**A systematic review: Impact of endocrine disrupting chemicals exposure on fecundity as measured by time to pregnancy**

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**Keywords**

Infertility, fecundity, time to pregnancy,EDCs,brominated flame retardants, POPs, hexabromocyclododecane, human, polybrominated diphenyl ethers, phthalates, and organophosphate flame retardants

**Abbreviations**

EDCs (environmental disrupting chemicals), BFR (brominated flame retardant), HBCD (hexabromocyclododecane), PBDE (polybrominated diphenyl ether), TBBPA (tetrabromobiphenol A), POPs (persistent organic pollutants), OPFRs (Organophosphates Flame Retardants), phthalates: MnBP (Mono-n-butyl phthalate), MBzP (Mono-ethyl phthalate), MMP (Mono-methyl phthalate), MEP (Mono-ethyl phthalate), MCHP (Mono-cyclo-hexyl phthalate), MiNP (Mono-isononyl phthalate), MCP (Mono-(3-carboxypropyl) phthalate), MEHP (Mono-(2-ethylhexyl) phthalate), MEOHP (Mono-(2-ethyl-5-oxo-hexyl) phthalate), IVF (in vitro fertilization), DE (Deiphenyl Ethers), PRISMA (the Preferred Reporting Items for Systematic Reviews and Meta-Analyses), OR (Odd Ratios), NOS (Newcastle-Ottawa Quality Assessment Scale), WCRF (World Cancer Research Fund), BMI (Body Mass Index), FR (Fecundity ratios), CI (Confidence Interval), SD (Standard Deviation)

 **Abstract**

**Background:** Emerging scientific evidence suggests that exposure to environmental pollutants is associated with negative effects on fecundity as measured by time to pregnancy (TTP).

**Objectives:** To conduct a systematic review of the literature on the association between selected endocrine disrupting chemicals (EDCs), and fecundity as measured by TTP in humans. Compounds included in this review are: brominated flame retardants (BFRs) such as hexabromocyclododecane, tetrabromobiphenol A and polybrominated diphenyl ethers; organophosphates flame retardants (OPFRs); and phthalates.

**Methods:** Scopus, MEDLINE via Ebscohost and EMBASE databases were searched for articles exploring the relationships between selected EDCs and fecundity as measured by time to pregnancy. We assessed the quality of included studies and evidence for causality was graded using the criteria developed by the World Cancer Research Fund.

**Results:** 14 studies of 191 full-text articles assessed for eligibility were included for qualitative synthesis. Five studies examined BFRs and 10 studies examined phthalates. Among the fourteen, one study assessed both BFRs and phthalates. There were no studies which investigated fecundity as measured by TTP on HBCD, TBBPA, or OPFRs. We recorded plausible fecundity outcomes as measured by TTP related to some of these EDCs. BFRs or phthalates increased TTP. However, results were inconsistent.

**Conclusion:** We recorded mostly weak associations between exposure to selected EDCs and fecundity. However, evidence was considered limited to conclude a causal relationship due to inconsistency of results. The health risks posed by these chemicals in exposed populations are only beginning to be recognised and prospective measurement of the environmental effects of the chemicals in large cohort studies are urgently needed to confirm these relationships and inform policies aimed at exposure prevention.

**1. Introduction**

Endocrine disrupting chemicals (EDCs) are chemicals that can interfere with normal endocrine systems or hormones. EDCs can lead to the interruption of normal hormone activity, especially in the endocrine system, and the normal signal then does not work properly (Johnston 2016). EDCs constitute a vast array of chemicals commonly found in our homes and food, and they are substantial contributors to global burden of disease (Attina et al., 2016). These chemicals enter the environment and human food chain through emission during manufacture or processing, volatilization and/ or leaching from applications and/ or through disposal, and so become available for human uptake (Gore et al., 2015). EDCs to be targeted in this study include: brominated flame retardants (BFRs)- polybrominated diphenyl ether (PBDEs); [hexabromocyclododecane](https://en.wikipedia.org/wiki/Hexabromocyclododecane) (HBCD); tetrabromobiphenol A (TBBPA), because they have been widely usedin industrial and consumer products, including electronic products, building materials and textiles and emerging chemicals, such as phthalates, and organophosphate flame retardants (OPFRs) (Table 1). In numerous countries including Australia, biomonitoring programs have demonstrated that these chemicals are detectable in the human population. For some chemicals, concentrations in Australia are higher than those in Europe where more stringent control measures have been implemented (Harden et al., 2007; Toms et al., 2008; Toms et al., 2009).

