Causal inference in multi-cohort studies using the target trial approach

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Abstract
Longitudinal cohort studies provide the opportunity to examine causal effects of complex exposures on long-term health outcomes. Utilizing data from multiple cohorts has the potential to add further benefit by improving precision of estimates through data pooling and allowing examination of effect heterogeneity across contexts. However, the interpretation of findings can be complicated by biases that may be compounded when pooling data or may contribute to discrepant findings when analyses are replicated across cohorts. Here we extend the “target trial” framework, already well established as a powerful tool for causal inference in single-cohort studies, to address the specific challenges that can arise in the multi-cohort setting. The approach considers the target trial as a central point of reference, as opposed to comparing one study to another. This enables clear definition of the target estimand and systematic consideration of sources of bias within each cohort and additional sources of bias arising from data pooling. Consequently, analyses can be designed to reduce these biases and the resulting findings appropriately interpreted. We use a case study to demonstrate the approach and its potential to strengthen causal inference in multi-cohort studies through improved analysis design and clarity in the interpretation of findings.

Key words
causal inference; target trial; bias; multi-cohort; data pooling; replication; cohort study
Causal inference, understood as the examination of the impact of potential interventions (1), is a common goal in health research, where the ultimate intent is to inform future action that will improve patient or population outcomes. Randomized controlled trials (RCTs) are considered the “gold standard” for causal inference, however, it is often not feasible, ethical, or timely to implement an RCT design (2). For example, in child and adolescent health, there is a pressing need to identify targets for preventive intervention (3) to counter risk associated with seminal factors such as obesity (4), mental disorders (5), and allergic diseases (6), that can track forward to adult non-communicable diseases (7-11). However, this cannot feasibly be achieved in an RCT, because of the long time-frame for outcomes and ethical issues in randomizing critical determinants like childhood adversity or poverty.

Observational longitudinal cohort studies offer a viable alternative to address these causal questions (12-14). Relative to RCTs, observational studies may suffer from higher risks of bias, most notably confounding bias, which may threaten the validity of causal inferences. However, recent methodological advances have developed improved understanding of types of bias as well as methods to tackle them that are now widely used in cohort research (1). In particular, the “target trial” approach (2) is a powerful tool for the planning and interpretation of causal analyses in observational data, including single-cohort studies (15-19).

Even so, single-cohort studies may present further limitations in terms of sample size, in particular to investigate rare events and exposures (20) as well as effect heterogeneity among subgroups (effect modification). Also, the potential specificity of effects in terms of setting, population and epoch may hinder the generalizability of findings to other contexts. Furthermore, it is possible that estimated effect sizes from single cohorts, even in large well-designed studies, may be exaggerated (12) and not replicable when investigations are repeated in new data (21).

To address these limitations, the use of individual-level data from multiple independent cohorts has become increasingly widespread (22-31). More precise estimation of a single causal effect can be achieved by the integration of harmonized data from multiple cohorts into a single dataset, on which analyses are performed directly (pooled-data analysis, also known as one-step individual participant meta-analysis) (20, 32), or by synthesis of effect estimates obtained from analyses of individual-level data from each cohort separately (two-step individual participant meta-analysis) (33, 34). Furthermore, investigation of effect
heterogeneity across contexts may be achieved by obtaining and comparing estimates in each cohort separately (*replication of analyses with comparison*), by random-effects two-stage individual participant meta-analysis, or by pooled-data analysis including specification of a cohort interaction term. We have recently summarized these various approaches elsewhere (35).

Crucially, as with any causal problem, the reliability of causal inferences arising from the application of any of these analytic approaches is dependent on a thorough understanding and tackling of sources of bias. Compared with single-cohort studies, undertaking causal inference in multi-cohort studies faces additional challenges in this regard. When pooling data, biases that arise in each study may be compounded and new biases introduced. Meanwhile, when investigating effect heterogeneity across contexts represented by a specific difference between cohorts (e.g., geographical location), interpretation may be complicated as different biases within each study may contribute to differences in estimated effects. This makes clear causal thinking, which includes clear definition of the target estimand and systematic consideration of sources of bias, critically important in the conduct of multi-cohort studies. There is currently little guidance on how to do this and the need for thorough planning of causal analyses when combining data from multiple sources has recently been highlighted, to assist in the understanding of what can be achieved with the data at hand prior to analysis (36).

The aim of this paper is to propose how the target trial framework can be extended to address the specific challenges that can arise in the conduct of causal inference in the multi-cohort setting. We review the approach in single-cohort studies, describe the extension to multi-cohort studies, and then use a case study to demonstrate its value in understanding potential biases associated with multi-cohort studies to inform analysis design and interpretation. We conclude by offering some guidance for planning analyses to address identified biases.

**The target trial framework: single-cohort studies**

The target trial approach involves specifying the ideal randomised experiment that would hypothetically be implemented to answer the research question of interest (2). A detailed description of this hypothetical randomised trial is developed by specifying the key protocol components of eligibility criteria, treatment strategies, assignment procedures, follow-up period, and outcome measures. This clear articulation of the target trial yields refined
definitions of the research question and corresponding estimand, which represents the causal effect to be estimated (15).

