Received: 21 September 2023 | Revised: 17 November 2023 | Accepted: 24 November 2023

DOI: 10.1111/ppe.13026

SPECIAL ISSUE ARTICLE

Paediatric and Perinatal Epidemiology WILEY

# Bias analyses to investigate the impact of differential participation: Application to a birth defects case-control study

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## Abstract

**Background:** Certain associations observed in the National Birth Defects Prevention Study (NBDPS) contrasted with other research or were from areas with mixed findings, including no decrease in odds of spina bifida with periconceptional folic acid supplementation, moderately increased cleft palate odds with ondansetron use and reduced hypospadias odds with maternal smoking.

**Objectives:** To investigate the plausibility and extent of differential participation to produce effect estimates observed in NBDPS.

**Methods:** We searched the literature for factors related to these exposures and participation and conducted deterministic quantitative bias analyses. We estimated case-control participation and expected exposure prevalence based on internal and external reports, respectively. For the folic acid-spina bifida and ondansetron-cleft palate analyses, we hypothesized the true odds ratio (OR) based on prior studies and quantified the degree of exposure over- (or under-) representation to produce the crude OR (cOR) in NBDPS. For the smoking-hypospadias analysis, we estimated the extent of selection bias needed to nullify the association as well as the maximum potential harmful OR.

**Results:** Under our assumptions (participation, exposure prevalence, true OR), there was overrepresentation of folic acid use and underrepresentation of ondansetron use and smoking among participants. Folic acid-exposed spina bifida cases would need to have been ≥1.2× more likely to participate than exposed controls to yield the observed null cOR. Ondansetron-exposed cleft palate cases would need to have been 1.6× more likely to participate than exposed controls if the true OR is null. Smoking-exposed hypospadias cases would need to have been ≥1.2 times less likely to participate than exposed controls for the association to falsely appear protective (upper bound of selection bias adjusted smoking-hypospadias OR = 2.02).

**Conclusions:** Differential participation could partly explain certain associations observed in NBDPS, but questions remain about why. Potential impacts of other systematic errors (e.g. exposure misclassification) could be informed by additional research.

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**Funding information**
Centers for Disease Control and Prevention

**KEYWORDS**
bias, congenital abnormalities, differential participation, quantitative bias analysis, selection bias, sensitivity analysis

## 1 | BACKGROUND

Epidemiological research is prone to errors that impact precision, validity and interpretation of effect estimates. Commonly, investigators quantify random error, but systematic errors, notably selection bias, are rarely quantified.[1] Quantitative bias analysis can be useful to understand the extent of bias needed to explain an association or quantify what the estimate would have been had bias been absent, given a set of assumptions.[2–4]

Quantitative bias analysis has been applied to several findings[5–12] from the National Birth Defects Prevention Study (NBDPS), a US population-based case-control study (1997–2011) of risk factors (including medications) for birth defects.[13,14] These investigations included selection bias from missing terminations and stillbirths, finding the bias' strength depended on exposure prevalence and strength of associations among the exposure, outcome and likelihood of foetal loss/termination.[5,6] Questions remain about the potential impacts of differential participation, a common concern in case-control studies, under the theoretical motivation that individuals affected by the outcome may be more motivated to participate, which could bias associations if exposure status is also related to participation.[15]

The overarching objective of our bias analyses was to investigate plausibility and to quantify the extent of differential participation needed to produce three previously published NBDPS associations. We selected findings that contrasted with other studies or where the research has been mixed: (i) no decrease in odds of spina bifida with consistent periconceptional folic acid supplementation[16]; (ii) moderately increased cleft palate odds with early pregnancy use of ondansetron (a medication for nausea and vomiting)[17]; and (iii) reduced hypospadias odds with active maternal cigarette smoking during early pregnancy.[18]

### Synopsis

#### Study question

Could differential participation by mothers of cases versus controls explain certain associations in the National Birth Defects Prevention Study (NBDPS)?

#### What is already known

Several associations have been observed in NBDPS that contrasted with other research or were from areas of study with mixed findings: no decrease in odds of spina bifida with periconceptional folic acid supplementation; moderately increased cleft palate odds with ondansetron use and reduced hypospadias odds with maternal smoking. Factors common to these exposures and participation may have contributed to selection bias.

