Study Protocol

P3-C3-009

DARWIN EU® – Utilisation of commonly used benzodiazepines during pregnancy and the incidence of pregnancy losses

09/04/2025

Version 2.0

Contents

Study title	DARWIN EU® – Utilisation of commonly used benzodiazepines during pregnancy and the incidence of pregnancy losses
Protocol version	V2.0
Date	09/04/2025
EU PAS number	EUPAS1000000536
Active substance	Benzodiazepines (therapeutic drug class N05BA, N05CD) Selective serotonin reuptake inhibitors (SSRIs, therapeutic drug class N06AB) Selective noradrenaline reuptake inhibitors (SNRIs, therapeutic drug class included within "other antidepressants", N06AX) Z-hypnotics (therapeutic drug class N05CF) Melatonin (therapeutic drug class N05CH)
Medicinal product	NA
Research question and objectives	The specific study objectives are the following: 1. To characterise users of benzodiazepine and alternative treatments during pregnancy in terms of demographics, prior medications, history of mental illness and other comorbidities. 2. To characterise treatments with benzodiazepine and alternative treatments during pregnancy in terms of duration, posology, and indication of prescription during pregnancy. 3. To describe the prevalence of benzodiazepine and alternative treatments' use during pregnancy. 4. To describe trajectories of prescription fills for benzodiazepine and alternative treatments throughout the year before pregnancy, pregnancy period, and the first month after pregnancy end date. 5. To estimate the incidence of pregnancy loss among all pregnancies and in benzodiazepines and alternative treatments users during pregnancy (when numbers allow). 6. To characterise individuals with pregnancy loss in terms of demographics, comorbidities, and treatments of interest. Objectives 1, 2, 5, and 6 will also include stratifications by age categories and pregnancy periods. When counts allow, objectives 2, 3, 4 and 5 will be stratified by each benzodiazepine active ingredient, grouped by benzodiazepines' half-life (short and long acting). When referring to alternative treatments, each alternative treatment will be reported separately (except for objective 4). Exploratory objectives (to assess the suitability of potential negative controls for subsequent analyses) 7. To estimate the incidence rates of potential negative control outcomes (e.g., musculoskeletal injuries, skin conditions, urinary tract infection) in benzodiazepine and alternative treatment users.
Country(ies) of study	Norway, Spain
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LIST OF ABBREVIATIONS

CDM	Common Data Model
DARWIN EU®	Data Analysis and Real-World Interrogation Network European Union
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
GW	Gestational Week
LMP	Last menstrual period
MBRN	Medical Birth Registry of Norway
NLHR	Norwegian Linked Health Registry data
OHDSI	Observational Health Data Sciences and Informatics
OMOP	Observational Medical Outcomes Partnership
PET	Pregnancy Extension Table
PSUSA	Periodic Safety Update Report Single Assessments
PRAC	Pharmacovigilance Risk Assessment Committee
RWE	Real-World Evidence
SIDIAP	Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària
SNRI	Selective noradrenaline reuptake inhibitors
SSRIs	Selective serotonin reuptake inhibitors
UiO	University of Oslo

1. TITLE

DARWIN EU® – Utilisation of commonly used benzodiazepines during pregnancy and the incidence of pregnancy losses

2. RESPONSIBLE PARTIES – STUDY TEAM

Study team role	Names	Organisation
Principal Investigator(s)	Julieta Politi Talita Duarte-Salles	Erasmus MC
Study Project Manager	Natasha Yefimenko	Erasmus MC
Data Scientist	Maarten van Kessel Ioanna Nika Cesar Barbosa Ross Williams Ger Inberg	Erasmus MC
Epidemiologist	Julieta Politi Talita Duarte-Salles Berta Raventós	Erasmus MC
Data Partner*	**Names**	**Organisation**
SIDIAP	Agustina Giuliodori Picco Anna Palomar Cros Irene López-Sánchez Laura Granés González	IDIAPJGol
NLHR	Saeed Hayati Nhung Trinh Hedvig Nordeng Maren Mackenzie Olson	University of Oslo

*Data partners' role is only to execute code at their data source, review and approve their results. They do not have an investigator role. Data analysts/programmers do not have an investigator role and thus declaration of interests (DOI) for them is not needed.

3. ABSTRACT

Title

DARWIN EU® – Utilisation of commonly used benzodiazepines during pregnancy and the incidence of pregnancy losses

Rationale and background

Benzodiazepines are commonly prescribed for their anxiolytic, hypnotic, and sedative effects. Despite the use of benzodiazepines during pregnancy, there is limited evidence to support their use during this period or to favour their use over alternative treatments that may provide similar symptom relief with differing safety profiles. Understanding the patterns of benzodiazepine use during pregnancy in Europe, together with the rates of pregnancy losses, is essential for evaluating safety and effectiveness. Despite detailed pregnancy information in many data sources, pregnancy episodes in electronic health record (EHR) data are often inconsistently coded across sources.

As part of the upcoming benzodiazepines periodic safety update report single assessment (PSUSA), the Pharmacovigilance Risk Assessment Committee (PRAC) has requested real-world evidence (RWE) on the utilisation of commonly used benzodiazepines during pregnancy. Additionally, the background rates of pregnancy losses will be described to help contextualise the assessment of treatment safety during pregnancy. To date, two data partners within the DARWIN EU® Data Network have pre-processed pregnancy episodes and developed a Pregnancy Extension Table (PET). While the table has been successfully employed in other contexts, this study marks the first application of this table within the DARWIN EU® Data Network.

Research question and objectives

Research Question:
This study is meant to inform on the use of benzodiazepine among pregnant individuals and the feasibility of a risk-assessment study that investigates benzodiazepine use during pregnancy.

The specific study objectives are the following:

1. To characterise users of benzodiazepine and alternative treatments (SSRIs, SNRIs, Z-hypnotics, and Melatonin) during pregnancy in terms of demographics, prior medications, history of mental illness and other comorbidities.
2. To characterise treatments with benzodiazepine and alternative treatments during pregnancy in terms of duration, posology, and indication of prescription during pregnancy.
3. To describe the prevalence of benzodiazepine and alternative treatments' use during pregnancy
4. To describe trajectories of prescriptions fills for benzodiazepine and alternative treatments throughout the year before pregnancy, pregnancy period, and one month following pregnancy end date.
5. To estimate the incidence of pregnancy loss among all pregnancies and in benzodiazepines and alternative treatment users during pregnancy (when numbers allow).
6. To characterise individuals with pregnancy loss in terms of demographics, comorbidities, and prior medications.

Objectives 1, 2, 5, and 6 will also include stratification by age categories and pregnancy periods. When counts allow, objectives 2, 3, 4, and 5 will be stratified by each benzodiazepine active ingredient, grouped by benzodiazepines' half-life (short and long-acting). When referring to alternative treatments, each alternative treatment will be reported separately (except for objective 4).