The next step is to consider the assumptions under which one may emulate the target trial with the observational data available to obtain an unbiased estimate of the target causal effect. Bias refers to a discrepancy between the value of the causal effect that is estimated from a study (on average over replications) and its true value, that is, the value of the effect in the target trial. In emulating each of the target trial protocol components, there are corresponding analysis decisions to consider, each of which can reduce or introduce bias depending on the assumed causal relationships between the variables in the study. Construction of a causal diagram or directed acyclic graph (DAG) to describe these assumptions helps to identify potential biases (1). Specifically, there are three key causal biases that are important to consider: confounding bias, selection bias, and measurement bias. These are summarised in Table 1 and illustrated using DAGs in Figures 1A, 2A and 3A. These arise from biasing paths between exposure and outcome that introduce a non-causal association. With a detailed understanding of the potential sources of these three key causal biases, statistical analyses can be planned to: 1) best reduce or counter them; 2) avoid introducing new biases; and 3) allow for thoughtful interpretation of findings considering potentially remaining biases.

**The target trial framework: multi-cohort studies**

Here we focus on multi-cohort studies that aim either to obtain a more precise estimate of a causal effect via pooled-data analysis or to examine heterogeneity of a causal effect across contexts represented by specific differences between cohorts using replication of analyses.

The initial step (as in a single-cohort study) is to define the causal effect of interest by specifying the target trial. The next step is to consider emulating the target trial within each of the contributing cohort studies separately. Indeed, we propose that the critical point to the application of this framework in the multi-cohort setting is that the ideal hypothetical target trial provides the central point of reference for the consideration of sources of biases, as opposed to comparing one cohort to another which is the usual tendency. As detailed in the next section, taking this approach enables disentagling biases arising from different sources so that they may be appropriately addressed in analyses where possible and considered in the interpretation of findings.
When combining cohorts to improve precision there are two distinct sources of bias. Firstly, there may be causal biases in the emulation of the target trial in each cohort that are compounded. We refer to these as “within-cohort” biases. It is possible that these biases operate in the same or opposite directions so it can be difficult to determine in each instance whether combining multiple cohorts together results in higher or lower bias overall. Secondly, there may be additional biases that are introduced through systematic differences between cohorts in terms of study features such as calendar period, geographic location, recruitment and assessment methods, and personnel. We refer to these as “across-cohort” biases. As illustrated in the next section (see also Figures 1B, 2B, 3B), these biases may take the form of confounding bias due to additional common causes of exposure and outcome, selection bias due to additional common causes of study participation or missing data and the outcome, and measurement bias due to measurement error being different between the cohorts, which may be exacerbated in the harmonisation process required to create a single integrated dataset.

When investigating effect heterogeneity, the research question aims to examine the difference in a causal effect across contexts represented by a specific difference between cohorts (e.g., geographical location). It follows that the factor defining the different contexts to which the question relates does not form part of the definition of the causal effect, that is, of the description of the target trial. All other aspects that are not the focus of the research question should form part of the causal effect definition (e.g., population of a specific calendar period) and thus differences between the cohorts in these aspects will be identified as within-cohort biases relative to the target trial. An estimated difference in the causal effects across cohorts may thus arise due to an actual difference in the causal effect across contexts or it may arise due to different within-cohort biases in each target trial emulation. Alternatively, it may be due to random variability. Unfortunately, it is often extremely challenging to pinpoint the source of discrepancies. Use of the target trial framework will help to identify and thus plan to minimise within-cohort biases as much as possible, and outline potential remaining biases to inform interpretation.

Example case study
We consider the published multi-cohort study by Spry et al. (37), which examines the extent to which preconception maternal mental health in both adolescence and young adulthood affects children’s early life behavioural outcomes.

Data sources
Data from two Australian prospective longitudinal cohort studies were utilized.

The Victorian Intergenerational Health Cohort Study (VIHCS) is a prospective study of preconception predictors of infant and child health (38). It arose from an existing cohort study, VAHCS (39), which commenced in 1992 and recruited a sample of Victorian mid-secondary school students (N=1943; 1000 female). Participants were assessed six-monthly during adolescence and three times in young adulthood. Between 2006 and 2013, VAHCS participants (aged 29–35 years) were screened six-monthly for pregnancies. Participants reporting a pregnancy or recently born infant were invited to participate in VIHCS, and asked to complete telephone interviews in trimester 3, 2 months and 1 year postpartum for each infant born during screening, with follow-up assessments now continuing into offspring childhood and adolescence.

The Australian Temperament Project, Generation 3 (ATPG3) study is an ongoing prospective study of infants born to a long-running population-based cohort (40). The original study (ATP) commenced in 1983 and has followed the social and emotional health and development of the main cohort (Generation 2, N=2443) since they were 4–8 months old, along with their parents (Generation 1). The original sample was recruited through maternal and child health centres in urban and rural local government areas in Victoria. Families were invited to complete mail surveys every 1–2 years until 19–20 years of age and every 4 years thereafter (41). Recruitment of the Generation 3 infant offspring occurred via six-monthly screening for pregnancies between 2012 and 2018, when participants were aged 29–35 years. Interviews were conducted in the third trimester, 2 months and 1 year postpartum, with follow-up assessments now continuing into offspring childhood.

Objectives of multi-cohort design & published findings
The study aimed to obtain precise estimates of the causal effects of preconception maternal mental health problems in both adolescence and young adulthood on infant emotional reactivity at 1 year postpartum, for which a pooled-data analysis of the two cohorts was
performed. This was reported as the primary finding, while replication of analyses were conducted as secondary analyses. As expected, pooled-data analysis achieved superior precision and when the cohorts were considered separately, some degree of discrepancy between the causal effect estimates was observed (Table 2).