#### What the study adds

This manuscript serves as an example for exploring plausibility and extent of differential participation in case-control studies of perinatal outcomes, notably when controls were selected from an underlying source population with population-level exposure estimates.

## 2 | METHODS

### 2.1 | Case-control selection

Pregnancies affected by major structural birth defects were identified from surveillance systems (1997–2011 unless otherwise noted) in Arkansas (1998–2011), Iowa, New Jersey (1998–2002) and Utah (2003–2011) and select counties in California, Georgia, Massachusetts, New York, North Carolina (2003–2011) and Texas, including live births, stillbirths (nine sites) and elective terminations (eight sites). Medical records of cases were screened by clinical experts for eligibility and classification. Live-born infants without a major structural defect, randomly sampled from hospital records or birth certificates with the same delivery timing and catchment areas as the cases, were screened for eligibility to be controls. Study staff contacted mothers of eligible cases and controls to obtain consent to participate. Study staff tracked the proportion of eligible cases in each defect group and eligible controls who participated. Reasons for non-participation included refusal by the mother or inability to consent/interview within the protocol-defined time period.[13]

### 2.2 | Outcomes

For our three bias analyses, case groups were defined based on the aforementioned publications.[16–18] Specifically, these were individuals diagnosed with (i) spina bifida (part of the spinal cord and nerves are exposed through an opening in the back); (ii) cleft palate without cleft lip (an opening at the roof of the mouth) or (iii) hypospadias (the

opening of the urethra is not at the tip of the penis). The smoking-hypospadias analysis included only male cases and controls.

## 2.3 | Exposures

Participants completed a structured telephone interview between 6 weeks and 2 years after the estimated date of delivery, which captured information on a variety of exposures.

The exposure window for each analysis corresponded to the etiologically relevant timing for that specific defect occurrence. Detailed descriptions of the exposures are documented in the NBDPS publications.[16–18] In brief, for the folic acid-spina bifida analysis, the exposure period of interest was 3 months before through the first month of pregnancy. Exposed mothers reported "consistent" (at least half of the time period) use of a folic acid-containing supplement, including multi- and prenatal vitamins and single-component products. Unexposed mothers did not use these products or initiated use in pregnancy. For the ondansetron-cleft palate and smoking-hypospadias bias analyses, the exposure period of interest was the first trimester of pregnancy. The ondansetron-cleft palate analysis was restricted to mothers who reported nausea and vomiting. Exposed mothers reported first-trimester treatment with ondansetron (with or without other prescription antiemetics). Unexposed mothers were untreated with ondansetron (i.e. treated with other prescription medications or untreated individuals). For the smoking-hypospadias analysis, exposed mothers reported active cigarette smoking in the first trimester. Unexposed mothers reported no active smoking or passive smoke exposure during pregnancy.

## 2.4 | Statistical analyses

We reviewed the literature to determine if there are factors commonly related to NBDPS participation (based on an internal study of NBDPS controls compared with all eligible controls and the general US population by Cogswell et al.[19]) and the likelihood of the exposures of interest (based on external US-based studies of pregnant people[20–23]). The evidence supported that certain common factors, including maternal age, education, race/ethnicity, multi-gestation (e.g. twins) and pregnancy planning, were related to both NBDPS participation and at least one of these exposures,[19–23] which could, in theory, have contributed to selection bias. We employed a simplified causal diagram to visually depict the theorized relationships between the exposure, outcome and these other factors and how they could result in selection bias via collider stratification (Figure 1).