Exploratory objectives (to assess their suitability for subsequent analyses)

7. To estimate the incidence rates of potential negative control outcomes (e.g., musculoskeletal injuries, skin conditions, urinary tract infection) in benzodiazepine and alternative treatment users.

Methods

Study design

- Cohort study design.

Study Period

From 01/01/2010 until 31/12/2023 or the first and last date of data availability in each database.

Population

Individuals of female sex (at birth) and at least one year of prior database history, with a pregnancy episode during the study period (2010-2023) and a pregnancy start date on or before December 31, 2022.

For specific objectives, the following nested cohorts will be defined as follows:

Objectives 1, 2, and 3: Individuals exposed to drugs of interest during pregnancy. For objectives 1 and 2, only the first exposure episode will be considered.

Objective 4: Individuals exposed to drugs of interest at any point during the year preceding pregnancy, pregnancy, or within 1 month following pregnancy end date.

Objective 5: No subset will be required. The main analysis will not impose any exclusion for a prior history of pregnancy, while additional analysis will:
1. exclude anyone with a pregnancy history in the year before the pregnancy start date.
2. exclude anyone with an unknown pregnancy outcome.

Variables

Indications

Indications will be derived from data by the presence of at least one diagnostic code for the following conditions (assessed using different time windows relative to index date): anxiety disorders, sleep disorders, including insomnia, and history of mental illness (a more general group of " mental illnesses" [including depression, bipolar disorder, schizophrenia and psychotic disorders, excluding anxiety and insomnia/sleep disorders]).

Exposures of interest

Benzodiazepines (at the active ingredient level). Prescription of any benzodiazepine, defined as the presence of any RxNorm codes.

Alternative treatments:

Selective serotonin reuptake inhibitors (SSRI) (at the active ingredient level). Prescription of any SSRI, defined as the presence of any RxNorm codes.

Selective noradrenaline reuptake inhibitors (at the active ingredient level). Prescription of any SNRI, defined as the presence of any RxNorm codes.

Z-hypnotics (at the active ingredient level). Prescription of any Z-hypnotic, defined as the presence of any RxNorm codes.

Melatonin (at the active ingredient level). Prescription of Melatonin, defined as the presence of any RxNorm codes.

Outcomes of interest

This study will describe the characteristics of benzodiazepine and alternative treatment users, treatment patterns (including first treatment era duration and dose), and indications. The prevalence of benzodiazepine and alternative treatment use will be estimated. Prescription trajectories categorised as restarting, switching, restarting with switching to another, or discontinuation will be assessed.
The incidence of pregnancy loss (miscarriage and stillbirth combined, and separately) will be estimated, and individuals who experience these events will be characterised. Additionally, the study will estimate the incidence of potential negative control outcomes, such as musculoskeletal injuries, skin conditions, and urinary tract infections, among benzodiazepine and alternative treatment users.

Relevant covariates

Pregnancy start and end date, gestational week, pregnancy year (pregnancy start date), age groups **(**≤24, 25-29**,** 30-34, ≥35), selected conditions and medications, number of healthcare visits, prior pregnancies, and pregnancy period (<20, >20 weeks).

Follow up

The index dates and follow-up will be different for the cohorts of interest and objectives and will consist of:

- For the general population cohort, individuals will be followed from the first date of eligibility criteria fulfilment (pregnancy start date), whereas for the benzodiazepine and alternative treatment nested cohorts, follow-up will start at the date of first treatment initiation during pregnancy (index date). Follow-up will end with pregnancy end date (irrespective of the outcome), or censoring (due to loss to follow-up, death), whichever occurs first.
- For objective 4, follow-up will begin at the first initiation of the treatment of interest (index date) within the period contained within the year prior to pregnancy start, pregnancy, and one month following pregnancy end date, or censoring (due to loss to follow-up, death), whichever occurs first.

Data sources

The following data sources, which have already implemented the PET, will be used for this study.

1. Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària (SIDIAP), Spain.
2. The Norwegian Linked Health Registry data (NLHR), Norway.

Statistical analysis

Characterisation of users of benzodiazepines and alternative treatments during pregnancy and individuals experiencing pregnancy losses will be done using *PatientProfiles* and *CohortCharacteristics* R package. For objectives 2 and 4, we will use the *DrugUtilisation* and *TreatmentPatterns* R packages to characterise benzodiazepines and alternative treatments during pregnancy, including counts (%) for each class, duration of treatment, prior medication use, and trajectories of prescription fill. For the calculation of the prevalence of benzodiazepines and alternative treatments during pregnancy, and the incidence rates of the outcomes of interest, the *IncidencePrevalence* R package will be used. Rates will be reported with the 95% Poisson confidence intervals. A minimum cell counts of 5 will be used when reporting results, with any smaller count reported as "<5". All analyses will be reported by country/database, overall and stratified by age groups and pregnancy period, when possible (minimum cell count reached). No meta-analysis will be performed.

4. AMENDMENTS AND UPDATES

None.

5. MILESTONES

Study milestones and deliverables	Planned dates*
Draft Study Protocol	February 2025
Final Study Protocol	March 2025
Creation of Test code	February - March 2025
Execution of Test Analytical Code on the data	March 2025
Creation of Analytical code	February – March 2025
Execution of Analytical Code on the data	April - June 2025
Draft Study Report	June – July 2025
Final Study Report	July - August 2025

*The proposed timelines may vary due to the need for IRB approvals and assessment of the use of DARWIN tools on the Pregnancy Extension Table.

6. RATIONALE AND BACKGROUND

Anxiety disorders affect up to 15% of pregnant individuals, and in some, pharmacological treatment may be required to ensure maternal well-being. (1) Additionally, for some, anxiety may also arise from previous negative pregnancy experiences, such as pregnancy loss, which can increase the need for therapeutic interventions. Sleep disorders are also prevalent in pregnancy, which can exacerbate anxiety symptoms, as well as limit maternal well-being and require treatment. Common sleep disorders during pregnancy include insomnia, sleep-disordered breathing, and restless legs syndrome.(2, 3)

Benzodiazepines are commonly prescribed for their anxiolytic, hypnotic, and sedative effects. In the United States, a record of benzodiazepine dispensation was identified in approximately 2% of completed pregnancies between 2007-2015, of which 60% had a diagnosis suggestive of anxiety disorders. (4) Among pregnant individuals with anxiety disorders, it is estimated that up to 25% receive prescriptions for benzodiazepines or benzodiazepine-like hypnotics (Z-hypnotics).(5, 6) However, benzodiazepines have been associated with an increased risk of preterm birth, low Apgar score, neonatal intensive care unit admission, and respiratory distress syndrome in the infant. (7, 8) Additionally, several studies suggest an association between benzodiazepine use during pregnancy and an increased risk of miscarriage. (9-14)