Infertility is a worldwide public health issue, with 9% of the global population seeking costly fertility care, such as in vitro fertilization (IVF) (WHO 2016). Exposure to environmental chemicals such as the selected EDCs have been suggested as a possible cause of infertility because of the harmful impact on reproductive and endocrine systems (Caserta et al., 2008). Fecundity is the ability to reproduce offspring. Time to pregnancy (TTP), which refers to the number of menstrual cycles required to conceive, is a good method to estimate fecundity or the probability that a pregnancy will occur during a menstrual cycle (Baird et al., 1986).

Animal and human studies have shown that the selected EDCs may interfere with the body’s endocrine system, producing adverse developmental, reproductive, neurological, and immune effects (Jin et al., 2010; Romani et al., 2014; Berger et al., 2014; Gore et al., 2015; Bhaskar et al., 2017; Pradhan, Olsson & Jass 2018). Many studies have previously examined adverse reproductive disorders and exposure to the selected EDCs (Kim et al., 2014; Bach et al., 2015). For example, Di(2-ethylhexyl) phthalate (DEHP) and diethyl phthalate (DEP) disrupt fecundity (reduced fecundity) in Caenorhabditis elegans (Pradhan, Olsson & Jass 2018). Yet, the relationship between fecundity measured by TTP is not well understood.

**Table 1. Overview of the chemical names and associated abbreviations discussed in this systematic review**

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| **Chemical name** | **Chemical abbreviation** |
| **Brominated Flame Retardants (BFRs)** |
| Polybrominated diphenyl ethers | PBDE\* |
| Hexabromocyclododecane | HBCD |
| Tetrabromobiphenol A | TBBPA |
| **Phthalates** |
| Mono-n-butyl phthalate (MnBP) : | MnBP |
| Mono-ethyl phthalate | MBzP |
| Mono-methyl phthalate | MMP |
| Mono-ethyl phthalate | MEP |
| Mono-cyclo-hexyl phthalate | MCHP |
| Mono-isononyl phthalate | MiNP |
| Mono-(3-carboxypropyl) phthalate | MCP |
| Mono-(2-ethylhexyl) phthalate | MEHP |
| Mono-(2-ethyl-5-oxo-hexyl) phthalate | MEOHP |
| **Organophosphate Flame Retardants (OPFRs)** |
| Bis(1-chloro-2-propoyl) phosphate  | BCIPP |
| Bis(1,3-dichloro-2-propyl) phosphate | BDCIPP |
| Diphenyl phosphate  | DPHP |
| Isopropylphenyl phenyl phosphate | Ip-PPP |
| Tert-butylphenyl phenyl phosphate | Tb-PPP |
| Tris(1, 3-dichloro-2-propyl) phosphate  | TDCPP |
| tiriphenyl phosphate | TPP |

\*there are 209 PBDE congeners with common congeners BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-209

The aim of the current study is to conduct a systematic review, and provide a definitive summary of current epidemiological evidence of an association between human fecundity as measured by TTP and exposure to selected, BFRs, OPFRs, and phthalates.

**2. Method**

The review was carried out following general recommendations from The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), an evidence-based minimum set of items for processing and reporting of results in systematic reviews and meta-analyses (Moher et al., 2009). Evidence for causality, in the relationship between exposure to EDCs and fecundity and TTP, was evaluated within the Bradford-Hill framework (Hill 1965). The data extraction form included a quality assessment tool to rate the methodological quality of each study, adapted from the Newcastle-Ottawa scale (NOS) for observational studies (Wells et al., accessed on May 2018).