**Application of the target trial approach to the case study**

Table 3 outlines a proposed target trial and corresponding emulation strategies for the two cohorts (VIHCS and ATPG3) based on the analysis approach described in Spry et al. (37). Considering each protocol component in turn, we first identify the within-cohort biases (e.g., Figures 1A, 2A, 3A), which may have been compounded in the pooled-data analysis and may explain observed discrepancies in the replication of analyses. We then describe additional across-cohort biases that may have arisen when combining the two cohorts in a pooled-data analysis, the study’s primary analysis approach (e.g., Figures 1B, 2B, 3B). A second illustration based on another case study (42) is presented in the Supplementary Material, to highlight different aspects of the application of the approach when undertaking replication of analyses.

**Eligibility criteria**

In the target trial, the population of interest is defined as adolescent females (13 years) in Australia. Both VAHCS and ATP were designed to recruit close-to-representative samples of participants but despite this, there is potential for selection bias within each cohort due to non-participation of sampled individuals. Additionally, both cohorts only captured births when mothers were aged 29–35 years. The DAG in Figure 2A depicts the structure of this selection bias, with maternal age at birth a common cause of study participation and the outcome. Analyses of either cohort will be restricted to participants, which is represented in the DAG as conditioning on study participation. This would lead to a biasing path between the exposure and the outcome via maternal age, potentially introducing selection bias unless the analysis approach specifically addresses this (see next section).

Given the long-term intergenerational nature of these studies, and despite high retention rates, there is also potential for selection bias in each cohort due to missing data in any variable relevant to the analysis, for example due to study dropout, if the analysis is restricted to participants without missing data (the so-called “complete cases”). This could be depicted in a DAG similar to that in Figure 2A with the study participation node replaced with a
“complete case” indicator. Alternative approaches to handling missing data can avoid this (next section).

There is potential for additional across-cohort biases due to systematic between-cohort differences in factors that predict both study participation and the outcome. For example, the two cohorts utilized different recruitment strategies: VAHCS in adolescence through secondary schools and ATP in infancy through maternal child health services. Different factors during these distinct life stages may have influenced family decisions to participate. Furthermore, other between-cohort differences such as period of recruitment for example, may be predictors of the outcome. Together, this creates a further biasing path between exposure and outcome that is introduced by the restriction to participants (Figure 2B). Cohort differences in factors that are common causes of missing data and the outcome, such as study personnel and assessment tools/methods, may introduce additional selection bias if the analyses are restricted to complete cases, which again could be depicted in a similar DAG to Figure 2B.

**Treatment strategies**

The exposure of interest is preconception maternal mental health problems in adolescence (age 13–18 years) and young adulthood (age 19–29 years). When thinking about the target trial, this is an example of an imprecisely-defined intervention. While an important and worthwhile intervention target, it is not clear by what intervention we might be able to change mental health problems. It could be medication, individual therapy and/or population-level interventions. This lack of precise articulation within the target trial complicates interpretation of findings and the selection of confounders (43). This is a common issue in studies asking complex causal questions of this nature, which reinforces the need for clear causal thinking and in particular the benefits of using the target trial framework (15).

Beyond the issue of imprecisely-defined interventions, measuring mental health problems is inherently challenging. It is a nebulous construct, that is not directly observable and can manifest differently over contexts and time (44). Both cohorts utilized well-regarded assessment tools with high internal reliability and construct validity for the population of interest. However, the best such tools can hope to achieve is to approximate the intended underlying construct of mental health problems, making some degree of measurement error unavoidable. Additionally, different measurement tools were used across waves in VIHCS,
and it is possible that these capture overlapping but slightly discrepant constructs (for example, emphasising different dimensions of internalising symptomology). Further to that, measurement of the exposure relies on mother self-report and although this perspective is valuable, perceived symptoms may be downplayed in reporting due to feelings of guilt, shame or embarrassment (45) leading to potential measurement bias within each cohort. This is illustrated in Figure 3A, where there is a biasing path between the exposure and the outcome via the measured exposure.

The risk of measurement bias is increased in the pooled-data analysis due to the necessary process of harmonisation to obtain a unified dichotomous indicator from the different assessment tools used to measure mental health problems in ATPG3 and VIHCS. This inevitably requires some loss of information, hence the resulting measure in each study may have increased and potentially discrepant measurement error, which could introduce across-cohort measurement bias due to differential misclassification. Reassuringly, the prevalence of preconception maternal mental health problems was consistent across cohorts suggesting that it is plausible that they are, overall, both capturing a common construct. Even so, the potential for additional across-cohort measurement bias remains an issue as it could be that, beyond issues introduced by harmonisation, the factors affecting measurement error differ systematically across the cohorts in a way that introduces further bias (Figure 3B).