Details specific to each analysis are provided below, in the Table 1 and in Appendix S1. From each original NBDPS publication,[16–18] we extracted the participants' exposure distribution, and we calculated the crude exposure-outcome odds ratio (OR). The crude and covariate-adjusted ORs from the publications[16–18] were similar, so for simplicity, we focused on the crude ORs. We estimated the proportions of case and control participants in each NBDPS analysis among all eligible cases and controls based on unpublished internal reports maintained by study staff who tracked participation. We estimated the expected exposure distribution among mothers of all eligible controls (participants and non-participants combined) based on exposure prevalence estimates in the same time window (periconception for folic acid, first trimester for ondansetron and smoking) from external US population-based reports.[22–25] To

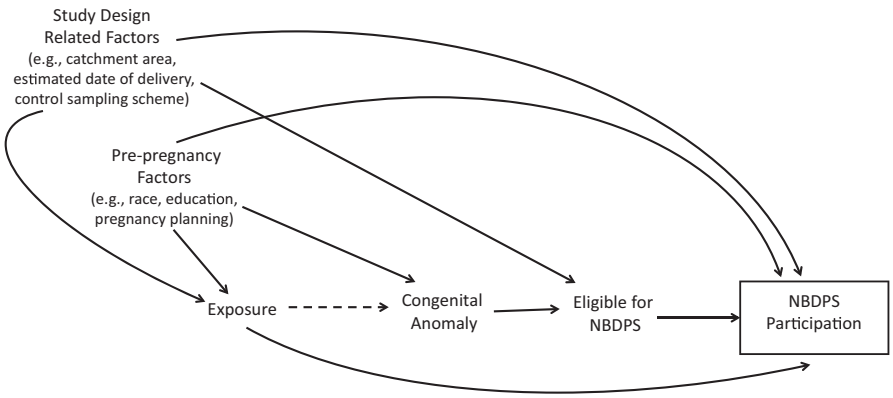


**FIGURE 1** Causal diagram of potential selection mechanism due to differential participation in the National Birth Defects Prevention Study (NBDPS). In this simplified directed acyclic graph (causal diagram), we describe a potential mechanism that could have led to selection bias of the association between early pregnancy exposures and birth defect occurrence in offspring in NBDPS. The "eligible for NBDPS" node indicates selection/eligibility to participate in the study based on case-control status (i.e. whether a pregnancy resulted in the defect of interest or was not affected by a structural birth defect). The "NBDPS participation" node indicates individuals that ultimately participated. The box around participation indicates conditioning on participation. The dashed arrow indicates a modified (biased) association between the exposure and the birth defect in offspring due to conditioning on participation (a collider). Certain factors related to the study design are related to the determination of eligibility of cases and controls (e.g. the State's participation in NBDPS, the control sampling scheme for a given study centre, the timing of the delivery). These factors may also be related to likelihood of being exposed and/or participation. All pre-pregnancy factors are grouped together in the figure because there is not a clear understanding of the relative importance of each pre-pregnancy factor in birth defect aetiology. Likely, these pre-pregnancy factors are proxies for the increased likelihood of being exposed to the actual biological teratogens that are the more direct causes of birth defects.

**TABLE 1** Observed, assumed and bias analysis estimates from three exposure-birth defect associations in the National Birth Defects Prevention Study.