YR, NW and LT completed quality assessment independently, and disagreements were resolved by discussion. The total quality score for each study was obtained from the sum of the quality scores for individual assessment items. The total quality score was converted to a proportional quality score by dividing by the maximum possible score (the total quality score divided by the maximum score possible) (S1 Quality assessment). We used the criteria for grading evidence developed by the World Cancer Research Fund (WCRF) (WCRF 1997) as a guideline. The WCRF criteria are included in the Supplementary S2. Insufficient studies of the same exposures for the same classes of chemicals and differences in study designs made it difficult to combine studies into a meta-analysis.

**2.1. Inclusion and exclusion criteria**

This systematic review incorporated epidemiological studies exploring exposure to EDCs (BFRs, PBDEs, HBCD, TBBPA, OPFRs, and phthalates) with fecundity or TTP. Retrospective and prospective cohort studies, cross-sectional and case-control studies meeting the following inclusion criteria: (1) reported original, empirical research published in a peer reviewed journal; (2) considered exposure to selected EDCs as potential risk factors for male and female fecundity; ‘time to pregnancy’ was used as a sensitive marker of fecundability (the probability of pregnancy during a single menstrual cycle) (Rachootin 1982); (3) reported effect size or convertible (such as odds ratio, risk ratio, hazard ratio or beta coefficient); and (4) examined in human.

**2.2. Search strategy description**

The electronic databases Scopus, MEDLINE via Ebscohost and EMBASE were searched for all studies published until 1st June 2018. The search strategy was developed in consultation with a research librarian and the following keywords were included: endocrine disrupting chemicals, infertility, fecundity, time to pregnancy, persistent organic pollutants, brominated flame retardants, hexabromocyclododecane, human, polybrominated diphenyl ethers, and tetrabromobisphenol A, organophosphate flame retardants, and phthalates. The synonyms of chemicals were also included in the search (S3 review protocol). A full list of search terms used can be found in the Supplementary Information. The reference lists of included articles were also screened by titles and abstracts for other relevant articles. The search was not restricted to the English language.

**2.3. Data collection**

Initial search results were collected. An additional 5 studies were identified through screening reference lists. The remaining records and those identified through screening reference lists were then screened for inclusion and exclusion criteria by titles and abstracts after duplicates were removed. The full-text article of any study that appeared to meet the inclusion criteria was retrieved for closer examination. Three reviewers (YR, NW and LT) independently assessed identical sets of articles for eligibility (see S3 review protocol). Disagreements were resolved by consensus (YR, NW, LT, and RP). A standardized data extraction form was developed and data retrieved included: publication details (year of publication, and country where study was conducted), methodological characteristics such as sample size and study design, exposure and outcome measures, type of EDCs and chemical analysis, gender and age of study population, matrix used (blood, follicular fluid, semen samples, air or job exposure assessment), health outcome assessment and effect size with standard errors or 95% confidence intervals. Study authors were contacted when additional information was required. Risk of bias of included studies was assessed by focusing on a set of methodological issues (S1). These included whether or not the sample was representative of the population; study design; ascertainment of exposure (whether objective markers of exposure were used and individual biological samples were taken or whether samples were pooled); assessment of health outcome (clinical diagnosis vs self-reported) and whether appropriate methods to control confounding were included (S3).

**3. Results**

**3.1. Study selection**

In total, 2,845 potentially relevant articles were identified from the search of databases, and 5 additional studies were identified from reference lists. Duplicate records were excluded (n=1,136). A total of 1,714 records were screened after duplicates were removed, and added additional records (n=5). Of 191 full-text articles assessed for eligibility, 14 studies were included in the qualitative synthesis (Figure 1).

**Figure 1.** Flow diagram showing process of study selection for inclusion in systematic review

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**3.2. Study characteristics**

Most of the included studies were cohort studies (n=11). One was a cross-sectional study, and one was a case-control study (Tables 2, 3). Studies meeting the inclusion criteria were published between 2002 and 2018. Of the fourteen studies identified, 8 were from North America, 4 were from Europe and 1 was from China (Gao et al., 2011). Sample sizes (individual samples) ranged from 120 (Den Hond et al., 2015) to 3, 719 (Burdort et al., 2011). Most studies examined phthalates (n=10), and PBDEs (n=5). Among the 14 included studies, one study examined both PBDEs, HBCD and phthalates (Den Hond et al., 2015). There were no papers examining TBBPA and fecundity. Study characteristics are presented across Tables 2 and 3 and the results were categorised into two different types of selected EDCs groups: (1) BFRs, with PBDE, HBCD, and TBBPA, and (2) Phthalates. Based on our inclusion criteria, there were no studies, which examined and OPFR exposure and fecundity as measure by TTP.