Assignment procedures
In the target trial, individuals would be randomized to experiencing mental health problems or not, creating two balanced groups that would prevent confounding bias in the estimation of the causal effect. Clearly, however, even if we had defined a specific intervention to achieve this, it would be unethical. Focusing on the available data from each cohort, a number of measured confounders were identified, based on prior evidence in the literature, representing socioeconomic circumstances (education, family composition) and adolescent smoking. As common causes of both the exposure and the outcome, these measured confounders induce a biasing path between them (Figure 1A). Even after adjusting for these confounders, there remains potential for further confounding bias due to biasing paths from the exposure to the outcome via unmeasured confounders (also depicted in Figure 1A). Spry et al. (37) considered genetic susceptibility, stressful life events, family violence or trauma, and perceived social support to be potential sources of unmeasured confounding. There is also potential for residual confounding bias within each cohort due to measurement error in the
use of proxies for the confounders. Family variables such as high-school completion and divorce were used as proxies for socioeconomic circumstances in the absence of more direct measures. Additionally, inaccurate reporting of confounders that reveal sensitive information such as smoking history or family divorce is a potential source of within-cohort measurement bias.

There is potential for exacerbation of within-cohort confounding bias due to measurement error in the necessary harmonisation of confounder variables. For example, the categories used to measure maternal education were not aligned between VIHCS and ATPG3 hence there is some loss of information in the blunt harmonised measure of “never completed” versus “ever completed” high school. Finally, some confounders may be available in only a subset of cohorts so cannot be included in the adjustment set, increasing the potential for confounding bias.

Additional across-cohort confounding biases may arise in the pooled-data analysis due to additional biasing pathways between exposure and outcome because of systematic between-cohort differences in design aspects such as calendar period, geographic location, and study team (Figure 1B). For example, recruitment of mothers and their infants took place in 2006–2013 for VIHCS and in 2012–2018 for ATPG3; period effects on exposure and outcome assessment could induce additional confounding bias.

*Follow-up period*

Consistent with the target trial, follow-up in both cohorts commenced when mothers were in adolescence and concluded at 1 year postpartum. There is potential for within-cohort measurement error (and thus bias) as a result of the exposure and outcome measurements not being taken at exactly the same time-points for all participants (e.g., Figure 3A).

Additional across-cohort measurement bias is possible due to systematic between-cohort differences in the timing of measurements of exposure, confounders and outcome (e.g., Figure 3B). For example, mothers in ATPG3 were slightly younger at each of the three assessments of mental health problems in young adulthood and there was also considerable variation between (and within) cohorts in maternal age at birth and consequently at 1 year postpartum.
**Outcome measure**

Both cohorts used the Short Temperament Scale for Toddlers (STST) via maternal report at 1 year postpartum with a mean score of ≥4 indicative of heightened infant emotional reactivity, the primary outcome defined in the target trial. As has already been identified for the exposure and confounders, there is potential for measurement bias in the use of STST as a proxy for the intended construct of infant emotional reactivity. Measurement error in the outcome could be depicted in Figure 3A by adding a node for “measured outcome \([Y^*]\)” and an arrow from the true outcome \([Y]\) to \(Y^*\). Measurement bias could arise due to common predictors of measurement error in the exposure \([X_i^*]\) and outcome \([Y^*]\). Measurement bias due to inaccurate reporting is also an issue here. Spry et al. (37) identified that maternal report of infant outcomes may be affected by a mother’s mental state such that depressed mothers perceive their infant as more reactive. This could also be depicted in Figure 3A by further adding an arrow from the true exposure \([X]\) to \(Y^*\).

As with measurement of the exposure and confounders, there is potential for additional across-cohort bias in the pooled-data analysis due to systematic between-cohort differences in measurement of the outcome. This is less of a concern here, however, since heightened infant emotional reactivity was measured consistently in both cohorts albeit the distribution of maternal age when the child was 1 year old varied across cohorts (see follow-up period).

**Guidance for planning and reporting analyses to address the identified biases**

Once potential sources of within- and across-cohort biases have been clearly identified, the next step is to plan an analysis approach that will best counter them. It will not be possible to completely mitigate all causal biases, but it is important to plan an analysis strategy that will diminish real causal bias threats as much as possible and not introduce new ones, for example, through conditioning on a “collider” (see Table 1).

**Selection bias**

Missing data methods such as multiple imputation (46) and inverse probability weighting (47) are widely utilized for countering selection bias due to study participation, loss to follow-up and other missing data in study variables. Modern implementations of multiple imputation (48, 49) provide a flexible approach to handle multivariable missingness problems, allowing specification of complex imputation models including all analysis variables in addition to predictors of incomplete variables, particularly if they are also
predictors of missingness, such as maternal age in Figure 2A. In pooled analyses, the cohort indicator (i.e., a variable indicating which cohort each participant comes from) is one such variable (Figure 2B), and as such should be included as a covariate in multiple imputation. Alternatively, when conducting replication of analyses, it is recommended that multiple imputation be performed on each cohort separately (50), particularly when cohorts represent distinct populations, settings and/or time periods. This was the approach taken by Spry et al. (37) and allows imputation models to be optimally tailored to each cohort.

Confounding bias
There are two commonly used classes of analytic approaches for addressing confounding bias: conditioning-based methods (e.g., multivariable outcome regression) and standardisation-based methods (or “G-methods”, e.g., IPW, g-computation) (1). All these methods require modelling either the outcome or the exposure based on the selected confounder set. So-called “doubly-robust” methods use models for both processes and reduce the risk of model misspecification bias due to their good performance when at least one of the models is consistently estimated and because they can be coupled with machine learning (51). Regardless of the analytic method, when performing a pooled-data analysis, it is critical to include the cohort indicator as an additional confounder in order to address any across-cohort confounding bias due to systematic differences in study features (Figure 1B). This was the approach taken in Spry et al. (37).