| | | Exposure-birth defect association | | | | | |
|---|---|---|---|---|---|---|---|
| | | Folic acid-spina bifida[16] | | | Ondansetron-cleft palate[17] | | Smoking-hypospadias[18] |
| | | Main | Sens 1 | Sens 2 | Main | Null Sens | Upper bound |
| Observed estimates from NBDPS | | | | | | | |
| N, Pr (participating \| all cases) | | 385, 55.6% | | | 418, 68.4% | | 2130, 64.3% |
| N, Pr (participating \| all controls) | | 3691, 57.8% | | | 3267, 63.3% | | 4666, 63.5% |
| N, Pr (exposed \| participating cases) | | 97, 25.2% | | | 40, 9.6% | | 333, 15.6% |
| N, Pr (exposed \| participating controls) | | 965, 26.1% | | | 212, 6.5% | | 887, 19.0% |
| Crude OR (95% CI) | | 0.95 (0.75, 1.21) | | | 1.52 (1,07, 2.17) | | 0.79 (0.69, 0.91) |
| Assumptions based on external (non-NBDPS) studies | | | | | | | |
| True OR | | 0.70 | | | 1.29 | 1.00 | 1.88 |
| Selection OR | | 1.36 | | | 1.18 | 1.52 | 0.39 |
| Pr exposed \| all controls | | 25.0% | 19.0% | 40.0% | 11.0% | | 14.0% |
| Bias analysis estimates | | | | | | | |
| Pr participated \| exposed cases i.e., selection fraction $\alpha$ | Pr participated \| exposed controls i.e., selection fraction $\gamma$ | 73.9% 60.4% | 93.9% 74.1% | 44.0% 37.8% | 47.4% 37.3% | 59.1% 37.3% | 40.2% 86.2% |
| Pr participated \| unexposed cases i.e., selection fraction $\beta$ | Pr participated \| unexposed controls i.e., selection fraction $\delta$ | 51.3% 56.9% | 48.9% 52.7% | 61.1% 71.1% | 71.8% 66.5% | 69.6% 66.5% | 72.3% 59.8% |
| Selection fraction ratio in cases | | 1.44 | 1.92 | 0.72 | 0.66 | 0.85 | 0.56 |
| Selection fraction ratio in controls | | 1.06 | 1.41 | 0.53 | 0.56 | 0.56 | 1.44 |
| Minimum number of additional individuals with a particular exposure-outcome combination that would have needed to participate to result in a selection OR of 1.0 (no bias) | | 31 unexposed cases (to shift $\beta$ from 0.51 to 0.57) and 216 exposed controls (to shift $\gamma$ from 0.60 to 0.74) | 22 unexposed cases (to shift $\beta$ from 0.49 to 0.53) and 258 exposed controls (to shift $\gamma$ from 0.80 to 1.0) | 47 unexposed cases (to shift $\beta$ from 0.61 to 0.71) and 158 exposed controls (to shift $\gamma$ from 0.38 to 0.44) | 57 exposed controls (to shift $\gamma$ from 0.37 to 0.47) and 243 unexposed controls (to shift $\delta$ from 0.67 to 0.72) | 124 exposed controls (to shift $\gamma$ from 0.37 to 0.59) and 142 unexposed controls (to shift $\delta$ from 0.66 to 0.70) | 381 exposed cases (to shift $\alpha$ from 0.40 to 0.86) and 790 unexposed controls (to shift $\delta$ from 0.60 to 0.72) |

Abbreviations: CI, confidence interval; Main, main bias analysis; NBDPS, National Birth Defects Prevention Study; N, number; Null Sens, nullification sensitivity analysis: assumed true OR is 1.0; OR, odds ratio; Pr, proportion; Sens 1, sensitivity analysis 1: assumed minimum reported prevalence of folic acid supplementation among all controls; Sens 2, sensitivity analysis 2: assumed maximum reported prevalence of folic acid supplementation among all controls, Upper bound of bias analysis results (all results found in Table S1), $\alpha$ proportion of exposed cases that participated, $\beta$ proportion of unexposed cases that participated, $\gamma$ proportion of exposed controls that participated, $\delta$ proportion of unexposed controls that participated.

hypothesize what the true ORs might be for the folic acid-spina bifida and ondansetron-cleft palate analyses, we relied on estimates from other (external) studies that were theoretically less prone to selection bias.[20,26–29] For the smoking-hypospadias analysis, since previous findings have been more mixed, we took a slightly different approach. Given the constraints of the observed data and expected smoking prevalence among US pregnancies,[22] we calculated what the true (unobserved) OR might be under a variety of differential participation scenarios.

Under our assumptions, we calculated the selection OR, selection fractions for exposed cases (α), unexposed cases (β), exposed controls (γ) and unexposed controls (δ) and selection fraction ratios (for cases α/β, for controls γ/δ; refer to Figure 2). Details of these calculations and an example are provided in the Appendix S1. A selection fraction ratio >1 implies a higher-than-expected exposure prevalence among participants in that outcome group (cases or controls) compared with the expected exposure prevalence in the base population (herein referred to as "overrepresentation"), whereas a ratio <1 implies a lower than expected exposure prevalence (herein referred to as "underrepresentation"). We also compared the selection fractions among the exposed cases versus the exposed controls to quantify how much more likely exposed cases (or controls) were to participate. Lastly, we calculated the minimum number of additional individuals with a particular exposure-outcome combination that would have needed to participate to result in a selection OR of 1.0 (no bias). Our inferences relied on our assumptions regarding the participation proportions among the eligible cases and controls, the exposure prevalence among all eligible participants and (except for the smoking-hypospadias analysis) the hypothesized true OR.