**3.4. Results**

**3.4.1. Brominated Flame Retardants**

In total, 5 studies met our criteria, and were evaluated in this review. Among these, one study examined both PBDEs and HBCD (Den Hond et al., 2015), one study examined both TTP and other female reproductive function (Gao et al., 2016). As shown in Table 2, retrospective and prospective cohort studies were the most common study design (Harley et al., 2010; Chevrier et al., 2013; Buck Louis et al., 2013; Gao et al., 2016). There was one case-control study (Den Hond et al., 2015). Studies by Gao et al. (2016), and Den Hond et al. (2015) were included in both Tables 2 and 3 as they both investigated BFRs and phthalates. All studies included in this review analysed BFR chemical concentrations in blood (n=5). Of these, three epidemiological studies in females for TTP or fecundability were extracted (Harley et al., 2010; Chevier et al., 2013; Gao et al., 2016). One epidemiological study examined both PBDEs and HBCD and subfertility (Den Hond et al., 2015). One epidemiological study examined selected BFRs and fecundity in couples (Buck Louis et al., 2013). Quality of assessment of included studies ranged from 6/10 to 9/10 (S1-2 table).

**Table 2. Study Characteristics examining human fecundity and BFRs**

**Females**

In a study of 223 predominantly Mexican immigrant women in a farmworker, low-income community in the United States (US), increased concentrations of PBDEs in serum were associated with a longer TTP in pregnant women (Harley et al., 2010). After controlling for confounders, increased maternal PBDE concentrations of BDE – 100 (geometric mean: 2.8 ng/g lipid) and BDE - 153 (geometric mean: 2.5 ng/g lipid) were associated with a 40% decrease (OR = 0.6, 95% CI = 0.4 - 0.9) and 50% decrease (OR = 0.5, 95% CI = 0.3 – 0.8), respectively. In this study, fecundability Odds Ratio (FOR) means the odds of achieving pregnancy in each month, and FOR <1 indicate longer TTP. BDE - 47 and BDE - 99 were also associated with reductions in ORs, but did not meet the defined level of statistical significance, for example, the p -value was smaller than 0.05. Maternal total PBDE concentrations were also related to decreased OR in TTP (OR = 0.7, 95% CI = 0.5 – 1.0) (Harley et al., 2010). In this study, PBDE levels were only measured in women, not in partners, and arguably, it may be difficult to generalise the study results of this farmworker community to other populations (Harley et al., 2010). Gao et al. (2016) reported that a longer TTP was associated with increased BDE – 28 (adjusted OR = 1.34, 95% CI = 1.03-1.76) after controlling for confounders, including parental age, maternal education level, parental occupations, mother’s passive smoke history (during pregnancy), father’s smoke history (during pregnancy), father’s alcohol consumption (during pregnancy), mother’s BMI, parity and family income. In Cox proportional hazards models, ORs were included as dichotomous variables, and results were presented as ORs (Gao et al., 2016). If a fecundability OR was less than 1, it means longer TTP or decreased fecundability. In a separate study, the association between fecundability and BDE – 209 in cord blood was not statistically significant (OR = 0.96, 95% CI = 0.72-1.29) (Chevrier et al., 2013).

**Males and couples**

In males, the study found that the OR for subfertility was 7.22 (95% CI = 1.03-50.6) in men with detectable serum levels of BDE – 209, but for other PBDEs, or HBCD examined in this study did not show statistically significant OR changes (Den Hond et al., 2015). In this study, ORs for subfertility were included as continuous variables. One study examined PBDE exposure in couples and identified no association between PBDE – 138 and fecundability (Buck Louis et al., 2013).

**3.4.2. Phthalates**

**Table 3.** **Study Characteristics examining phthalates exposure and time to pregnancy (N=10)**

In total, ten studies met the criteria, and were included in this review. Table 3 presents the study characteristics examining phthalates exposure and TTP. Among the ten studies, five studies showed that phthalate exposure was associated with TTP (Burdorf et al., 2011; Buck Louis et al., 2014; Specht et al., 2015; Velez et al., 2015; Thomsen et al., 2017).