Despite the use of benzodiazepines during pregnancy, limited evidence directly compares their safety to alternative pharmacologic treatments that can offer similar symptom relief with potentially reduced risks. Guidelines on managing these disorders during pregnancy stress the importance of psychosocial and structured psychological interventions, reserving pharmacological treatment for moderate or severe symptoms. (15-17) Additionally, recommendations in the context of anxiety seem to favour the use of

some selective serotonin reuptake inhibitors (SSRIs), reserving benzodiazepines as support while awaiting the onset of SSRIs effect or very short treatments, and favour the use of short-acting versus long-acting benzodiazepines.(16, 17) Still, guidelines stress that the quality of evidence to support their use during pregnancy is low or very low.(16, 17) For sleep disorders, doxylamine, a drug that has not shown an increased risk for congenital malformations when used during pregnancy, is proposed as a safe option during pregnancy.(16)

One of the primary challenges in studying pregnancy outcomes using electronic health records (EHRs) is outcome misclassification. Pregnancy episodes in EHR data are often inconsistently coded across individuals and data sources, making it difficult to identify episodes and outcomes (e.g., pregnancy loss, including stillbirth and miscarriage) accurately. This inconsistency can compromise study validity by leading to misclassified or missing outcomes. However, the use of validated algorithms—which integrate multiple data sources (e.g., diagnostic codes, procedure codes, lab results, registries, and primary/secondary care records)—alongside standardised coding conventions (e.g., harmonising data across sources) may improve the reliability of pregnancy episode data.(18-20) Another challenge relating to existing pharmacoepidemiologic pregnancy studies is indication bias (confounding by indication), as benzodiazepines may be prescribed because of ongoing symptoms associated with the risk of pregnancy loss; alternatively, the underlying health disorder motivating the prescription may itself be associated with the outcome. Moreover, individual differences in disease severity may influence both treatment decisions and outcomes. While an active-comparator study could provide a more rigorous assessment of the relative risks and benefits of benzodiazepines compared to alternative treatments, the present study will provide insights into the feasibility of conducting a comparative effectiveness study within the DARWIN EU® Data Network.

Background rates for pregnancy losses in the general population vary significantly across studies, likely due to differences in population characteristics and gestational week-age thresholds (e.g., <20 vs <24 weeks). In Europe, stillbirth rates are generally estimated at 3 per 1,000 births, equivalent to approximately 1 in 160 deliveries, when using <24 weeks threshold.(21, 22) Miscarriage, defined as spontaneous pregnancy loss occurring between 8 and 20 weeks of gestation, has been reported at a rate of approximately 140 per 1,000 pregnancies (equivalent to approximately 1 per 7 pregnancies). (22, 23)

As part of the upcoming benzodiazepines periodic safety update report single assessments (PSUSA), the Pharmacovigilance Risk Assessment Committee (PRAC) has requested real-world evidence (RWE) on the utilisation of commonly used benzodiazepines during pregnancy. Additionally, background rates of pregnancy losses (including miscarriages and stillbirths) will be described to assist in contextualising the assessment of treatment safety during pregnancy. In order to fulfil this study's objectives, pregnancy episodes need to be identified, which must be derived first. This requires additional data preprocessing by data partners. To date, two data partners within the DARWIN EU® Data Network have already pre-processed pregnancy episodes and developed a Pregnancy Extension Table (PET).(20) While the table has been successfully employed in other contexts, this study marks its first application within the DARWIN EU® Data Network.

7. RESEARCH QUESTION AND OBJECTIVES

Research Question: This study is meant to inform on the use of benzodiazepine among pregnant individuals and the feasibility of a risk-assessment study that investigates benzodiazepine use during pregnancy.

The specific study objectives are the following (**Table 1**):

1. To characterise users of benzodiazepine and alternative treatments (SSRIs, SNRIs, Z-hypnotics, and Melatonin) during pregnancy in terms of demographics, prior medications, history of mental illness and other comorbidities.
2. To characterise treatments with benzodiazepine and alternative treatments during pregnancy in terms of duration, posology, and indication of prescription during pregnancy.
3. To describe the prevalence of benzodiazepine and alternative treatments' use during pregnancy
4. To describe trajectories of prescriptions fills for benzodiazepine and alternative treatments throughout the year before pregnancy, pregnancy period, and one month following pregnancy end date.
5. To estimate the incidence of pregnancy loss among all pregnancies and in benzodiazepines and alternative treatment users during pregnancy (when numbers allow).
6. To characterise individuals with pregnancy loss in terms of demographics, comorbidities, and treatments of interest.

Objectives 1, 2, 5, and 6 will also include stratifications by age categories and pregnancy periods (independently). When counts allow, objectives 2, 3, 4 and 5 will be stratified by each benzodiazepine active ingredient, grouped by benzodiazepines' half-life (short and long acting). When referring to alternative treatments, each alternative treatment will be reported separately (except for objective 4).

Exploratory objective (to assess their suitability for subsequent analyses)

7. To estimate the incidence rates of potential negative control outcomes (e.g., musculoskeletal injuries, skin conditions, urinary tract infection) in benzodiazepine and alternative treatment users.

Table 1. Primary and secondary research questions and objectives.

A. Primary research question and objective (objectives 1-4).

Objective:	To describe **the use of benzodiazepines and alternative treatments during pregnancy**, characterising users, treatments in terms of duration and dose, prevalence of use, and trajectories of prescriptions (overall and by pregnancy period: <20 and >= 20 weeks)
Hypothesis:	NA
Population *(mention key inclusion-exclusion criteria):*	Individuals of female sex (at birth) with a pregnancy episode during the study period (2010-2023, or last data availability), a pregnancy start date on or before December 31, 2022, and at least one year of prior database history at pregnancy start date. For specific objectives, the following nested cohort will be defined as follows: Objectives 1, 2, 3: Individuals exposed to drugs of interest during pregnancy. For objectives 1 and 2, only first exposure during pregnancy will be considered. Objective 4: Individuals exposed to drugs of interest during pregnancy, the year preceding pregnancy, or 1 month following pregnancy end date.
Exposure:	Benzodiazepines and alternative treatments (SSRIs, SNRIs, Z-hypnotics, and Melatonin)
Comparator:	NA