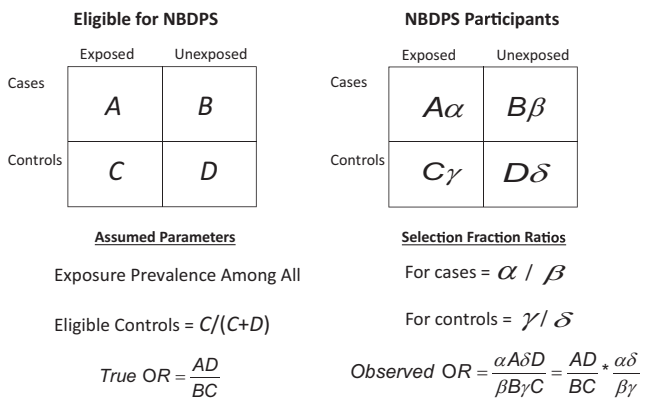


**FIGURE 2** 2×2 tables representing the exposure distributions among the base population (i.e. all individuals eligible for the National Birth Defects Prevention Study (NBDPS)) versus the subset who participated and the calculations for the bias analyses. α proportion of exposed cases that participated, β proportion of unexposed cases that participated, γ proportion of exposed controls that participated, δ proportion of unexposed controls that participated.

### 2.4.1 | Folic acid and spina bifida

In the NBDPS investigation of 385 spina bifida cases and 3691 controls (1998–2003 deliveries), consistent periconceptional folic acid supplementation was reported by 25.2% and 26.1% of mothers of cases and controls, respectively.[16] This equated to a null association between periconceptional folic acid supplementation and spina bifida (crude OR 0.95, 95% CI 0.75, 1.21).[16] The other US investigation conducted post-mandatory folic acid fortification of grain products also found a null association but was also a case-control design and may be subject to similar biases.[30] These null findings contradicted a wealth of pre-fortification research, including randomized trials, that supported maternal periconceptional folic acid intake reduces spina bifida risk by at least 30%.[26–29] While fortification has improved folate status in the US population, serum sample studies suggest that the average US woman of reproductive age does not obtain sufficient folic acid from fortified products alone.[31,32] For this reason, the US Preventive Services Task Force still recommends that all women with reproductive potential supplement with ≥400 μg of folic acid daily.[33] Thus, we explored whether differential participation might explain the null NBDPS finding. We estimated that 55.6% and 57.8% of all eligible spina bifida cases and controls, respectively, were included in NBDPS analysis. In the main analysis, we assumed 25.0% of all eligible controls were periconceptional folic acid supplementers based on US reports.[23,24] We assumed the true folic acid-spina bifida OR is ≤0.70,[26–29] focusing on the weakest association (true OR = 0.70).

### 2.4.2 | Ondansetron and cleft palate

The NBDPS investigation (2005–2011 deliveries) found among women who reported nausea and vomiting during the first trimester of pregnancy, 9.6% of 418 case mothers and 6.5% of 3267 control mothers used ondansetron in the first trimester.[17] Ondansetron use was associated with moderately increased cleft palate odds (crude OR 1.52, 95% CI 1.07, 2.17).[17] We estimated that 68.4% and 63.3% of all eligible cleft palate cases and controls, respectively, were included in the NBDPS analysis. There was a slight challenge in estimating the expected prevalence of ondansetron use because US-based reports noted an increase in ondansetron use over time, notably after 2006 when oral ondansetron was approved by the FDA.[21,25,34] Based on a large US-based administrative claims study, we averaged across 2011–2015, assuming that first-trimester ondansetron use in all eligible controls was 11.0%.[25] Most other studies of ondasetron and cleft palate have also supported an increased risk, but some suggested a weaker association.[20,35,36] For the main analysis, we assumed that the true ondansetron-cleft palate OR was 1.29, as observed in a large Medicaid-based cohort study of >1.8 million pregnancies (2000–2013) that was unlikely to be biased by differential participation.[20]

