracted data independently whilst another (MY) checked the extracted data from all eligible papers.

2.7. Statistical analysis

Study outcomes were summarized using narrative and quantitative methods. Because of the heterogeneity in type of the studies which were included, different methods of assessing

exposure to phthalates, different scales for detecting autism and also using different statistics for summarizing the results, meta-analysis could not be used to combine the results of included studies.

3. Result

3.1. Bibliographic search

A total of 2539 records have resulted from the combined database searches. After duplicates were removed, 1861 of them were excluded in the initial screening of manuscript titles and abstracts. Then, by screening the full texts of the remaining 74 articles according to the inclusion/exclusion criteria, seven studies were regarded as relevant to the objectives of this review. Five studies (three case-controls and two cohorts) (Braun et al., 2014; Kardas et al., 2015; Miodovnik et al., 2011; Stein et al., 2013; Testa et al., 2012) which were related to analyzing biochemical markers in association with the autism and phthalates exposure, were selected (Fig. 1). However, of those seven, two studies (Larsson et al., 2009a; Philippat et al., 2015) were excluded as they did not use bio-monitoring approach (direct measurement). One of them was a Swedish cohort study on association of ASD and type of flooring material as polyvinyl chloride (PVC) (Larsson et al., 2009b). Larsson et al. (2009a, 2009b) did not directly implicate the evaluation of possible link between phthalates exposure and autism. Thus, phthalate metabolites were not measured in biological samples of the patients. Indeed, assessment of the exposure to phthalate was based indirectly on the questionnaire data and eventually, they reported that ASD was significantly associated with PVC as flooring material (in the parent's bedroom). In the second investigation, Philippat et al. (2015) studied phthalate concentrations in the house dust in association with the risk of developing ASD or developmental delay (DD). Participants were a subset of children from the case–control study of CHARGE (Childhood Autism Risks from Genetics and the Environment). Similar to the pervious article, in this study phthalate metabolites were not measured in biological samples of children with ASD. Instead, Philippat et al., measured the concentration of five phthalate esters in the dust collected from the child's home using a high volume small surface sampler. This study reported that detection frequency of phthalates in the home dust was 63% for DMP, 92% for DEP and 99% for DEHP, DBP and BBzP. However, none of the dust phthalate concentrations was associated with the risk of ASD. In addition, they found no association of vinyl flooring with the diagnosis of ASD (Philippat et al., 2015). One of the possible explanations is that, vinyl flooring was found in 37% of residences in CHARGE study as compared to the Sweden cohort in which vinyl flooring has been reported in 52% of the children's bedrooms and 45% of the parents' bedrooms. However, due to inconsistency in method of autism and phthalate exposure measurement we had to eliminate this study.

It is to be note that, there was one article in the United States and Mexico involving 71 deciduous teeth, primarily molars and canines (without cavities or fillings), from children with ASD who were chosen from IRB-approved pilot studies on autism through the University of Texas Health Science Center (UTHSCSA) (Palmer et al., 2015). The mentioned article has not assessed any association between exposure to phthalate and ASD. Instead, this study objective was to evaluate the use of deciduous teeth as a new biological sample for measuring early life exposure to semi-volatile organic chemical metabolites such as monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), and mono-2-ethylhexyl phthalate (MEHP) in autistic children. In this report, detection rates of all phthalate monoester metabolites except MBzP (0–6%) were between 36–100% (Palmer et al., 2015). This report provided evidence that deciduous teeth can be used as a useful medium for measurement of early life exposure to organic contaminants biomarkers in epidemiological case-control studies. However, they did not provide any information regarding the relationship between early life exposure to these chemicals and ASD.

3.2. Narrative analysis

We reviewed various aspects of the included studies and presented the results narratively. As mentioned earlier, due to the large variation in the type of studies and methods used to assess exposure and outcome, we could not combine the results using Meta-analysis.

3.3. Overview of the type of study

Tables 1–5 show the characteristics of studies included in this review. Across the relevant studies, three were case-control studies (Kardas et al., 2015; Stein et al., 2013; Testa et al., 2012) and the other two were prospective cohort studies (Braun et al., 2014; Miodovnik et al., 2011).