In particular, maternal phthalate exposure was related to prolonged TTP (> 6 months) (OR = 2.16, 95%CI = 1.01 – 4.57) when adjusted for age, education, minority, parity, smoking and alcohol use (Burdorf et al., 2011). However, this study did not specify the phthalate metabolites explored, and used only job-exposure matrix to assess levels of phthalates. Similar findings in women with MEP (Monoethyl phthalate, lower phthalate metabolites) exposure was confirmed by a Danish cohort study (Thomsen et al., 2017). In the study of Thomsen et al (2017), decreased fecundity was related to higher urinary concentration of MEP (ranged 21.5-1,920 ng/ml). There was also a longer TTP (FOR < 1) for men who were exposed to some phthalates in the USA LIFE (Longitudinal Investigation of Fertility and the Environment) study (FOR (fecundability Odds Ratio) = 0.80, 95%CI=0.70 - 0.93 with MMP (Mono-methyl phthalate) exposure; FOR = 0.82, 95%CI 0.70 – 0.97 with MBP (Mono-n-butyl phthalate) exposure; FOR = 0.77, 95%CI 0.65 – 0.92 with MBzP (Monobenzyl phthalate) exposure) (Buck Louis et al., 2014).

Conversely, two studies showed evidence that shorter TTP is associated with some phthalate exposure. For example, increased FR (fecundity ratio) was evident with high levels of DEHP exposure (Fecundity Ratio = 1.14, 95%CI = 1.0 – 1.30) in serum (Specht et al., 2015). A similar result was reported by Velez et al. (2015) showing fecundability ORs were greater than 1 in individuals exposed to phthalates (MBP, MBzP) compare to individuals that were not exposed through analysis of urine samples, which means that exposure to phthalates is associated with shorter TTP.

Some phthalates were not associated with TTP. In an occupational study, Modigh et al. (2002) reported that paternal DEHP exposure does not affect prolonged TTP (median months of TTP: 2-3 months) among couples with a mean exposure level of <0.5 mg/m3 in air. No statistically significant associations were identified between women exposed to DiNP (Diisononyl phthalate) and TTP in women. High serum levels of DiNP in Greenland women was not associated with a longer TTP (Specht et al., 2015). In women, unlike men of the LIFE study, exposure to phthalates as determined in urine samples are not related to TTP (Buck Louis et al., 2014), and exposure to phthalates as determined in semen analysis are also not associated with couple fecundability (Buck Louis et al., 2018). MBP, MBzP or MBHP exposure were also not associated with a longer TTP (Thomsen et al., 2017). In one study (Jukic et al., 2016), there was no associations between phthalate exposure and fecundability, estimated by time to clinical pregnancy when adjusted for age, age at menarche, current smoking, alcohol intake, BMI, caffeine consumption, and education. Den Hond et al. (2015) also found that male subfertility is not associated with phthalates exposure as determined by urine analysis.

A number of large prospective cohort studies (n=8) assessed the association between phthalate exposure and TTP. Among the eight cohort studies, one study conducted a cross-sectional analysis within a cohort. Two studies, among 10 identified phthalates studies, used a case-control study design (Den Hond et al., 2015), and a cross-sectional study design (Specht et al., 2015). All included studies were adjusted for confounders, although the confounders were varied (Table 3). Five studies included in the phthalates review used urine as a biomarker for phthalates exposure assessment, while two studies used serum (Specht et al., 2015) or air (Modigh et al., 2002). Two studies, Burdorf et al. (2011), and Snijder et al. (2011) conducted occupational exposure assessment, and self-reported exposure alone. Only one study used semen samples to assess chemical exposure. Questionnaires (n=5) or interview (n=5) were preferred by authors to collect pregnancy data. Three studies used a clinical pregnancy test to confirm pregnancy (Buck Louis et al., 2014: Jukic et al., 2016; Buck Louis et al., 2018). Chemical concentrations ranged from <0.1 mg/m3 air (Modigh et al., 2002) to 225 ng/ml urine (Thomsen et al., 2017). Burdorf et al. (2011), and Snijder et al. (2011) did not show chemical concentrations.