Outcome:	Objective 1: Characteristics of benzodiazepine and alternative treatment users. Objective 2: Benzodiazepine and alternative treatment first era's treatment duration, persistence and indication. Objective 3: Prevalence of benzodiazepine and alternative treatment's use. Objective 4: Prescription trajectories of benzodiazepine and alternative treatments use, categorised into: Restarting the same treatment, switching to a different treatment, restarting the same treatment while also switching to another, discontinuing treatment altogether (neither the original treatment nor any potential switch).
Time *(when follow up begins and ends):*	For objective 2, follow-up will start on the date of first treatment initiation during pregnancy, and end with end of exposure (first continuous exposure episode to the drug), end of pregnancy, or censoring (due to loss to follow-up, death), whichever occurs first. For objective 3, follow-up will start on pregnancy start date and the end of follow up will be defined by pregnancy end date, or censoring (due to loss to follow-up, death), whichever occurs first. For exposures with multiple drug eras, all exposure periods during pregnancy will be included. For objective 4, follow-up will start one year before pregnancy start date and the end of follow-up will be one month after the pregnancy end date, or censoring (due to loss to follow-up, death), whichever occurs first. For exposures with multiple drug eras, all exposure periods during this period will be included to describe trajectories of prescriptions.
Setting:	Primary and secondary care, Outpatient
Main measure of effect:	Counts, means, medians, proportions, percentages, prevalence, standardized mean difference

B. Secondary research question 2 and objective (objectives 5 and 6).

Objective:	To describe **the incidence of pregnancy loss overall and by benzodiazepines and alternative treatments of interest during pregnancy,** and to characterise individuals with pregnancy losses in terms of demographics, comorbidities, and treatments of interest
Hypothesis:	NA
Population *(mention key inclusion-exclusion criteria):*	Individuals of female sex (at birth) with a pregnancy episode during the study period (2010-2023), a pregnancy start date on or before December 31, 2022, and at least one year of prior database history at pregnancy start date.
Exposure:	Benzodiazepines and alternative treatments (SSRIs, SNRIs, Z-hypnotics, and Melatonin)
Comparator:	NA
Outcome:	Pregnancy loss, (e.g., involuntary miscarriage, stillbirth).

Time *(when follow up begins and ends):*	Objectives 5: Follow up will start at pregnancy start date, and end with pregnancy end date, or censoring (due to loss to follow-up, death), whichever occurs first. Objective 6: Index date for characterisation will the date of pregnancy loss.
Setting:	Primary and secondary care, Outpatient
Main measure of effect:	Incidence rates, prevalence, counts, proportions, means, medians

C. Exploratory research question and objective (objective 7).

Objective:	To describe the incidence of potential negative control outcomes (e.g., musculoskeletal injuries, skin conditions, urinary tract infection) by benzodiazepines and alternative treatments of interest during pregnancy.
Hypothesis:	NA
Population *(mention key inclusion-exclusion criteria):*	Individuals of female sex (at birth) with a pregnancy episode during the study period (2010-2023), a pregnancy start date on or before December 31, 2022, and at least one year of prior database history at pregnancy start date.
Exposure:	Benzodiazepines and alternative treatments (SSRIs, SNRIs, Z-hypnotics, and Melatonin)
Comparator:	NA
Outcome:	potential negative control outcomes (e.g., musculoskeletal injuries, skin conditions, urinary tract infection)
Time *(when follow up begins and ends):*	Follow up will start at pregnancy start date, and end with occurrence of the outcome, or censoring (due to loss to follow-up, death), whichever occurs first.
Setting:	Primary and secondary care, Outpatient
Main measure of effect:	Incidence rates, counts

8. RESEARCH METHODS

8.1 Study type and study design

Study types and designs included in this study are detailed in **Table 2**

- Patient-level DUS: To characterise benzodiazepine and alternative treatment users during pregnancy (Objectives 1 and 2).
- Population-level DUS: To describe the exposure to benzodiazepine and alternative treatment during pregnancy and to describe changes in prescription trajectories within the year before pregnancy, the pregnancy period, and one month after pregnancy end date (Objectives 3 and 4).
- Population-level descriptive epidemiology: To describe the incidence of pregnancy loss (Objective 5). To describe the incidence of potential negative controls (objective 7).
- Patient-level characterisation: To characterise individuals with pregnancy loss (Objective 6).

Table 2. Description of potential study types and related study designs.

Study type	Study design	Study classification
Population-level DUS	Population-level cohort	Off the shelf
Patient-level DUS	Drug/s user cohort	Off the shelf
Population-level descriptive epidemiology	Population-level cohort	Off the shelf
Patient-level characterisation	Cohort analysis	Off the shelf

8.2 Study setting and data sources

This study needs to identify pregnancy episodes, which must be derived first. This requires additional data pre-processing by data partners. To date, two data partners in the DARWIN EU® Data Network have already pre-processed pregnancy episodes and developed a PET.(20) These two data partners are considered fit for this study's purpose (**Table 3)**:

1. The Norwegian Linked Health Registry data (NLHR), Norway.
2. Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària (SIDIAP), Spain.

Table 3. Description of the selected data sources.

Country	Name of Database	Justification for Inclusion	Health Care setting	Type of Data	Number of active subjects	Data lock for the last update
Norway	NLHR	Possibility to fulfil study objectives due to the availability of the pregnancy extension table and sufficient counts on the exposure and outcomes of interest. This database will participate with their 2 available CDMs.	Primary care, specialist care, and hospital care. Norwegian birth registry.	Registry	6.94 M	2024-06-30
Spain	SIDIAP	Possibility to fulfil study objectives due to the availability of the pregnancy extension table and sufficient counts on the exposure and outcomes of interest	Primary care, hospital discharge	EHR	5.95 M	2023-06-30

*Active Persons is the maximum number of persons in an observation period in the last 6 months.

SIDIAP=Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària. NLHR=The Norwegian Linked Health Registry data (NLHR). CDM=Common Data Model.

The Norwegian Linked Health Registry data (NLHR), Norway

Norway has a universal public health care system consisting of primary and specialist health care services covering a population of approximately 5.4 million inhabitants. Many population-based health registries were established in the 1960s with use of unique personal identifiers facilitating linkage between registries. Data in these health registries are used for health analysis, health statistics, improving the quality of healthcare, research, administration and emergency preparedness. Data has been harmonized across the following registries: the Medical Birth Registry of Norway (MBRN), the Norwegian Prescription Registry (NorPD), the Norwegian Patient Registry (NPR), Norway Control and Payment of Health Reimbursement (KUHR), the Norwegian Surveillance System for Communicable Diseases (MSIS), the Norwegian Immunisation Registry (SYSVAK), the National Death Registry, and the National Registry (NR). Linkage between the registries was facilitated using project-specific person ID generated from unique personal identification assigned at birth or immigration for all legal residents in Norway. In brief: MBRN stores information about the pregnancy, the mother, father and child. NPR records diagnosis in secondary care (e.g., hospital). KUHR contains information about diagnosis and contact in primary care (e.g., GPs and outpatient specialists). NorPD recorded all medications dispensed outside of hospitals. MSIS collects test results of communicable diseases (e.g., Sars-Cov-2). SYSVAK recorded vaccinations. Notifications of pregnancies and pregnancy outcomes are mandatory in Norway > gestational week (GW) 12. However, the UiO Pregnancy algorithm improves the detection and dating of early non-live pregnancy outcomes that would have gone unnoticed if relying solely on the medical birth registry information by linking MBRN records with hospital and primary care records. (19)