### 2.4.3 | Smoking and hypospadias

The NBDPS investigation of 2130 moderate/severe hypospadias cases and 4666 controls (1997–2011 deliveries) observed that first trimester smoking was reported among 15.6% and 19.0% of case and control mothers, respectively, which equated to a decreased odds of hypospadias (crude OR 0.79, 95% CI 0.69, 0.91).[18] These results were slightly stronger than a pooled estimate from a meta-analysis of 15 studies of hypospadias and maternal smoking during pregnancy (pooled OR 0.90, 95% CI 0.85, 0.95)[29] and were inconsistent with a large cohort that found a slightly increased hypospadias risk with first-trimester smoking (relative risk 1.1, 95% CI 1.0, 1.2).[37,38] The latter finding is in line with research finding that smoking increases risks for most birth defects.[37,38] Based on publicly available estimates from the Pregnancy Risk Assessment Monitoring System (40 states, 2000–2010),[22] we assumed first-trimester maternal smoking prevalence fell between 14.0% and 25.0%. Using these bounds, we varied the selection fractions for cases and controls differentially with respect to exposure, keeping the overall participation percentages as estimated (i.e. 64.3% for hypospadias cases and 63.5% for male controls), and determined the upper bound of the exposure-outcome OR that could be observed (i.e. if the prevalence of smoking among all case mothers and all control mothers was 25% and 14% respectively). We focused on the upper bound to determine the highest increase in hypospadias odds that smoking could impose under our assumptions.

### 2.4.4 | Missing data

The original analyses excluded <10% of participants (e.g. due to missing data on the exposures of interest). Since our approach involved conducting bias analyses using the original findings as published, we did not take any additional measures to account for missing data specifically.

### 2.4.5 | Sensitivity analyses

In sensitivity analyses of the folic acid-spina bifida association, we changed the expected exposure prevalence among all eligible controls to 19.0% and 40.0% (the lowest and highest estimates, respectively, from the literature).[23,24] Regarding the ondansetron-cleft palate analysis, a few external studies reported weaker and even inverse associations.[36,39] While these studies may be susceptible to biases, in a nullification sensitivity analysis, we investigated the degree of selection bias needed to fully explain the NBDPS association (i.e. assumed true OR = 1.00). The sharp null was also the lower bound of the 95% confidence interval observed in the cohort on which our main bias analysis was based.[20] For the smoking-hypospadias analysis, since the observed exposure distribution within the case and control participants was within the bound based on external estimates, we conducted nullification sensitivity analyses, assuming the observed smoking prevalence among the mothers of either the cases or controls was representative of the smoking prevalence among all eligible cases and controls. Lastly, we estimated the lower bound of the smoking-hypospadias selection bias-adjusted OR that could be observed (i.e. if the prevalence of smoking among all case mothers and all control mothers was 14% and 25% respectively).

## 2.5 | Ethics approval

NBDPS was approved by the institutional review boards for the study centres and the US Centers for Disease Control and Prevention. Mothers of the cases and controls provided informed consent to participate in NBDPS.

# 3 | RESULTS

## 3.1 | Folic acid and spina bifida

Under our assumptions for the main and first sensitivity analyses of the spina bifida association, there would need to have been overrepresentation of folic acid supplementation among the participants, but more so among case mothers (Table 1). Folic acid-exposed spina bifida cases would need to have been at least 1.2× more likely to participate than folic acid-exposed controls to explain the null OR observed in NBDPS if the true OR is actually ≤0.70. Under our assumptions for the main analysis, there would have been no selection bias if 247 more individuals had participated. In our second sensitivity analysis (where we assumed a higher prevalence of folic acid supplementation), there would need to have been underrepresentation of folic acid supplementation, more so among control mothers.

## 3.2 | Ondansetron and cleft palate

Under our assumptions, there would need to have been an underrepresentation of ondansetron use among the participants, but more so among control mothers (Table 1). If the true OR is 1.3, ondansetron-exposed cases would need to have been 1.3× more likely to participate than ondansetron-exposed controls, and there would need to have been 300 more individuals participating in the study to result in no selection bias. If the true OR is null, ondansetron-exposed cases would need to have been 1.6× more likely to participate than ondansetron-exposed controls.