Measurement bias
Addressing sources of measurement bias in cohort studies is complex, particularly in multi-cohort studies where harmonisation of measures to create a single integrated data set is required and may entail simplification of measures (e.g., collapsing categories) to find a minimum common ground among the multiple cohorts. Specialised methods to handle measurement error such as “regression calibration” (52) exist, though these usually require strong assumptions and validation samples. As with confounding bias, inclusion of the cohort indicator in a pooled-data analysis will address across-cohort measurement biases due to systematic between-cohort differences (Figure 3B).

Sensitivity analysis
Sensitivity analyses play an important role in exploring the robustness of findings to key assumptions underpinning the chosen statistical analysis approach. This is particularly
relevant for multi-cohort studies where it may be of interest to explore the ramifications of data integration, by repeating the analysis under different harmonisation decisions. Formal approaches such as quantitative bias analysis (53) that quantify the direction and magnitude of systematic biases may also be valuable. These methods are increasingly recommended for individual cohort analyses and we recommend they also be applied to pooled-data analyses, with their application being guided by DAGs expanded with the cohort indicator (e.g., Figures 1B, 2B, 3B).

Pooled-data analysis vs. replication of analyses

As previously emphasised, while pooled-data analysis and replication of analyses target different aims (increased precision vs. examination of effect heterogeneity), the data available and how well the multiple datasets can be harmonised may limit possible use of the former. If there are fundamental aspects of the study designs that do not meaningfully align and will thus compound biases in a way that will be hard to assess, then replication of analyses that acknowledges these differences and consequently expresses caution in the interpretation of the results would be more appropriate. This is also a sensible approach if the data harmonisation process results in too great a loss of information from one or both cohorts resulting in increased risk of bias.

In replication of analyses it is possible to tailor analysis methods to each cohort individually to account for different study designs, sampling mechanisms and available confounders – to engage with each cohort ‘on its own terms’. This has the potential to better reduce biases within each cohort, however, it does introduce complexity in interpretation of the results when these are discrepant (32). As demonstrated in Spry et al. (37), even when pooled-data analyses are able to be performed as the primary analyses, it is good practice to also report cohort-specific results as sensitivity analyses to explore the consistency of findings across cohorts.

Reporting

Finally, the complexity and extensive data wrangling involved in multi-cohort approaches reinforce the need and value to undertake this work with an Open Science lens. Reporting of results should include a clear and detailed description of the analysis approach, including where possible provision of code. This promotes transparency of the assumptions made and
allows thorough consideration of study limitations, thus providing context for the appropriate interpretation of study findings.

**Conclusion**

The target trial is a powerful tool for improving the conduct of causal inference in observational studies, by enabling explicit definition of the target estimand and systematic assessment of potential sources of bias. We have described the application of this framework to multi-cohort studies, clarifying that the target trial is the reference point for identifying biases as opposed to comparing studies to each other. Using this approach, it is possible to identify biases within each cohort individually and those that may be introduced when combining data from multiple cohorts. Disentangling biases arising from different sources helps better harness the risk of bias in analyses and inform the interpretation of findings, in particular discrepant findings across cohorts. As such, use of the target trial framework in multi-cohort studies can help strengthen causal inferences through improved analysis design, transparency in the assumptions and clarity in reporting and interpretation.

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Table 1: A summary of the three key causal biases that are important to consider when working through a target trial emulation: confounding bias; selection bias; and measurement bias.

| Bias            | Description                                                                                                                                                                                                 | Target trial protocol component where bias can arise                                      |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| **Confounding bias** | Arises from differences between exposure groups in terms of individual, pre-exposure characteristics that are also related to the outcome. Formally, in a causal diagram or directed acyclic graph (DAG), confounding bias results from an open non-causal (biasing) “backdoor path” between the exposure and the outcome via a common cause, which represents an additional source of association between these (see Figure 1A for example). | Assignment procedures due to lack of randomisation of the treatment strategies             |
| **Selection bias** | Occurs when the sample used for analysis is not representative of the target population due to, for example, individuals with certain characteristics being more likely to not participate or be lost from the study over time. In a DAG, selection bias is represented as arising from a non-causal (biasing) path that becomes open after conditioning on a common effect of two variables (known as a “collider”), one of which is either the treatment or a cause of treatment, and the other is either the outcome or a cause of the outcome (see Figure 2A). | Eligibility criteria                                                                      |
| **Measurement bias** | Refers to bias that arises as a result of measurement error, which reflects a discrepancy between the measured value of a quantity and its true value. This includes misclassification in the case of categorical data. Measurement bias can be depicted in a DAG in many ways; Figure 3A provides an example of bias arising from measurement error in the exposure, with a non-causal (biasing) path that becomes open when using the measured exposure. | Treatment strategies (exposure measurement) Assignment procedures (confounder measurement) Follow-up period (timing of measurements) Outcome measures (outcome measurement) |
Table 2: Estimated causal effects of preconception maternal mental health problems in adolescence and young adulthood on offspring infant emotional reactivity in pooled analysis and replication of analyses reported in Spry et al. (37).