This database will participate with its 2 available CDMs, that is: MBRN (NLHR@ University of Oslo (UiO): PERINATAL CDM, covering all individuals with a pregnancy event gestational week (GW) >12 2008 – 2019) and NLHR (NLHR@UiO CDM dataset, covering the years 2018 to 2022). Both CDMs use the UiO pregnancy algorithm and cover the entire Norwegian population. However, they have different source populations, differing in the data source providing the linkage IDs (source registry), and in the data period covered. Specifically:

- **NLHR@UiO: PERINATAL CDM**, IDs come from the medical birth registry, covering all individuals with a pregnancy event > GW12, and covers the period 2008 – 2019.
- **NLHR@UiO CDM**, IDs come from the National Registry covering the entire Norwegian population and covers the period 2018-2022.

Overall, both can identify similar pregnancy events. However, the NLHR@UiO CDM has a greater ability to detect early pregnancies (<GW12), as its source population includes the entire Norwegian population. In contrast, the source population in the NLHR@UiO: PERINATAL CDM, is based on the MBRN, which only includes individuals with a pregnancy episode lasting beyond GW12. Although the UiO pregnancy algorithm improves the identification of pregnancy events <GW12, in this CDM, it can only improve detection for individuals present in the MBRN. Consequently, differences between the two CDMs are likely driven by the NLHR@UiO: PERINATAL CDM's lower ability to detect elective terminations, particularly among younger individuals, who are less likely to have had a prior pregnancy extending beyond GW12, and therefore less likely to appear in the MBRN.

This data source has been previously used to carry out pregnancy studies,(24) and has been recognised for its value in providing reliable and comprehensive data.(25)

Overview of different Norwegian CDM datasets to be used in this study:

Pregnancy algorithm)	Data source	Information	Years	Source study population	N pregnancies	Prevalence pregnancy outcomes					
						LB	SAB	T	SB	ECT	MO
UIO algorithm	NLHR@UiO, P1380	MBRN**, NPR KUHR	2018-2022	All females in Norway*	412 204	64.8	17.4	16.2	0.2	1.2	0.1
UIO algorithm	NLHR@UiO:PERINATAL P704	MBRN, NPR KUHR	2008-2018	Pregnancy episodes in MBRN	859 449	74.8	13.1	11.0	0.3	0.7	0.1

* All females (biological sex at birth) 15 – 55 years with a filled prescription in NorPD 2004-2020 (source population)
** MBRN data in NLHR@UiO (data delivery 2024) is not complete for 2023 (data delivery expected Q4 2025).
LB: Live births, SAB: spontaneous abortions, T: elective terminations, SB: stillbirths. ECT: ectopic pregnancies, MO: molar pregnancies. MBRN: medical birth registry, NPR: Patient registry, KUHR: Primary care registry.

Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària (SIDIAP), Spain (IDIAP Jordi Gol)

SIDIAP is collected from EHR records of individuals receiving primary care delivered through Primary Care teams consisting of GPs, nurses and non-clinical staff. It is a regional database covering the region of Catalonia. The Catalan Health Institute manages 286 out of 370 such PCTs with a coverage of 5.6M individuals out of 8M people that reside in the Catalan region (75%) and mapped to the Observational Medical Outcomes Partnership (OMOP) common data model (CDM).(26, 27) The database started to collect data in 2006 and is updated every six months. The mean follow-up is 15.5 years. The observation period for an individual can be the start of the database (2006) or when a person is assigned to a Catalan Health Institute primary care centre. The exit date can be when a person is transferred out to a primary care centre that does not pertain to the Catalan Health Institute, date of death, or date of end of follow-up in the database. The demographic composition within SIDIAP closely mirrors that of the broader Catalan population, encompassing a representative spectrum of geographic distribution, age, and sex proportions. SIDIAP obtains data from electronic health records. The dataset covers demographics, all-cause mortality, disease diagnoses, prescription and dispensation records of drugs, results of laboratory tests, socio-economic indicators, vaccination records, lifestyle information, parent-child linkage, and various clinical parameters. Diseases are classified under the International Classification of Diseases 10th revision (ICD-10). In this data source, prescription data offers more information compared to dispensation (such as dose, posology, and quantity).

Quality checks have been implemented, including central identification of duplicate patient IDs and visual inspection for temporal patterns in the registry of a certain variable. Furthermore, the data undergoes assessment for availability (longitudinally and reliability), plausibility (range checks and unusual values), and visualisation tools. Specifically, for biochemistry data, consistency for measurements taken in different laboratories is assessed, and unit conversion is undertaken when needed. SIDIAP is linked with numerous other databases. It integrates data from external sources, including laboratory biomarker data, drug prescription and dispensation records, hospital discharge records, mental health centres, and other specific disease registries. Drugs not prescribed in the GP setting might be underreported, and disease diagnoses made in specialist care settings are not included. Vital status (death date and cause) is collected through linkage with the civil registry. The main limitation is that SIDIAP covers only primary health care records. However, it is combined with data from various other sources through effective linkages. This study will use primary care data linked to hospital discharge data.

SIDIAP includes detailed information related to the perinatal period for approximately 40,000 deliveries per year. The quality of a wide number of data captured in SIDIAP has been demonstrated in validation studies, which include several diseases in the general population.(26) Additionally, this database has been previously used in drug utilisation studies in pregnancy. (28)

8.3 Study period and follow-up

From 01/01/2010 until 31/12/2023 or the first and last date of data availability in each database.

8.4 Follow-up

The start of follow-up will vary according to each study objective, as follows:

Patient-level DUS (Objectives 1 and 2):

The index date for objectives 1 and 2 will be the date of the first prescription of benzodiazepine or alternative treatments during pregnancy. When a prescription starts before pregnancy start date but overlaps in days of supply with the pregnancy start date, the index date will be considered the pregnancy start date (date of the last menstrual period [LMP]) (this applies to all objectives in which exposure to benzodiazepine or alternative treatments during pregnancy is assessed).

For objective 2, the end of follow-up will be defined as the end of the first drug era (allowing a **gap of 30 days** between prescriptions), pregnancy end date, or censoring (due to loss to follow-up, death), whichever occurs first.