## 3.3 | Smoking and hypospadias

Under our assumptions, the selection bias adjusted ORs moved towards or across the null in scenarios where smoking-exposed cases were ≥1.2× less likely to participate than unexposed cases (see

Appendix S1). If the smoking prevalence among all eligible mothers of cases and controls was 25.0% and 14.0%, respectively, the upper bound of the selection bias adjusted OR would be 2.02. This extreme selection bias scenario would have required smoking-exposed cases to be >2× less likely to participate than smoking-exposed controls.

## 4 | COMMENT

### 4.1 | Principal findings

We observed possible overrepresentation of folic acid supplementation and underrepresentation of ondansetron use and smoking among NBDPS participants. Based on our assumptions, folic acid-exposed spina bifida cases would need to have been more likely to participate than folic acid-exposed controls to shift the hypothesized protective true OR to null (observed in NBDPS). Similarly, for the ondansetron-cleft palate analysis, exposed cases would need to have been more likely to participate than exposed controls, but with more extreme differential participation, to yield an inflated ondansetron-cleft palate association in NBDPS. Lastly, smoking-exposed hypospadias cases would need to have been less likely to participate than smoking-exposed controls for the association observed in NBPDS to falsely appear protective; a null association seemed plausible as it would require minimal selection bias, but much more extreme selection bias would be needed to mask the maximum possible harmful OR under our assumptions.

### 4.2 | Strengths of the study

The NBDPS case classification process was rigorous,[40] reducing the potential for outcome misclassification. We capitalized on existing internal NBDPS data to estimate the proportion of eligible people who were included in each analysis.

### 4.3 | Limitations of the data

Our inferences depend on the accuracy and representativeness of the data informing the bias analyses. Since NBDPS controls were randomly sampled from the general populations of the state-based study centres, we assumed external US-based data from overlapping time periods would be representative of their expected exposure prevalence, but we did not account for differences within subgroups (e.g. state-specific) or over time. While we aimed to select large-scale, rigorously designed studies that may be less prone to selection bias, other errors may affect their estimates. To address this uncertainty, we applied a multidimensional approach, but still we evaluated only a limited number of scenarios. Our current analysis does not explore whether associations observed in NBDPS could be attributed to exposure misclassification due to misreporting (e.g. misremembering the start of folic acid supplementation or medication(s) taken to treat nausea and vomiting). A major challenge is identifying appropriate bias parameters (e.g. sensitivity and specificity). There is a lack of validation data, introducing a high degree of uncertainty. Misclassification bias analyses for folic acid supplementation and ondansetron use would most likely be informed by expert opinion and constraints of the data. External validation data exist to estimate the degree of error in smoking reporting, but to our knowledge, the data are severely lacking with respect to differential reporting based on pregnancy outcomes. Another bias analysis of NBDPS investigating misreporting of smoking when studying associations with cleft defects reported that credible interval widths nearly doubled in part due to this uncertainly,[12] highlighting the need for more validation studies. Lastly, there are few well-established strong risk factors for birth defects, so adjustment for measured confounders did not lead to meaningful changes in effect estimates in the original NBDPS studies.[16–18] However, this does not rule out the possibility of bias from unmeasured factors.

### 4.4 | Interpretation

There are several reasons why exposed persons may be more (or less) likely to participate in research. Another pregnancy study with self-selection observed that individuals with greater potential to adhere to clinical guidance were more likely to participate.[41] Individuals with risk factors for severe nausea and vomiting in pregnancy (and so more likely to use ondansetron, e.g., multiples and certain pre-existing medical conditions)[42] may have been less likely to participate, as observed by Cogswell et al. among NBDPS controls.[19] NBDPS maternal smoking was towards the lower range of US estimates, possibly because mothers who smoke might have been less willing to participate due to feelings of stigma or guilt. Our analysis demonstrated how non-participation of 300 or fewer individuals has the potential to explain certain associations observed in NBDPS. Still, although some differential participation is possible, it is not clear why pregnant people with certain exposures and offspring affected by a birth defect would be much more (or less) likely to participate. More research is needed to understand if such motivations actually exist, and if so, whether researchers can combat these biases by means of other designs or whether factors that drive these differences can be measured and accounted for in analysis to yield less biased effect estimates.