In all of the included studies, phthalate exposure was mainly measured by the examining biomarkers in biological samples; for instance, HPLC electrospray ionization MS (HPLC-ESI-MS), and isotope dilution-liquid chromatography mass spectrometry–mass spectrometry (ID-LC-MSMS) were applied in order to measure the concentration of phthalate in urine and serum samples (Tables 1 and 2). In addition, autism characteristics in participants were assessed using different methods. As shown in Table 1, selected cohort studies (Braun et al., 2014; Miodovnik et al., 2011) used the Social Responsiveness Scale (SRS). The SRS is a well-validated tool for quantitative autism spectrum assessment particularly for social impairment identification while it is highly correlated with gold standard diagnostic instruments such as the autism diagnostic observation schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R) (Daniels et al., 2012). It should be noted that in these cohort studies, the mean change in SRS score was considered as an indicator of the severity of autistic behavior. However, in case-control studies (Kardas et al., 2015; Stein et al., 2013; Testa et al., 2012), ASD were assessed by gold standard tools including ADOS or ADI-R and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV TR) criteria(Table 2).

3.4. Quality assessment

Based on the quality assessment procedure of the five selected studies, using the mentioned criteria, the researchers found that only one study (Braun et al., 2014) illustrated high quality. The other four studies had medium quality in terms of predefined quality assessment criteria (Table 3).

3.5. Effects of quantified exposure to phthalate on autism

Miodovnik et al. (2011) conducted a multiethnic cohort study on 404 primiparous women who delivered at Mount Sinai Hospital between May 1998 and July 2002, with follow-ups to 2009. In this study, they focused on social behavior of the school-aged children with respect to the prenatal exposure to phthalates. Through linkage between maternal exposure to phthalates and children who were diagnosed with ASD in the New York City, at the last childhood evaluation, when the child was between the ages of 7 and 9 ($n = 137$), a survival analysis of the time to diagnosis in children was used to estimate the incidence of ASD according to the SRS completed by mothers. In this article, the maternal spot urine samples were analyzed by 10 phthalate's metabolites and

Table 1
Summary of cohort studies' characteristics and exposure-outcome assessment methodology.

Study	Location	Autism data source	Birth years	Sample size	Sex/age	Exposure measurement	Exposure timing	Outcome assessment
Miodovnik et al. (2011)	New York City, New York, USA	The Mount Sinai Children's Environmental Health Study	1998–2002	137	Gender not specified/4–9 years of age	Laboratory analyzed for 10 individual high and low molecular phthalate metabolites* in Maternal spot urine samples were collected during pregnancy	Mid-late pregnancy between 25 and 40 weeks (mean of 31.2 weeks)	Social Responsiveness Scale (SRS) when child was 7–9 years
Braun et al. (2014)	Cincinnati, Ohio metropolitan area, USA	The Health Outcomes and Measures of the Environment (HOME) Study	2003–2006	222	Gender not specified/4 and 5 years of age	8 Phthalate metabolites** measured in twice maternal urine samples with sensitive and specific gas chromatography mass spectrometry and creatinine standardized	Pregnancy around 16 and 26 weeks of gestation	Social Responsiveness Scale (SRS) at ages 4 and 5 years

*HMW phthalates=DEHP metabolites [MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; and MEHP, mono(2-ethylhexyl) phthalate]; MBzP, monobenzyl phthalate; and MCPP, mono(3-carboxypropyl) phthalate.
LMW phthalates=MMP, monomethyl phthalate; MEP, monoethyl phthalate; MBP, monobutyl phthalate; and MiBP, mono-isobutyl phthalate.

** MBP, mono-n-butyl-phthalate; MBzP, monobenzyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate.

Table 2
Summary of Case-Control studies' characteristics and exposure-outcome assessment methodology of studies included in the review.