Population-level DUS (Objectives 3 and 4):

For objective 3, follow-up will start on the pregnancy start date, and the end of follow-up will be defined by the pregnancy end date or censoring (due to loss to follow-up, death), whichever occurs first.

For objective 4, follow-up will start one year before the pregnancy start date, and the end of follow-up will be defined one month after the pregnancy end date or censoring (due to loss to follow-up, death), whichever occurs first. For exposures with multiple drug eras, all exposure periods during this period will be included to describe prescription trajectories.

For both objectives, all exposure periods during follow-up will be included in the case of multiple drug eras.

Population-level descriptive epidemiology (Objectives 5 and 7) and patient-level characterisation (Objectives 6):

Objective 5: Follow-up will start with pregnancy start date and end with pregnancy end date, or censoring (due to loss to follow-up, death), whichever occurs first. For rates by treatment type, follow-up will start at the date of the first prescription to the drug of interest, and the end of follow-up will be defined as the pregnancy end date or censoring (due to loss to follow-up, death), whichever occurs first

Objective 6: The Index date for characterisation will be the date of pregnancy loss.

Objective 7: Follow-up will start with the pregnancy start date and end with the observance of potential negative control outcome date, or censoring (due to loss to follow-up, death), whichever occurs first.

Table 4. Operational definition of time 0 (index date) and other primary time anchors.

Study population name(s)	Time Anchor Description (e.g. time 0)	Number of entries	Type of entry	Washout window	Care Setting[1]	Code Type[2]	Incident with respect to…
Overall pregnant population	Pregnancy start date	Multiple (individuals with multiple pregnancies are allowed to contribute multiple times)	n/a	[-1, pregnancy episode start date]	IP, OP, OT	*	n/a
Pregnant individuals exposed to benzodiazepines or alternative treatments (objectives 1, 2, and 7)	Date of first prescription of benzodiazepines or alternative treatments during pregnancy	Single (with regards to pregnancy episode)	Incident	[-pregnancy start date, -1]	IP, OP, OT	*, RxNorm	Exposure
Pregnant individuals exposed to benzodiazepines or alternative treatments (objective 3)	Pregnancy start date, prescription of benzodiazepines or alternative treatments during pregnancy	Multiple (all drug eras)	Prevalent	n/a	IP, OP, OT	*, RxNorm	n/a

Study population name(s)	Time Anchor Description (e.g. time 0)	Number of entries	Type of entry	Washout window	Care Setting[1]	Code Type[2]	Incident with respect to…
Pregnant individuals exposed to benzodiazepines one year before, during pregnancy, and up to 1 month after (objective 4)	-365 days from pregnancy start date, date of first prescription of benzodiazepines during [-365 days pregnancy start date, +30 days pregnancy end date]	Multiple (all drug eras)	Prevalent	n/a	IP, OP, OT	*, RxNorm	Exposure

1 IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable. * From pregnancy extension table.

8.5 Study population with inclusion and exclusion criteria

Participants in the study will be required to fulfil the following criteria:

Inclusion criteria (

Table 5):

1) Observation period within the study period (1st January 2010-31st December 2023, or latest data availability).
2) Female sex at birth.
3) At least one year of prior history recorded before start of pregnancy episode.
4) A pregnancy episode recorded during the study period (defined by the pregnancy start date), with a pregnancy start date on or before December 31, 2022 (to allow sufficient time between index date and last date of database data availability to cover a full-term pregnancy).(29)
5) Pregnancy end date follows pregnancy start date (in time).
6) Pregnancy duration (in days) is greater than 1 but less than 308 days (equivalent to 44 weeks of gestation).

Exclusion criteria (**Table 6**):

1) Molar and ectopic pregnancies will be excluded (concept ids: 439083 and 437611).

Disease Epidemiology (objective 5): Two approaches will be used:

- No exclusion for prior history of pregnancy
- Excluding anyone with a history of pregnancy in the -365 days.
- Excluding anyone with an unknown pregnancy outcome.

Table 5. Operational definitions of inclusion criteria.

Criterion	Details	Order of application	Assessment window	Care Settings[1]	Code Type	Applied to study populations:
Observation period during the study period	All individuals present in the period 01/01/2010-31/12/2023 (or last available date)	After	n/a	IP, OP, OT	n/a	All study populations
Female sex at birth	Study participants will be required to have female sex at birth	Prior	n/a	IP, OP, OT	n/a	All study populations
Prior database history	365 days of prior history observed before contributing observation time is required	Prior	[-365, 0]	IP, OP, OT	n/a	All study populations
Pregnancy episode during the study period, with start date one year before study end date	A pregnancy episode recorded during study period with pregnancy start date before 31/12/2022	Prior	01/01/2010-31/12/2023	IP, OP, OT	*	All study populations
Pregnancy end date is after pregnancy start date	Pregnancy end date > Pregnancy start date	Prior	01/01/2010-31/12/2022	IP, OP, OT	*	All study populations
Pregnancy length in days (duration)	Pregnancy in days >1 and <308 days	Prior	01/01/2010-31/12/2022	IP, OP, OT	*	All study populations

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable. * From pregnancy extension table.

[2] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

Table 6. Operational definitions of exclusion criteria.

Criterion	Details	Order of application	Assessment window	Care Settings[1]	Code Type	Applied to study populations:
Molar and ectopic pregnancies	Pregnancy episodes representing molar or ectopic pregnancy will be excluded	Prior	[pregnancy start date, pregnancy end date]	IP, OP, OT	SNOMED	All study populations
No prior pregnancy history in the year to the current episode (objective 5)	No prior history of pregnancy in the year prior to pregnancy start date	Prior	[-365, -1] from pregnancy start date	IP, OP, OT	*	Overall pregnant population in objective 5
Complete case scenario – known pregnancy outcome requirement (objective 5)	Exclusion of pregnancies with unknown pregnancy outcomes	Prior	Pregnancy end date	IP, OP, OT	*	Overall pregnant population in objective 5

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable. * From pregnancy extension table.

2 Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

8.6 Variables

The preliminary concept/code lists used for identifying exposure/s and/or outcomes are included as Supplementary Documents in **Appendix I (Appendix I. Table 1, Appendix I. Table 2, Appendix I. Table 3)**. These will be refined during the study execution following the DARWIN EU® Phenotyping standard processes, which involve the review of code lists by clinical experts and the review of phenotypes after their execution in the participating databases.

8.6.1 Indication/s

Indications will be derived from data by identifying at least one diagnostic code (code list is specified in **Appendix I, Appendix I. Table 2**). The following conditions are of interest:

- Anxiety disorders
- Sleep disorders, including insomnia
- History of mental illness (a more general group of "history of mental illness" will be described [including depression, bipolar disorder, schizophrenia and psychotic disorders, excluding anxiety and insomnia/sleep disorders]).