### 4.5 | Conclusions

These deterministic bias analyses serve as examples of how one might conduct initial investigations of bias from differential participation in case-control studies of perinatal outcomes when the controls were selected from a base population with population-level exposure estimates. Despite limitations, a simplistic approach like ours allows for an evidence-informed examination of multiple scenarios of potential selection bias and quantification

of the possible impacts on the effect estimates, which could be more informative than qualitative speculation (the most common current practice). Our bias parameters, causal diagram and results may be useful to inform future bias analyses of these associations, including those that quantify the impact of multiple plausible biases at once, other factors related to the bias mechanisms and uncertainty from assumptions.

## AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization of the research. JMP led the development of the methodology. JMP, JCK, NA and MDP conductedthe investigation, including review of the literature and the bias analyses. MEW, AFO, LHS, MMH, ECA, PAR, AHH, SEP and GMS provided oversight and expert input. JMP led the writing of the original draft paper with support from JCK, NA and MDP. All authors reviewed and edited the paper and approved the final version.

## ACKNOWLEDGEMENTS

Coding of drug information in the National Birth Defects Prevention Study used the Slone Drug Dictionary under licence from the Slone Epidemiology Center of Boston University. We thank the following collaborators for their input in the original design of these bias analyses and their support of this work: Suzan Carmichael, Anne-Marie Darling, Dominique Heinke, Rebecca Liberman, Eirini Nestoridi, Martha Werler and Mahsa Yazdy.

## FUNDING INFORMATION

This project was supported through Centers for Disease Control and Prevention (CDC) cooperative agreements under PA #96043, PA #02081, FOA #DD09-001, FOA #DD13-003, NOFO #DD18-001 and NOFO #DD23-001 to the Centers for Birth Defects Research and Prevention participating in the National Birth Defects Prevention Study (NBDPS) and/or the Birth Defects Study To Evaluate Pregnancy exposureS (BD-STEPS).

## CONFLICT OF INTEREST STATEMENT

GMS has provided expert testimony in litigation involving ondansetron. MEW is faculty affiliated with, and JCK is a fellow through, the Center for Pharmacoepidemiology at the University of North Carolina at Chapel Hill, which is funded by unrestricted donations from industry partners (AbbVie, Boehringer Ingleheim, GSK, Takeda, UCB, Sarepta, Astellas). The findings and conclusions in this report are those of the authors and do not necessarily represent the official positions of the Centers for Disease Control and Prevention, the North Carolina Department of Health and Human Services Division of Public Health or the California Department of Public Health.

## DATA AVAILABILITY STATEMENT

Data described in the manuscript are those from the publications: Mosley BS, Cleves MA, Siega-Riz AM, et al. Neural Tube Defects and Maternal Folate Intake among Pregnancies Conceived after Folic Acid Fortification in the United States. *Am J Epidemiol*. 2009;169(1):9–17; Parker SE, Van Bennekom C, Anderka M, Mitchell AA, National Birth Defects Prevention S. Ondansetron for Treatment of Nausea and Vomiting of Pregnancy and the Risk of Specific Birth Defects. *Obstet Gynecol*. 2018;132(2):385–394; and Carmichael SL, Ma C, Shaw GM, National Birth Defects Prevention S. Maternal Smoking, Alcohol, and Caffeine Exposures and Risk of Hypospadias. *Birth Defects Res*. 2017;109(14):1127–1133. The process for accessing the data and codebooks from the National Birth Defects Prevention Study is described at https://www.cdc.gov/ncbddd/birthdefects/nbdps-public-access-procedures.html.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Petersen JM, Kahrs JC, Adrien N, et al. Bias analyses to investigate the impact of differential participation: Application to a birth defects case-control study. *Paediatr Perinat Epidemiol*. 2024;38:535-543. doi:10.1111/ppe.13026