Study	Location	Autism data source	Population	Sex (%)/age	Exposure measurement	Outcome assessment
Testa et al. (2012)	Siena, Italy	Staff of Children Neuropsychiatric Department	48 with autism 45 healthy controls	36boys, 12girls/age at examination: 11 ± 5 years 25 boys, 20 girls/age at examination: 12 ± 5 year	Urinary concentrations of the primary and secondary metabolites of DEHP [di-(2-ethylhexyl) phthalate] by HPLC-ESI-MS (HPLC electrospray ionization MS), was applied to urine spot sample.	All the patients with ASD, diagnosed by Diagnostic and Statistical Manual of mental disorders (DSM IV) and evaluated using ADOS (autism diagnostic observation schedule), ABC (autism behavior checklist) and CARS (childhood autism rating scale) scores entered the study.
Stein et al. (2013)	USA	Pediatric Neurology and Pediatrics clinical practices at University of Medicine and Dentistry of New Jersey (UMDNJ), New Jersey Medical School	50 children with autism 53 healthy controls	76% boys/age at examination: 10.26 ± 3.83 55% girls/10.74 ± 4.03	The concentration of free phthalates and total phthalates in the collected spot urine samples between 10:00 a.m. and 4:00 p.m. was measured by isotope dilution-liquid chromatography mass spectrometry–mass spectrometry (ID-LC-MSMS) using minor modifications.	All autistic subjects were under the care of the pediatric neurologist and the diagnoses were made by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV TR); 52% of the subjects were further confirmed by Autism Diagnostic Interview-Revised, and/or Autism Diagnostic Observation Scale-Generic criteria.
Kardas et al. (2015)	Kayseri, Turkey	Children's Hospital of Erciyes University Medical School (Kayseri, Turkey) between May, 2012 and May, 2013.	48 children with autism 41 healthy subjects	27 boys, 21 girls/age at examination: 7.54+2.92 24 boys, 17 girls/age at examination: 7.47+2.79	MEHP and DEHP concentrations were determined by using high performance liquid chromatography in serum sample.	The diagnosis of autism was made according to the criteria of the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and the Autism Behavior Checklist.

Table 3
Outcome and quality assessment of studies, limitations and benefits of articles.

References	Results	Strengths of study	Limitations of study	Quality
Testa et al. (2012)	Indicate that specific DEHP metabolites are statistically significantly increased in autistic children.	– Patients with Rett syndrome, X- fragile syndrome, inborn errors of metabolism, 21 trisomy, tuberous sclerosis and gene microdeletions were excluded from the present study. – Urinary creatinine was measured in the children mg/kg per 24 h) and data unadjusted for creatinine.	– Single spot urine specimens were collected in the morning. – Medical history of patient did not screen. – Important perinatal and neonatal potential confounders being appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest has not been investigated. – Genetic data on subjects or their parents has not been collected.	Medium
Stein et al. (2013)	There are an association between phthalate metabolism and autism. So that, the degree of glucuronidation was lower with the autistic group. Although this study shows a compromised phthalate metabolite glucuronidation pathway, this does not necessarily mean that phthalates are directly linked to ASD.	– Medical history and comorbidity data were collected in the autistic subjects. – All subjects were carefully screened for signs of infection or inter-current illness on the day of specimen acquisition, and subjects with acute illness were excluded. – The dietary intake history within 24 h of sampling was recorded, including that of medication and vitamin intake. – Urinary concentration data are frequently normalized to, or controlled for creatinine to control for differences in urine dilution. – Multiple regression analyses were conducted in which, sex, age, BMI, and creatinine were entered simultaneously as covariates with the metabolite indices	– This study included ASD children with or without comorbidity. – Single spot urine specimens were collected – Time of urine sample collection was wide (between 10:00 a.m. and 4:00 p.m). – Genetic data on subjects or their parents has not been collected. – Important perinatal and neonatal potential confounders being appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest has not been investigated.	Medium
Kardas et al. (2015)	In this study, serum MEHP and DEHP levels were found to be significantly higher in the autism spectrum disorder group when compared to healthy controls ($P = 0.000$).	– Patients with pervasive developmental disorder-not otherwise specified, Asperger's syndrome, Rett syndrome, or Childhood disintegrative disorders were excluded. – Patients were excluded if they had genetic disorders, including chromosomal abnormalities, fragile X syndrome, tuberous sclerosis, and neurofibromatosis type 1. – Subjects with acute infection were excluded. – Plastic products have not been used during blood sampling. – Relationship between variables include age, gender, residence and duration of breast feeding with phthalates have been evaluated in both groups.	– Single spot serum specimens were collected, while the time and condition of sampling was not mentioned. – Secondary metabolites did not measured in other biological sample. – Genetic data on subjects or their parents has not been collected. – Medical history of patient did not screen.	Medium
Miodovnik et al. (2011)	maternal urinary MEP concentrations during pregnancy were associated with higher SRS scores in 7- to 9-year-old children	– Urinary concentrations of the biomarkers were examined both as micrograms per liter and corrected for urine dilution as micrograms per gram creatinine (mg/gC). – The following were considered as potential confounders or covariates[a].	– Outcome assessment did not rely on the clinical diagnosis of autism but only on symptoms common to the disorder. – Single spot urine specimens were collected. – Mode of measurement of urinary concentration of phthalates has not been mentioned.	Medium
Braun et al. (2014)	A part of monoethyl phthalate (MEP) other biomarkers of phthalates exposure during pregnancy were not clearly associated with autistic behaviors in 4- and 5-year-old children in this cohort.	– The total (free plus conjugated) urinary concentrations of eight phthalate metabo- lites and bisphenol A (BPA) were creatinine-normalized in units of micrograms per gram creatinine to account for urine dilution. – The following potential confounding variables were adjusted[b]. – Depressive symptoms during the second trimester were measured with the Beck Depression Inventory-II.	– Outcome assessment did not rely on the clinical diagnosis of autism but only on symptoms common to the disorder.	High