As some of these conditions are likely chronic, the optimal period during which diagnostic codes will be recorded is unclear. In turn, conditions will be assessed using the following time windows:

- 1-month before and after index (-30, +30 days before index date)
- In the year prior and up to index (0, -365 days before index date)
- Any time before index date (0, -Inf).

8.6.2 Exposure/s

Exposures are described in **Table 7.**

Drugs of Interest: All Benzodiazepines (at the ingredient level). A benzodiazepine prescription is defined as the presence of any RxNorm codes, as specified in **Appendix I (Appendix I. Table 1)**.

For pregnancy loss and stillbirth outcomes, exposure will be assessed at any time during pregnancy. For miscarriage, exposure will be assessed between the pregnancy start date (LMP) and up to 20 weeks of pregnancy. Exposure will be defined as at least one prescription for any benzodiazepine from the pregnancy start date until the outcome date. Prescriptions for benzodiazepines before becoming pregnant with a prescription duration overlapping the pregnancy start date (LMP) will also be considered to be exposed during pregnancy, and the index date will be defined as the pregnancy start date.

For some objectives, results will be provided for each benzodiazepine active ingredient, which will be dichotomously grouped based on duration of action, namely short-acting (half-life ≤24 hours) or long-acting (half-life > 24 h) benzodiazepines (**Appendix I**).

Alternative treatments:

- SSRIs
- SNRIs
- Z-hypnotics
- Melatonin

For objective 4, alternative treatments will be defined as a composite exposure, indicating the presence of any of the three selected alternative treatments (SSRI, SNRIs, Z-hypnotics, and melatonin), whether individually or combined.

Table 7. Operational definitions of exposure.

Exposure group name(s)	Details	Washout window	Assessment Window	Care Setting[1]	Code Type	Applied to study populations	Incident with respect to…
Benzodiazepines	Exposure to Benzodiazepines	n/a except for objectives 2, 5, and 7 [pregnancy start date, first prescription date], to reflect the first use during pregnancy.	Assessment windows vary by objective: Objectives 1-3, 5, and 7 [Pregnancy start date – pregnancy end date] Objective 4 [-365 days from pregnancy start date, +30 days from pregnancy end date]	IP, OP, OT	RxNorm	All	For objective 2, 5, and 7, exposure will be incident with regards to each drug class during pregnancy period (Benzodiazepines and alternative treatments). Individuals can contribute to multiple exposure cohorts over time.
SSRI	Exposure to SSRI		[Pregnancy start date – pregnancy end date]	IP, OP, OT	RxNorm	All	
SNRI	Exposure to SNRI		[Pregnancy start date – pregnancy end date]	IP, OP, OT	RxNorm	All	
Z-hypnotics	Exposure to Z-hypnotics		[Pregnancy start date – pregnancy end date]	IP, OP, OT	RxNorm	All	
Melatonin	Exposure to Melatonin		[Pregnancy start date – pregnancy end date]	IP, OP, OT	RxNorm	All	
Alternative treatments	Exposure to any of the alternative treatments of interest: SSRIs, SNRIs, Z-hypnotics, or Melatonin	n/a	Objective 4 [-365 days from pregnancy start date, +30 days from pregnancy end date]	IP, OP, OT	RxNorm	Pregnant individuals exposed to benzodiazepines one year before, during pregnancy, and up to 1 month after (objective 4)	n/a

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable

[2] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

8.6.3 Outcome/s

Study outcomes are specified in **Table 8** and described for each objective below.

Objective 1: Characteristics of benzodiazepine and alternative treatment users.

Objective 2: Benzodiazepine and alternative treatment first era's treatment duration, posology (dose), and indication.

Objective 3: Prevalence of benzodiazepine and alternative treatments use.

Objective 4: Prescription trajectories of benzodiazepine and alternative treatments use, categorised into:

- Restarting the same treatment.
- Switching to a different treatment.
- Restarting the same treatment while also switching to another.
- Discontinuing treatment altogether (neither the original treatment nor any potential switch), relative to the entire period analysed.

Objectives 5:

Pregnancy loss: Date of pregnancy end date, comprising any of the following events: miscarriage/abortion (spontaneous), stillbirth (death of foetus, foetal loss, intrauterine death, dead born), as recorded in the PET. (20) Additionally, miscarriage and stillbirth will be assessed separately.

Objective 6: Characteristics of individuals with pregnancy loss.

Objective 7: Incidence of potential negative control outcomes among benzodiazepine and alternative treatment users during pregnancy. The outcome will be based on the presence of at least one (SNOMED) code to identify the following conditions:

- musculoskeletal injuries (any fracture, tendinitis, carpal tunnel syndrome)
- skin conditions
- urinary tract infection

Table 8. Operational definitions of outcome.

Outcome name	Details	Primary outcome?	Type of outcome	Washout window	Care Settings[1]	Code Type	Applied to study populations
Benzodiazepine and alternative treatment prescription	A prescription with a benzodiazepine active ingredient	Primary	Count	n/a	IP, OP, OT	RxNorm	Pregnant individuals (objectives 1-4)
Pregnancy loss	Combines involuntary miscarriage and stillbirth	Primary	Count	Objective 5: No washout and a one-year washout relative to pregnancy start date [-365, -1]	IP, OP, OT	*	Pregnant individuals (Objective 5)

Outcome name	Details	Primary outcome?	Type of outcome	Washout window	Care Settings[1]	Code Type	Applied to study populations
Miscarriage (spontaneous abortion)	Involuntary miscarriage, recorded <20 weeks of GW	Primary	Count	Objective 5: No washout and a one-year washout relative to pregnancy start date [-365, -1]	IP, OP, OT	*	Pregnant individuals (Objective 5)
Stillbirth	Foetal loss recorded >20 weeks GW	Primary	Count	Objective 5: No washout and a one-year washout relative to pregnancy start date [-365, -1]	IP, OP, OT	*	Pregnant individuals (Objective 5)
Potential negative control outcomes	musculoskeletal injuries, skin conditions, urinary tract infections	Secondary	Count	For musculoskeletal injuries, skin condition: [-Inf, -1] For urinary tract infections: [pregnancy start date, -1]	IP, OP, OT	SNOMED	Pregnant individuals (objective 7)

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable. GW = gestational weeks. *From Pregnancy extension table.
[2] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

8.6.4 Other covariates, including confounders, effect modifiers and other variables *(where relevant)*

The study covariates are described conceptually, and the context or rationale for the choices are provided in this section. The operational definition of the covariates is described in **Table 9**. Preliminary code lists are available in **Appendix I. Table 3**.