Three studies(Modigh et al., 2002; Burdorf et al., 2011; Snijder et al., 2012) found a health effect of TTP from occupational exposure. Subjects were included from three plants with DEHP exposure (Modigh et al, 2002), whereas Burdorf et al. (2011) did not show specific job titles that assessed chemical exposure by self-reported chemical exposure, and a job-exposure matrix (JEM). For these studies, there was no association between paternal DEHP exposure and TTP in couples, while maternal phthalates exposure was associated with prolonged TTP (OR = 2.16, 95%CI = 1.01 – 4.57), which means 6- 12 months or > 12 months of TTP.

**4. Quality assessment results of included studies (S1-2)**

The quality assessment (QA) score of the observational studies included in our systematic review ranged from 6 to 10 (possible scores range from 0 to 10). In this QA, we scored ‘1’ or ‘0’ depending on the answer of 10 questions: representativeness of the populations, ascertainment of exposure to the EDCs, selection of the non-exposed cohort/controls, case definition for fecundity measured by TTP, assessment of EDCs exposure, aassessment of outcome, adequacy of follow-up of cohorts (where relevant) or response rate, appropriate statistical analysis, appropriate methods to control confounding, source of funding declared). All studies scored as ‘1’ for comparability with regards to age, which we considered the most important factor for adjustment, because our study eligibility criteria required, as a minimum, adjust­ment for age and sex. In addition, all studies scored as ‘1’ for representative of population, source of funding declared and appropriate methods to control confounding. Evaluation of the non-respondent rate was the question with the lowest number of scores, accounting for about 80% of the non-respondent rate that is selection bias.

**4. Discussion**

This paper provides the most comprehensive critical analysis of association between selected EDCs, and human fecundity and TTP through a systematic review. A systematic search of the literature identified 14 papers. Evidence for a causal relationship between exposure to the EDCs and fecundity as measured by TTP was evaluated within the Bradford-Hill framework (Hill 1965), focusing on the six Bradford Hill Criteria of temporality, strength and consistency of the associations, dose–response relationship, biological plausibility and the consideration of alternative explanations. The limitations of using the Bradford Hill framework in a study of environmental pollutants and health effects are acknowledged (Fedak et al., 2015). As this framework is frequently used with other systematic reviews to evaluate causal relationships (Grant et al., 2013, Kim et al., 2014, Boniface, Scannell & Marlow 2017, Degelman, M., & Herman, K. 2017) it was considered an appropriate method to facilitate inter-study comparison. We used the grading system developed by the World Cancer Research Fund (WCRF 1997) as used in the Global Burden of Disease study as a guideline for evaluation of the level of evidence (Norman et al., 2012).

**4.1 Temporality**

This review included cohort studies, cross-sectional studies (Specht et al., 2015) and a case-control study (Den Hond et al., 2015) and showed an association between the selected EDCs and human fecundity effects. Data was obtained from participants from pregnant women (eg. Harley et al., 2010; Chevier et al., 2013; Gao et al., 2016) or fertility clinics (eg. Den Hond et al., 2015). One study recruited participants from couples discontinuing contraception for pregnancy purposes (Buck Louis et al., 2013). Prospective studies showed a temporal relationship for human fecundity effects. In the studies included in this review, many did not control for pre-existing reproductive history that could affect human fertility although all studies controlled for confounders. Nevertheless, one study found that when controlling for pre-existing history of gynecologic conditions, longer TTP was associated with increasing levels of BDEs (Harley et al., 2010). Importantly, oral contraceptives used before attempting pregnancy were considered as a potential confounder in some studies (Harley et al., 2010; Buck Louis et al., 2013; Buck Louis et al., 2014; Chevrier et al., 2013).