|                        | Odds ratio | 95% CI  |
|------------------------|------------|---------|
| **Pooled data analysis** |            |         |
| No preconception maternal mental health problems (unexposed) |            |         |
| Preconception maternal mental health problems (exposed) |            |         |
| In adolescence only    | 1.3        | (0.9, 2.0) |
| In young adulthood only| 1.3        | (0.7, 2.1) |
| In adolescence and young adulthood | 2.1        | (1.4, 3.1) |
| **Replication of analyses** |            |         |
| VIHCS                  |            |         |
| No preconception maternal mental health problems (unexposed) |            |         |
| Preconception maternal mental health problems (exposed) |            |         |
| In adolescence only    | 1.2        | (0.6, 2.3) |
| In young adulthood only| 1.8        | (0.8, 3.9) |
| In adolescence and young adulthood | 2.4        | (1.3, 4.2) |
| ATPG3                  |            |         |
| No preconception maternal mental health problems (unexposed) |            |         |
| Preconception maternal mental health problems (exposed) |            |         |
| In adolescence only    | 1.5        | (0.8, 2.7) |
| In young adulthood only| 0.9        | (0.4, 2.0) |
| In adolescence and young adulthood | 1.9        | (1.1, 3.4) |
Table 3: Proposed target trial and emulation implicit in the statistical analysis approach of Spry et al. (37), focusing solely on the causal effect of preconception maternal mental health problems in young adulthood on offspring infant emotional reactivity.

| Protocol component | Target trial | VIHCS | Emulation | ATPG3 |
|--------------------|--------------|-------|-----------|-------|
| **A. Eligibility criteria** | TARGET POPULATION: Young adolescent females (13 years of age) in Australia and their subsequently-born infants | SAMPLE SELECTION: Female VAHCS study participants (close-to representative sample of 1992 Victorian mid-secondary school students), who subsequently reported pregnancy or recently born infant between 2006 and 2013 (29–35 years) when screened. All participants were retained in the sample regardless of missing data via use of multiple imputation. | SAMPLE SELECTION: Female ATP study participants (recruited through rural and urban Victorian Maternal and Child Health centres at 4–8 months of age in 1983), who subsequently reported pregnancy or recently born infant between 2012 and 2018 (29–35 years) when screened. All participants were retained in the sample regardless of missing data via use of multiple imputation. |
| **B. Treatment strategies** | TREATMENT ARMS: | TREATMENT/EXPOSURE MEASURE: | TREATMENT/EXPOSURE MEASURE: |
| | 1. Preconception maternal mental health problems in adolescence (13–18 years) only | Intervention arms: | Intervention arm: |
| | 2. Preconception maternal mental health problems in young adulthood (19–29 years) only | | 1. The presence of any mental health problems at ≥1 wave in adolescence (ATP waves 10–12) |
| | 3. Preconception maternal mental health problems in adolescence (13–18 years) and young adulthood (19–29 years) | Comparator arm: | 2. The presence of any mental health problems at ≥1 wave in young adulthood (ATP waves 13–15) |
| | Comparator arm: | No mental health problems (VAHCS waves 2–9) | Comparator arm: |
| | No preconception maternal mental health | Mental health problem measure: | No mental health problems (ATP waves 10–15) |
| | | Waves 2–7: CIS-R ≥12 | Mental health problem measure: |
| | | Waves 8–9: GHQ-12 ≥3 | Wave 10: SMFQ ≥11 or RBPCS mean ≥1 |
| | | | Waves 11–12: SMFQ ≥11 or RCMA mean ≥1 |
| | | | Waves 13–15: DASS-21, Depression ≥7 or Anxiety ≥6 or Stress ≥10 |
problems in adolescence (13–18 years) or young adulthood (19–29 years)

| C. Assignment procedures | Randomisation at recruitment without blind assignment | Selection of confounders: Confounder (self-reported measure) |
|--------------------------|-----------------------------------------------------|----------------------------------------------------------------|
|                          |                                                     | • Mother’s parent’s high school completion (neither parent vs. at least one parent completed) |
|                          |                                                     | • Mother’s parent’s divorce/separation during or before adolescence (ever vs. never divorced/separated) |
|                          |                                                     | • Mother’s high school completion (ever vs. never completed) |
|                          |                                                     | • Mother’s adolescent smoking (daily smoking at \( \geq 1 \) adolescent wave vs. no daily smoking) |
|                          |                                                     | • Mother’s history of divorce/separation (ever vs. never divorced/separated) |
|                          |                                                     | Approach to adjustment: Regression |

| D. Follow-up period | Follow-up: At randomisation (mother aged 13 years) | Timing of measures: |
|---------------------|----------------------------------------------------|-------------------|
| Start: At randomisation (mother aged 13 years) | Start: VAHCS wave 2 (mother aged 14–15 years old) | Start: ATP wave 10 (mother aged 13–14 years old) |
| End: Child aged 1 year | End: VIHCS wave 3 (child aged 1 year old) | End: ATPG3 wave 3 (child aged 1 year old) |

| E. Outcome | Outcome: Offspring infant emotional reactivity | Outcome measure: STST via maternal report at 1 year postpartum, mean score \( \geq 4 \) |
|------------|-----------------------------------------------|-------------------------------------------------------------|

| F. Causal effects of interest | Odds ratio of risk of offspring infant emotional reactivity in each intervention arm relative to the comparator arm in the target population |