[a] Maternal age (continuous variable), maternal IQ, marital status at the time of follow-up (single caretaker versus living with both parents), maternal education (less than high school versus more than a high school), child race (non-Hispanic white, non-Hispanic black, or Hispanic), sex, child IQ, exact age at examination, and urinary creatinine.

[b] Maternal demographic and prenatal factors, including maternal age at delivery, race, marital status, education, parity, insurance status, employment, household income, and prenatal vitamin use were obtained using structured interviews and chart reviews conducted by trained research staff.

Table 4
Median maternal urinary concentration of phthalate metabolites in cohort studies and unadjusted and covariate adjusted associations (β [95% CI]) between SRS Total score in children and maternal gestational urinary phthalate concentrations.

Study		Median (Interquartile range)[a]	Unadjusted β [95% CI]	Adjusted model β [95% CI][b]
Miodovnik et al. (2011)	**MEP**	380 (137–1010)	–	1.38 (0.23, 2.53)
	MBP	36 (16–75)	–	1.37 (−0.43, 3.17)
		Median (95% median CI)[c]	**Unadjusted β [95% CI]**	**Adjusted model β [95% CI][d]**
Braun et al. (2014)	**MEP**	133 (25–1010)	1.3 (−1.1, 3.6)	−0.9 (−2.7, 1.0)
	MBP	26 (9.5–75)	0.8 (−1.7, 3.3)	−0.4 (−2.3, 1.5)

MBP-Mono-n-butyl phthalate, MEP-Monoethyl phthalate, μg/g creatinine.

[a] μg/ml.

[b] Adjusted for child race, sex, caretaker marital status and urinary creatinine.

[c] μg/g creatinine.

[d] Adjusted for maternal age (continuous, years), parity (0, 1–2, and 3+), prenatal vitamin use (daily, 1–6 times/week, and never or rarely), maternal IQ (continuous), depressive symptoms during pregnancy (continuous), HOME score (continuous), and gestational serum cotinine concentration (continuous log10 transformed).

Table 5
Comparisons of urinary excretion of secondary metabolites for DEHP in autistic and control groups in case-control studies.

Study	Size		Scale	Effect Median (95% median CI) in μg/ml[a]		P-value
	With autism	**Control**		**With autism**	**Control**	
Testa et al. (2012)			5-OH-MEHP	0.18 (0.037–0.399)	0.04 (0–0.124)	0.0224
	48	45	5-Oxo-MEHP	0.096 (0.04–0.17)	0.04 (0.015–0.079)	0.005
			MEHP	0.055 (0–0.11)	0.028 (0–0.059)	0.0312
	With autism	**Control**		**Effect** Median (25th, 75th) in μg/ml[a]		
Stein et al. (2013)	50	53	5-OH-MEHP	0.005 (0.002–0.014)	0.008 (0.004–0.012)	0.12
			5-Oxo-MEHP	0.003(0.001–0.006)	0.004 (0.002–0.006)	0.21
			MEHP	0.011 (0.009–0.014)	0.013 (0.009–0.018)	0.06
Kardas et al. (2015)			**Scale**	**Effect** Mean+SD in μg/ml		
	Non autism	**Control**				
	48	**41**	MEHP	0.47+0.14	0.29+0.05	.000
			DEHP	2.70+0.90	1.62+0.56	.000

MEHP monoethylhexylphthalate, 5-OH-MEHP 5-hydroxy-methylethylhexyl phthalate, 5-oxo MEHP 5-oxo-methylethylhexyl phthalate.