**Strength and consistency of the associations**

Consistency of associations was difficult to assess because there were differences in fecundity as measured by TTP outcomes. In particular, chemical types and concentrations were different. Some studies used concentrations of PBDE 209 (Chevrier et al 2013), PBDE 183 (Buck Louis et al. 2013), PBDEs or HBCD (Den Hond et al. 2015), and chemical concentrations were varied, ranging from 2.08 ng/g lipid to 14.9 ng/g lipid. Even total PBDEs are different across the included studies (Table 2, 3). Sample size varied from 120 to >501, and age ranges were also different from >18 age (Gao et al., 2016) to >50 years (Den Hond et al., 2015). We recorded mostly weak associations between exposure to selected EDCs and fecundity as measured by TTP according to WCRF guideline. We also noted inconsistent findings in studies assessing the effects of selected BFRs on fecundability. A prospective cohort study (Buck Louis et al., 2013), and a retrospective cohort study (Harley et al., 2010) reported that except for BDE 209, there was an association between PBDEs and fecundability (Chevrier et al., 2013). Due to inconsistent results from prospective studies, it was difficult to establish a temporal relationship between exposure to PBDEs and TTP.

Regarding phthalate exposure, shorter or longer TTP was associated with phthalate exposure (Burdorf et al, 2011; Buck Louis et al., 2014; Specht et al., 2015; Velez et al., 2015; Thomsen et al., 2017). The association from the included studies were consistent in similar study settings; cohort study, urinary matrix, large study sample size (ranged from 229 to 3, 719), although two studies assessed chemical assessment through a job exposure assessment tool and self-reported exposure (Burdorf et al., 2011; Snijder et al., 2011). Concentrations of phthalates were inconsistent across the included studies, ranged from <0.1 mg/m3 air (Modigh et al., 2002) to 225 g/ml urine (Thomsen et al., 2017). Two studies (Burdorf et al., 2011; Snijder et al., 2011) did not show chemical concentrations.

**Dose–response relationship**

In this review, meta-analysis was not conducted, thus we cannot assess overall dose-response relationship for fecundity measure by TTP. However, dose-response effect on the different EDC levels was evident (Specht et al., 2015). In Greenland women with highly exposed to Proxy-MEHP compared to lower levels exposure, shorter TTP was suggestive. Whilst concentrations of BFRs are generally higher in North American compared to Europe, the studies reviewed here did show a more causal relationships based on countries with high concentrations (eg. USA; Harley et al., 2010; China; Gao et al., 2016). Concentrations of phthalates are in general higher in the US compared to Europe, interestingly the studies with both higher (eg. Specht et al., 2015) and lower concentrations (eg. Modigh et al., 2012) show a relationship with TTP while those with the lower concentrations do not. It is important to note that a typical linear dose–response pattern is not observed for compounds that have endocrine-disrupting effects. Non-linear and low-dose associations are more typical of these compounds (Rhomberg & Goodman, 2012).

**4.2 Biological plausibility**

Although there is evidence of biological plausibility and demonstration of mechanisms of action for these chemicals in animal and in-vitro models (Moyer and Hixon 2012; Berger et al., 2014; Dobrzyńska 2016; Dominguez et al. 2016; Tavares et al. 2016), it is broadly recognized that these results may not necessarily translate to similar effects in humans. While BFRs, as one of the group of persistent organic pollutants, are endocrine disruptors with an especially strong effect from early life exposure (Caserta et al., 2008), it is difficult to definitively assess the consequences of simultaneous exposure to many chemicals as combined effects are not well-understood. Most studies investigated unique combinations of EDCs, but there might be other sources that influence on human fertility. While the possibility of unknown potential confounders in observational studies exists, in studies that accounted for confounding variables, investigators nevertheless recorded significant associations between exposure to EDCs and TTP in males and females. A precautionary approach towards exposure of both females and males seems warranted with long-term monitoring and ongoing surveillance to fully characterise risks to health. The major shortcomings of the existing research relates to the external validity of the studies analysed.