(VIHCS: Victorian Intergenerational Health Cohort Study; ATPG3: Australian Temperament Project Generation 3; VAHCS: Victorian Adolescent Health Cohort Study; ATP: Australian Temperament Project; CIS-R: Clinical Interview Schedule – Revised; GHQ-12: General Health Questionnaire; SMFQ: Short Mood and Feelings Questionnaire; RBPCS: Revised Behavior Problem Checklist Short Form; RCMAS: Revised Children’s Manifest Anxiety Scale; DASS-21: Depression Anxiety Stress Scales; STST: Short Temperament Scale for Toddlers).
Figure 1: Directed Acyclic Graphs (DAGs) depicting examples of: A) confounding bias in a single cohort (within-cohort confounding bias); and B) additional confounding biases in pooled analyses of multiple cohorts (across-cohort confounding bias).
Figure 2: Directed Acyclic Graphs (DAGs) depicting examples of: A) selection bias in a single cohort (within-cohort selection bias); and B) additional selection biases in pooled analyses of multiple cohorts (across-cohort selection bias).
Figure 3: Directed Acyclic Graphs (DAGs) depicting examples of: A) measurement bias in a single cohort (within-cohort measurement bias); and B) additional measurement biases in pooled analyses of multiple cohorts (across-cohort measurement bias). For the case of \( k \) cohorts, there could be up to \( k \) different exposure measures \( X_i^*, \ i = 1, ..., k \), from which the pooled exposure measure \( X^{**} \) is derived.
Causal inference in multi-cohort studies using the target trial approach:
Supplementary Material

Description of second case study
O’Connor et al. (1) aimed to investigate the extent to which exposure to adversity negatively impacts inflammation in mid to late childhood, where inflammation was proposed as a central mechanism through which exposure to childhood adversity translates to disease risk, in particular cardiovascular disease risk (2, 3).

Data sources
Data from two Australian prospective longitudinal cohort studies were utilized.

The Barwon Infant Study (BIS) is a population-derived birth cohort study (N=1074 infants) with antenatal recruitment (at approximately 15 weeks of pregnancy) during 2010–2013, conducted in the Barwon region of Victoria, Australia (4). The study was originally designed to explore the early life origins of a range of non-communicable diseases in the modern environment. Participants completed self-reported structured questionnaires as well as clinical and biological measurements at birth and at 1, 6, 9 and 12 months, and at 2 and 4 years, with a primary school (8–10 years) review under way in 2020–21. Data on inflammatory biomarkers were available for N =510 children at the four-year review. Ethical approval for this methodology was obtained from the Barwon Health Human Research Ethics Committee.

The Longitudinal Study of Australian Children (LSAC) is a nationally representative study of two cohorts, including a birth cohort (N=5107 infants), aiming to investigate a broad range of aspects of development and wellbeing over the lifecourse, with 9 waves of bi-annual data collection so far. In 2003–2004, a multistage cluster sampling design utilising the comprehensive national Medicare database was employed to select a sample that was broadly representative of all Australian children except those living in remote geographic areas (5). In 2015, a comprehensive, one-off physical health and biomarker module, known as the Child Health CheckPoint, was conducted for the birth cohort between waves 6 and 7, when children were 11–12 years of age (6). Approximately half (53%, N=1874 families) of the Wave 6
sample participated in the Child Health CheckPoint (7). The study is overseen by the Australian Institute of Family Studies human ethics review board.

**Objectives of multi-cohort design & published findings**
This study aimed to investigate heterogeneity in the causal effect of exposure to adversity on inflammation across the different endpoint time frames of mid-childhood (4 years) and late-childhood (11–12 years), for which replication of analyses were performed. Cohort-specific analyses were reported and small associations between exposure to adversity and increased inflammation were consistently observed across both cohorts, however, effects were imprecisely estimated.

**Application of the target trial framework**
Supplementary Table 1 shows how the target trial framework can be applied based on the statistical analysis approach described in O’Connor et al. (1). The paper examined multiple definitions of adversity including a binary exposure to each of several different types of adversity, a cumulative count of the types of adversities experienced, and initial timing of exposure to adversity. For simplicity, here we consider a binary indicator of exposure to any type of adversity. The table defines the target trial, outlines the proposed emulation strategies for each of BIS and LSAC separately and identifies potential remaining “within-cohort biases”. Given this case study focuses primarily on replication of analyses, we then discuss other aspects apart from remaining biases that may explain discrepant findings across cohorts.
**Supplementary Table 1:** Proposed target trial and emulation based on the statistical analysis approach described in O’Connor et al. (1) for considering the causal effect of exposure to adversity on inflammation in mid to late childhood.