[a] Mann–Whitney *U* Tests.

expressed as sum of the high-molecular-weight (>250 Da), namely, monoester metabolites (∑HMW) and low molecular-weight (<250 Da) known as monoester metabolites (∑LMW). The reason for this type of classification is that the phthalate metabolites within each grouping demonstrate similar molecular structure, biological activity and sources of exposure as the parent diester. Metabolites and methods of detection which have been used in the studies are also shown in Tables 1 and 3. Compared with the original birth cohort (n=404), the median urinary concentrations of the low and high molecular phthalates metabolites were similar in the women who returned for follow-ups (n=137). Accordingly, there were no significant differences with respect to median urine concentrations of phthalate metabolites between the original birth cohort and those at 7–9 year follow-ups. In addition, among the 137 children in follow-up stage of the cohort study, based on the SRS score, 106 children were in the normal range of social impairment, 25 children had mild social impairment and the other six children had scores higher than 75 which were strongly associated with a clinical diagnosis of ASD. Regarding the prenatal LMW phthalate urine biomarkers in relation to SRS T-scores at age 7–9 years, in thirty-one (22.6%) children who met the threshold level of "Mild to Moderate" (SRS T-score of 60–74) and "Severe" (SRS T-score ≥75) social impairment in the studied population, the LMW phthalate metabolite concentrations were 460 μg/L (n=25) and 1260 μg/L (n=6), respectively. It implied that relative to the healthy children with normal SRS T-score, children with mild and severe social impairment had highest concentrations of phthalates. In general, the scores of few children exceeded the cut-off value which identifies children with clinically significant social impairments. On one hand, any increase in the log-unit of LMW phthalate metabolite concentration was associated with higher SRS scores; and among the investigated LMW phthalate metabolites, only MEP was found to be statistically significant (Table 4). On the other hand, no consistent association between SRS scores and HMW phthalate metabolites was found. Furthermore, although a positive association between ASD and the LMW phthalate level was observed, statistical significant difference was not found (LMW phthalates, $p=0.09$; HMW phthalates, $p=0.54$).

The second study in which the phthalate exposure and its adverse birth outcomes were measured was conducted from March 2003 to January 2006 (Braun et al., 2014). In the prospective birth cohort study on mothers and their children participating in the Health Outcomes and Measures of the Environment (HOME), a study from Cincinnati Ohaio was aimed at assessing the association between low-level environmental chemical exposure and children's growth and development. Two spot urine samples were taken from each pregnant woman (n=389) who were between 16

and 26 weeks of their pregnancy (Table 1). The following 222 mother–child pairs (57%) completed the SRS when their children were 4 (n=184), 5 (n =205) years of age and at both 4 and 5 years of age (n =135). Of the entire 135 participants, the scores of only 22 children were higher than 60 (SRS score ≥60) and the rest were in the normal range. They also measured the concentrations of eight urinary phthalate metabolites expressed in μg/g creatinine which were similar among women with and without follow-ups when their children were 4 or 5 years old (Table 4). It should be mentioned that all the phthalate metabolites were associated with the autistic symptom scores.

In three case-control studies performed by Testa et al. (2012), Stein et al. (2013), and Kardas et al. (2015) the association of phthalates exposure to ASD were also investigated. In the study conducted by Testa et al. (2012) on an Italian sample, it was tried to evaluate the levels of the primary and secondary metabolites of DEHP in children with ASD by using innovative chemically reversed approach (Testa et al., 2012). This small nested case control study was conducted on 48 children with ASD and 45 children without ASD as the control group. As shown in Table 2, all the scores regarding 48 patients with ASD who were diagnosed by DSM-IV ( ) and evaluated using ADOS and CARS (Childhood Autism Rating Scale) were captured in the study. Healthy controls (HCs) were randomly chosen from outpatients who had no pathological symptoms. Determination method of urinary concentrations of DEHP metabolites (MEHP and 6-OH-MEHP [mono-(2-ethyl-6-hydroxyhexyl) 1,2-benzenedicarboxylate], 5-oxo-MEHP [mono-(2-ethyl-5-oxohexyl) 1,2-benzenedicarboxylate] and 5-OH-MEHP [mono-(2-ethyl-5-hydroxyhexyl) 1,2-benzenedicarboxylate]) is shown in Table 2. Table 5 shows results of the mentioned study. As shown in this table, urinary excretion of 5-oxo-MEHP ($P=0.005$), 5-OH-MEHP ($P=0.0224$) and MEHP ($P=0.0312$) was significantly higher in autistic patients, compared with the gender- and age-comparable HCs. This study demonstrated a strong association between phthalates exposure and risk of ASD.