**4. 3 The consideration of alternative explanations**

Different studies controlled for a range of possible confounders including socio-economic status, behavioural risk factors and exposure to other environmental toxicants, making comparisons difficult, although combining studies whilst controlling for different confounders can be effective for a meta-analyses. Residual confounding or unmeasured potential confounders may also affect study results. However, in the recent phthalate study, urine is commonly used to measure phthalate concentrations, but two studies used different exposure matrices (serum: Specht et al., 2015, seminal plasma and urine both; Buck Louis et al., 2018). In the study of Buck Louis et al. (2018), there were lower EDC concentrations in seminal plasma compared to in urine samples. However, there was no different study results from included studies when used different matrices. Using TTP data to infer fecundity may be biased, especially given the challenges in measuring self-reported TTP for up to 12 months of trying for pregnancy. Much of the available data on this topic relies on pregnant women (excluding women not achieving pregnancy, whose exposure may or may not differ to pregnant women) and retrospective reporting (which has been empirically demonstrated to have low validity with bi-directional reporting errors).

**4. 4 Assessment of evidence**

We used the grading system developed by the WCRF (1997) as a guideline to assess the level of evidence. Based on the WCRF (1997), we concluded that the evidence to establish a convincing causal relationship between selected EDCs and fecundity as measured by TTP was limited, but was suggestive of a direction of effect. Precautionary action should nevertheless be undertaken when there are credible threats of harm, despite residual scientific uncertainty about selected EDCs cause and fecundity or TTP effect relationships. Furthermore, imposing a high standard of evidence for establishing causality in environmental exposures may result in inappropriately ignoring important potential hazards (Watt and Carincross 2012). Conducting randomized controlled trials to examine chemical exposure and human health outcomes is not ethical. This review found the cross-sectional approach in prospective cohort study design (Snijder et al. 2011; Gao et al., 2016) to estimate fecundity. Cross-sectional studies alone are not considered adequate when identifying causation in this domain, because the study factor should not be measured at the same time point as the outcome. Thus, longitudinal cohort studies may be the most appropriate and highest level of evidence available. More well-designed research, and population-based data would be needed to support tentative associations, and correct for possible bias arising from study design (Smarr et al., 2017).

Although this review provides a summary of current research on human fecundity, it also found some limitations. While some negative findings were identified (eg. Chevrier et al., 2013), the review may nevertheless be subject to publication bias as publication of non-significant findings may depend on funding. Only statistically significant effects are reported in many studies, meaning effects that could not be validated statistically are often not considered or reported. There were inconsistencies in how exposure and outcomes were defined and measured across studies. Measurement bias with respect to health outcomes, particularly where outcomes were self-reported, may have affected the results. Since individuals cannot be randomly allocated to case groups, the influence of confounding variables cannot be fully evaluated. Finally, many studies recruited study populations from fertility-clinics (eg. Den Hond et al., 2015), workplaces (eg. Modigh et al., 2002), which indicate occupational exposure, or pregnant women (eg. Harley et al., 2010; Chevrier et al., 2013). On the other hand, the LIFE study (Buck Louis 2013; 2014; 2018) conducted their study on populations that were not known infertility or sterility diagnoses.

Prospective, long-term cohort studies are needed to study exposed individuals from the time of exposure to EDCs, measuring body burden and investigating fecundity among other health outcomes. These would include measurement in a range of biological samples, such as serum, urine, or semen such as Buck Louis et al (2013; 2014; 2018) which can replicate and confirm or deny the current findings. TTP measurement was used as the only indicator of fecundity in this review, and it is important that other human fecundity indicators be considered. Targeted research focused on the mechanism of action of the selected EDCs alone and in combination, would provide important evidence of their impact on reproductive outcomes. Attempts to understand safe levels of chemical exposure on the human body should be considered, as different levels of exposure to chemicals showed different OR results.

**5. Conclusion**

In conclusion, weak associations and inconsistent findings across studies precluded the establishment of a causal relationship when assessing the evidence of selected EDC exposure, and fecundity and TTP through conventional epidemiological approaches. There was evidence suggestive of a link between increased TTP and PBDE exposure. Evidence also exists on phthalates exposure and increased or decreased TTP. However, the results were not always consistent and an insufficient number of studies were identified. Replicated study results are needed to definitively establish a causal relationship between exposure and disease. This systematic review has revealed important data gaps regarding EDCs exposure and human fecundity as measured by TTP. Despite the limited number of studies, a precautionary approach towards exposure of both males and females seems warranted, and longitudinal surveillance is needed to characterise potential risks to fertility issue and provide important evidence for informing policy.

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**Conflict of Interest**

The authors declare no conflicts of interest.

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