| Protocol component | Target trial | BIS | Emulation | LSAC | Remaining within-cohort bias risks |
|--------------------|--------------|-----|-----------|------|-----------------------------------|
| **A. Eligibility criteria** | TARGET POPULATION: Australian infants at birth in early 2000s | SAMPLE SELECTION: BIS participants, who were recruited through pregnant women attending antenatal appointments at approximately 15 weeks during 2010–2013, in Barwon region of Victoria (south-east Australia). All BIS participants were retained in the sample regardless of missing data via use of multiple imputation. | SAMPLE SELECTION: LSAC participants who then participated in the Child Health CheckPoint, a one-off physical health assessment at 11–12 years. LSAC is a cohort of Australian infants aged 0–1 years in 2004 recruited through multi-stage cluster sampling of the comprehensive Medicare database. All CheckPoint participants were retained in the sample regardless of missing data via use of multiple imputation. | • Risk of selection bias due to each study’s sample selection strategy (e.g., calendar period, geographic location, recruitment procedure) capturing only a subset of the target population • Risk of selection bias due to non-participation: - In BIS, baseline cohort characteristics similar to AUS population, except a smaller proportion of families from non-English speaking backgrounds - In LSAC, baseline cohort characteristics broadly representative of AUS population, except a smaller proportion of children living in highly remote geographic areas • Risk of selection bias (in each of BIS and LSAC) due to loss to follow-up/missing data in any analysis variable; mitigated in both cohorts by use of multiple imputation on all missing data |
| B. Treatment strategies | TREATMENT ARMS: | TREATMENT/ EXPOSURE MEASURE: | TREATMENT/ EXPOSURE MEASURE: | • Risk of measurement bias due to: |
|-------------------------|----------------|-----------------------------|-----------------------------|----------------------------------|
|                         | Intervention arm: | Exposed to adversity at any measured time point(s) during childhood | Exposed to adversity at any measured time point(s) during childhood | - The use of imperfect measures of childhood adversity, e.g., parental mental illness measured in BIS using the Edinburgh Postnatal Depression score > 13 (depression likely) and in LSAC using K6 scale > 13 (high psychological distress) |
|                         | Comparator arm: | Never exposed to adversity during childhood | Never exposed to adversity during childhood | - The full range of adversity experienced during childhood not being adequately captured, e.g., racial discrimination |
|                         |                  | Adversity measured as parent-reported presence of any of seven adverse experiences: | Adversity measured as parent-reported presence of any of seven adverse experiences: | - Some adversities measured using proxies, for example, anger in parental responses scale used as a proxy for child maltreatment |
|                         |                  | - Parent legal problems | - Parent legal problems | - Family circumstances and experience of adversity may alter reporting |
|                         |                  | - Parent mental illness | - Parent mental illness | - Adversity indicators sometimes not including the full interval between waves, for example, responses were made in reference to the past 12 months even if waves were > 12 months apart, meaning some adverse experiences may not have been captured |
|                         |                  | - Parent substance abuse | - Parent substance abuse | - In LSAC, a change in scale of measurement for anger in parental responses (harsh parenting) between waves 2 and 3 |
|                         |                  | - Anger in parenting responses | - Anger in parenting responses | |
|                         |                  | - Separation/divorce | - Separation/divorce | |
|                         |                  | - Unsafe neighbourhood | - Unsafe neighbourhood | |
|                         |                  | - Family member death | - Family member death | |
| Each adversity measured at least once across the waves (but not at all waves): | Each adversity measured at each wave: | | |
| - W1 (1 month) | - W1 (0–1 years) | | |
| - W2 (6 months) | - W2 (2–3 years) | | |
| - W3 (12 months) | - W3 (4–5 years) | | |
| - W4 (2 years) | - W4 (6–7 years) | | |
| - W5 (4 years) | - W5 (8–9 years) | | |
| | - W6 (10–11 years) | | |
C. Assignment procedures

| Assignment procedures | Randomisation at recruitment (birth) without blind assignment |
|-----------------------|---------------------------------------------------------------|
| SELECTION OF CONFOUNDERS: | Confounder (self-reported measure) |
| • Child sex | |
| • Family socioeconomic position (composite of education and income, dichotomised bottom third vs. higher) | |
| • Young maternal age (below or above 23 years) | |
| • Indoor smoking (Y/N, same room as baby) | |
| • Ethnicity (Anglo/European, Ethnic minority) | |
| • BMI (continuous) at 4–5 years | |
| APPROACH TO ADJUSTMENT: | Regression |

D. Follow-up period

| FOLLOW-UP: | Start: At birth |
| Endpoint: Mid-to-late childhood |
| TIMING OF MEASURES: | Start: Wave 0, pregnancy |
| Ends: Wave 5, 4 years |

E. Outcome

| OUTCOME: | Inflammation |
| OUTCOME MEASURE: | Inflammatory markers (continuous, µg/ml): |
| OUTCOME MEASURE: | Inflammatory markers (continuous, µg/ml): |

- In BIS, adversity indicators of unsafe neighbourhood and anger in parenting responses measured at only one wave
- Risk of residual confounding bias due to unmeasured confounding
- Risk of measurement bias due to:
  - The use of proxies for confounders in the absence of more direct measures, e.g., a composite variable of education, occupation and income for socioeconomic position, a composite of language and country of birth for ethnicity, indoor smoking measured using Y/N same room as baby in BIS vs. Y/N any indoor smoking in LSAC
  - Inaccurate reporting of confounders that reveal sensitive information such as income, smoking history
- Risk of measurement bias due to the exposure not being taken at exactly the same time point for all participants
  Note: The difference in the time of outcome measurement is the key factor of interest in the research question, therefore it is not a bias per se but the source of difference to be assessed
- Risk of measurement bias due to:
|                          | hsCRP | hsCRP | Use of inflammatory markers as proxy measures of inflammation |
|--------------------------|-------|-------|---------------------------------------------------------------|
|                          | GlycA | GlycA |                                                              |

**F. Causal effect of interest**

Percentage difference in mean inflammation between intervention and comparator arms in the target population.
Discrepant findings across cohorts could be attributed to discrepant remaining within-cohort biases, detailed in Supplementary Table 1, chance or alternatively may be explained by an actual difference in the causal effect across the two time points at which the inflammation outcome was captured by the studies (mid-childhood at 4 years in BIS vs. late-childhood at 11–12 years in LSAC).

References (for supplementary material)

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