The other case-control study was aimed at comparing the efficiency of conjugation reactions of DEHP metabolites as a detoxification mechanism in a group of children with documented ASD against healthy children as a control group (Stein et al., 2013). In this study, random spot urine specimens were collected from 50 children with ASD and 53 age-matched HCs between 10:00 a.m. and 4:00 p.m. As shown in Table 2, all ASD subjects were under the care of the pediatric neurologist and the diagnosis was made by the DSM-IV TR. In this regard, 52% of the subjects were further confirmed by ADI-R, and/or ADOS. Control children were screened for medical and developmental disorders during their well-child visits in addition to chart review, and only those which were free of any chronic or recurrent medical disorders were considered healthy and included in the study. The metabolites measured were mono-2- ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5-oxo MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (5-OH MEHP) and mono-(2-ethyl-5-carboxypentyl phthalate (5-CX MEPP). Based on the results of this study, total MEHP, total 5-OH MEHP and total 5-oxo MEHP in ASD group were lower than those of the control group (Table 5); and, total 5-cx MEPP in ASD children were higher than those in the control group. However, there was no significant difference in urinary concentration of 5-cx MEPP between autistic group and control group. As 5-cx MEPP accounted for more than 90% of the total metabolites of DEHP, therefore, sum of the total amount of DEHP metabolites (free plus conjugated) was similar between case and control groups. In other words, although this study shows a compromised phthalate metabolite glucuronidation pathway, this does not necessarily mean that phthalates are directly linked to ASD. Perhaps, a decreased capacity for glucuronidation in ASD children caused a little higher amount of total free phthalates metabolites in their urine than healthy children (Alabdali et al., 2014; Stein et al., 2013).

The Third case control study was conducted by Kardas et al. (2015) on 48 children with ASD without comorbidity and 41 healthy subjects as controls in Turkey between May, 2012 and May, 2013 (Table 2). In this study, serum MEHP and DEHP levels were found to be significantly higher in the ASD group when compared to healthy controls (P =.000). No significant relationship was detected between gender, residence and duration of breast feeding and MEHP and DEHP within each group (Kardas et al., 2015).

All of these research studies have a number of methodological strengths and limitations regarding outcome-exposure assessment and confounding factors analysis which are summarized in Table 3.

4. Discussion

Over the last three decades, global concern about the public health risk factors attributed to the environmental pollution has been increasing. It can be said that like an iceberg which has only one-tenth of its volume visible, of the thousands of chemicals currently being used, only a small fraction has been identified as neurotoxicants for the human developmental processes. Moreover, many of these chemicals are likely to have stronger effects on fetuses and children, compared with adults. In fact, this visible part, which is the result of previously conducted studies in this field, may only be a small part of a bigger potential problem (Grandjean and Landrigan, 2006).

Although, several researches have been conducted to assess the effects of genetic, environmental and immunological factors on the development of ASD, there is still much to be done regarding the understanding of the ASD etiology (i. e. a phenomenon termed "etiological heterogeneity") (Kalkbrenner et al., 2014). Extensive research in rodent models has shown that phthalates primarily act as anti-androgens which impair testosterone production in Leydig cells (De_falco et al., 2015; Foster, 2005). Furthermore, it is proposed that phthalates may disrupt the endocrine system by interfering with thyroid homeostasis through various mechanisms, including alteration of transcriptional activity of the sodium/iodine symporter, inhibition of the binding of triiodothyronine (T3) to purified thyroid receptors, and inhibition of T3-induced cell proliferation (Boas et al., 2012; Ghisari and Bonefeld-Jorgensen, 2009). Likewise, involvement of phthalates in neurodevelopmental disorders is supported by animal studies that indicate the role of phthalate induced hypothyroidism in decreased intellectual capacity and development of ASD (Miodovnik et al., 2014b).

In this review, the researchers have brought together the existing body of evidence regarding the effects of phthalate exposures on ASD. Findings from this study may highlight the fact that so far, a limited number of studies attempted to assess the phthalate exposure during pregnancy and childhood as an ASD risk factor. Among the five retrieved studies on human subjects from three different countries, three were case-control, while the other two studies were cohort.

Cohort studies are very important as they show the full impact of gestational exposure to phthalates by measuring the maternal urinary concentrations of phthalate metabolites. During this critical period, even low doses of EDCs which may have little effect on adults can have devastating effects on the unborn, neonate and the child. Many substances easily penetrate the placenta during prenatal development and because the fetal blood-brain barrier is not fully formed, toxicants can enter this vital organ and interfere with its development. This can occur through direct toxicity or interference with a variety of cell-signaling mechanisms, including the endocrine system (Colborn, 2004).

In the both cohort studies, among the phthalate metabolites, high concentrations of MEP were found in all patients. However, Braun et al. (2014) reported that gestational MEP concentrations in either adjusted or unadjusted models for co-pollutant confounding had an inverse association with SRS scores (Table 3). The study showed that the beta coefficients for prenatal urine MEP, in relation to total SRS scores was −0.5 (95% confidence intervals −2.2, 1.3) in fully adjusted model as compared with the displayed betas which changed in SRS scores among children born to women with detectable vs. non-detectable levels of these chemicals [β= −0.2 (95% confidence intervals −1.9, 1.5)]. In contrast, as shown in Table 3, in the first prospective birth cohort of 137 mothers and their children, Miodovnik et al. (2011) reported that maternal urinary MEP concentrations during pregnancy were positively associated ($p < 0.05$) with severe social impairments (SRS ≥75) in 7 to 9 year-old children in adjusted model for race, sex, caretaker material status and urinary creatinine of the child [β=1.38 (0.23–2.53)]. Since in the investigated studies different target groups and confounding variables were presented to the model and different indices were reported as the central tendency and dispersion of results (i.e. median with quartiles and mean with confidence intervals), there was a high level of heterogeneity in the findings of the included studies and that prevented us from combining the results of these studies.

In addition to the hereditary factors, one of the most important points that should be taken into consideration is to avoid under or over estimation of the association between the exposure and neurodevelopment endpoints (Polańska et al., 2013). In the field of ASD particularly, the important risk factors reported by previously conducted studies were pregnancy and delivery complications, low birth weight, too small for gestational age, duration of maternal bleeding during pregnancy, maternal depression, umbilical-cord complications, excess gestational weight gain, maternal prenatal medication (psychiatric medication), being first born versus third or later (birth order), low 5-minute Apgar score, feeding difficulties, meconium aspiration, neonatal anemia, ABO or Rh incompatibility, hyper bilirubinemia and maternal diabetes before and during pregnancy (Gardener et al., 2009; Gardener et al., 2011; Guinchat et al., 2012; Lyall et al., 2014; Xu et al., 2014).

Moreover, it is well known that the prenatal period risk factors, including maternal and paternal ages, demographic variables, geographic coordinates, the socio-economic status, physical activity, smoking status (Smoking during pregnancy and second-hand exposure to smoke) and nutrient deficiencies, have also been reported to be potential risk factors for psychopathology and behavioral problems of the offspring (Dietert et al., 2011; Kolevzon et al., 2007; Sandin et al., 2012). It should also be taken into consideration that mothers' inadequate knowledge about environmental/lifestyle contaminants and greater exposure to environmental contaminants may be associated with ASD (Kim et al., 2010). However, in retrieved studies, only the prenatal risk factors were mentioned among the pre-, peri- and neonatal risk factors. In other words, the perinatal and neonatal risk factors have been ignored.

Since the critical period for the development of a human brain is believed to be between fourth week of pregnancy and the time of delivery, and considering that the sensitivity to damage extends up to early childhood (Schug et al., 2015), exposure to chemicals during critical windows of development can lead to neurodevelopmental diseases. However, all the windows are not equal. Regarding the two cohort studies, Braun et al. (2014) measured phthalates exposure two times at the second trimester, (Weeks 16 and 26), but Miodovnik et al. (2011) evaluated exposure to phthalates during the second and third trimesters of pregnancy (mean of 31.2 Weeks). Although, available evidence from neuroanatomical, animal and epidemiological studies show the prenatal and early postnatal origins of ASD, but the accurate critical windows of susceptibility to neurotoxicant chemicals for ASD have not been fully elucidated. Therefore, in order to provide a better insight for the specific effects of maternal phthalates exposure and to identify critical periods of exposure, it is of interest to assess the exposure to phthalates at several time points during and after pregnancy than only one time point exposure measurement.

In the cohort studies, the way of assessing the exposure to phthalates was based on the measurement of maternal urinary biomarkers. However, in the case control studies, the exposure measurement was based on comparison of the phthalate metabolite concentrations in the urine of ASD children with the control group. Regarding the evidence provided and methods of measuring the phthalates exposure, it can be argued that the case-control studies failed to obtain exact data on maternal exposures in pregnancy period.

Among the three case-control studies, Testa et al. (2011) reported that urinary concentrations of two DEHP metabolites (5-OHMEHP and 5-oxo-MEHP) in the ASD group were significantly higher than control group. Consistent with this study, Kardas et al. (2015) reported that serum MEHP and DEHP levels were significantly higher in the ASD group as compared to controls (Kardas et al., 2015). In contrast to previous case control studies, Stein et al. (2013) did not show any association between phthalates and ASD. However, they reported that despite similar phthalate exposure levels, ASD children had decreased glucuronidation of DEHP (i. e. measured by urinary metabolites) in comparison to the control group, Glucuronidation is notably a significant pathway involved in the metabolism of xenobiotics and lower glucuronidation might lead to a decreased detoxification capacity for phthalates (Stein et al., 2013). Generally, case-control and cross-sectional studies cannot determine the causal relationship regarding the issue at hand, because the temporal relationship between disease occurrence and exposure cannot be established (Song and Chung, 2010). In addition, a single case-control study in which a single biomarker is measured is not appropriate. In other words, biomarkers have usually short half-life and there is a long period between exposure to phthalates and its outcomes.

Since phthalates, as non-persistent compounds, have short biological half-lives, phthalate metabolites only represent exposure for a few days instead of the entire relevant period of development (Kalkbrenner et al., 2014). Therefore, a single urinary measure of biomarkers for phthalates does not suffice, and, it may not adequately reflect long-term exposure level to these chemicals. It is believed that in the case of phthalates, since ongoing exposure to these chemicals is expected, urinary phthalate metabolites appear to be stable over a period of days to months (Miodovnik et al., 2014b). However, a single measurement during pregnancy, delivery or childhood period may not be sufficient in determining the window at which the exposure occurred. In the evaluated studies, two-time urine sample measurements from pregnant women were taken only in one perspective cohort study (Braun et al., 2014).

Several concerns and limitations have emerged from this topic. First, the issue is related to the small number of studies with similar health endpoints. In addition to the paucity of evidence, the findings were inconsistent which this inconsistency could be explained by differences in the collection time of urine samples, instruments of measurement and health endpoints across the studies. The potential for misclassification of the exposure due to the short biological half-life of phthalates in humans is the next point. It is believed that single urinary concentrations can only reflect the recent exposures over the past 6–12 h. Therefore, a single spot urine sample cannot accurately classify long-term exposure (over weeks, months or years), since data shows that exposures are often episodic and vary over time (Braun et al., 2010).

In addition, many studies only assessed phthalate exposures during specific periods of time (e. g. during gestation (in cohort studies) or early childhood (in case-control studies)). Since there are several critical time periods during development, the precise timing at which the exposure to a toxicant is most disruptive is unclear. It is also of concern that the timing of spot urine collection was different in these studies. Therefore, this difference may account for the inconsistent results.

Finally, information from extensive body of literature on the association between phthalate exposure in prenatal and postnatal period and ASD is limited. Thus, the researchers could not apply meta-analysis as well as graphical or statistical methods to assess any publication- related biases. Therefore, there is need to conduct large, well-designed prospective cohort studies and in doing so, the relevant pre-, peri- and neonatal confounders and characterization of the exposure should be taken into consideration.

5. Conclusions

ASD is a serious neuro-developmental disorder with heterogeneity in the behavioral symptoms and it affects the functions and structures of the brain (Ratajczak, 2011). It is clear that there is no single or universal cause of ASD; rather, many environmental and genetic factors are likely to be involved. Although, over the last decade, potential contributions of environmental chemicals and conditions to the etiology of ASD have been the subject of current researches and speculations, the field is still at its early stages. Till now, a few studies support a potential role for phthalate exposures in relation to ASD. Considering the insufficiency of the number of identified studies and the heterogeneous methods used in these studies, it is difficult to provide a definite conclusion. In this review, the authors have provided a useful summary of existing research findings. This review reveals evidence showing a connection between exposure to phthalates and ASD. Nevertheless, results of the retrieved studies confirm the shortage of knowledge in this important area and confirm that the major limitations of the existing studies are related to both the exposure and outcome assessments. Therefore, the association between exposure to phthalates and ASD requires further well-designed pregnancy cohort studies in order to aid understanding and validation of the findings from population-based studies.

Conflict of interest

The authors declare that they have no actual or potential competing financial interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2016.08.021.

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