The following covariates will be used:

- Pregnancy year (pregnancy start date, year)
- Pregnancy week
- Age (in years) at pregnancy start date will be categorised as follows:
    - ≤24
    - 25-29
    - 30-34
    - ≥35

The following pre-specified conditions and prescriptions of interest (objectives 1 and 6), are to be presented in a summary of baseline characteristics at index (-1 to -Infinity, unless otherwise specified):

- **Conditions**:
    - Neurological disorders
        - Seizures/epilepsy

- Pregnancy history (before current pregnancy episode):
  - Live birth
  - Spontaneous abortion
  - Induced abortion
  - Stillbirth
- Cardiovascular disorders
  - Hypertension
- Endocrine disorders
  - BMI (pre-pregnancy)
  - Overweight (pre-pregnancy)
  - Obesity (pre-pregnancy)
  - Diabetes
  - Thyroid disorder
- Rheumatological disorders
  - Systemic lupus erythematosus
  - Antiphospholipid syndrome
- Haematological disorders
  - Sickle cell anaemia

- **Prescriptions** (during the year prior)
  - Anticoagulating agents: use of these agents can suggest a pro-thrombotic state, among which pregnancy losses are expected to be higher.
  - Central nervous system drugs (Other antidepressants [excluding SSRIs and SNRIs], antipsychotics, anti-epileptics): Use of these drugs that can suggest underlying mental health disorders.
  - Hydroxychloroquine: Use of this medication is recommended in individuals with autoimmune disorders during pregnancy. Autoimmune disorders can increase the risk of pregnancy loss.
- **Prescriptions for drugs of interest** (applicable to objective 6 and limited to the year prior)
  - Benzodiazepines
  - SSRIs
  - SNRIs
  - Z-hypnotics
  - Melatonin
- **Lifestyle factors**
  - Smoking (pre-pregnancy)
  - Alcohol use (pre-pregnancy)
- **Healthcare use** (during the year prior)
  - Number of visits before pregnancy (categorical)

Large-scale characterisation: Drugs (by RxNorm code) and conditions (by standard SNOMED code) within predefined windows will be assessed. Conditions and drugs used before the index date will be assessed using different time windows. While all conditions and medications above 1% will be recorded, only the 10 most frequent conditions and drugs will be described within the report.

The following time windows will be used for the large-scale characterisation:

- 365 days to 1 day before the index date

Stratification variables:

Main stratifications will include age categories and pregnancy period (<=20 weeks, >20 weeks), independently (Objectives 1, 2, and 5).

For some objectives, results will be stratified by continuous pregnancy week (when counts allow).

For some objectives (when counts allow), results will be stratified by each benzodiazepine active ingredient and grouped by benzodiazepines' half-life (short and long acting) (Objectives 2, 3, 4, and 5).

Table 9. Operational definitions of covariates.

Characteristic	Details	Type of variable	Assessment window	Care Settings[1]	Code Type	Applied to study populations
Pregnancy year	Pregnancy year (pregnancy start date)	Continuous	0 [Pregnancy start date]	n/a	n/a	All
Pregnancy week	Pregnancy week from pregnancy start date	Continuous	[Pregnancy start date, pregnancy end date]	IP, OP, OT	*	All
Age groups	(≤24, 25-29, 30-34, ≥35)	Categorical	0 [Pregnancy start date]	n/a	n/a	All
Maternal BMI	Maternal BMI before pregnancy	Continuous	[pre-pregnancy start date]	IP, OP, OT	*	All
Comorbidities	Pre-specified conditions of interest prior to index date (as specified in section 8.5.4)	Count, Binary	[-Inf, -1]	IP, OP, OT	SNOMED	All
Prior medication use	Pre-specified prescriptions (as specified in section 8.5.4)	Count, Binary	[-365, -1]	IP, OP, OT	RxNorm	All
Health Conditions, prescriptions	All history prior to the index date (for	Count, Binary	[-365, -1]	IP, OP, and OT	SNOMED	All

Characteristic	Details	Type of variable	Assessment window	Care Settings[1]	Code Type	Applied to study populations
	large-scale characterisation)					
Pregnancy history	Prior obstetric history related to the current pregnancy episode	Count, Binary, categorial	[pre-pregnancy start date]		*	
Lifestyle factors	Smoking and alcohol use	Count, Binary	[pre-pregnancy start date]	IP, OP, and OT	*	All
Healthcare use	Number of visits or hospitalizations before pregnancy (any) to serve as an indicator of disorder severity	Count, Binary	[-365, -1]	IP, OP, and OT	SNOMED	All

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable. *From Pregnancy extension table.
[2] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

8.7 Study size

As all objectives in this study are descriptive in nature, no sample size will be estimated.

8.8 Analysis

All analyses will be conducted separately for each database and will be carried out in a federated manner, allowing analyses to be run locally without sharing patient-level data (**Table 10**).

Before sharing the study package, test runs of the analytics will be performed on a subset of the data sources, and quality control checks will be performed. After all the tests are passed, the final package will be released in a version-controlled study repository for execution against all the participating data sources.

The data partners will locally execute the analytics against the OMOP CDM in R Studio and review and approve the default aggregated results. They will then be made available to the Principal Investigators and study team in a secure online repository (Data Transfer Zone). All results will be locked and timestamped for reproducibility and transparency.

Table 10. Description of study types and type of analysis.

Study type	Study classification	Type of analysis
Population Level DUS	Off-the-shelf	- Population-based incidence rates - Population-based prevalence of use of a drug/drug class - Summarise % of drug restart and switch (prescription trajectories)
Patient Level DUS	Off-the-shelf	- Characterisation of patient-level features - Frequency and % of indication/s - Estimation of minimum, p25, median, p75, and maximum initially prescribed or dispensed dose/strength - Estimation of minimum, p25, median, p75, and maximum treatment duration
Population-level descriptive epidemiology	Off-the-shelf	- Incidence rates of the condition of interest
Patient-level characterisation	Off-the-shelf	- Large-scale characterisation - Patient-level characteristics

Patient-level characteristics on/before index date

Characterisation of individuals treated with Benzodiazepines and alternative treatments (objective 1): We will use the R package "PatientsProfiles" and "CohortCharacteristics" for the patient-level characterisation of demographics and predefined clinical characteristics, as well as large-scale characterisation. The co-variates to be presented in a summary baseline characteristics table have been described in section 8.6.4 Other covariates, including age, comorbidities, and prior medication use based on predefined conditions and drugs. Comorbidities will be assessed at any time prior. Prior use of medications of interest will be described before the index date (365 days to 1 day before index date). The large-scale patient-level characterisation, including all records and the 10 most frequent pre-index concept-based characteristics available in the data, will be conducted to describe the medical history and prior medication use (365 days to 1 day before the index date). Differences in baseline prespecified characteristics (see section 8.6.4) between benzodiazepines and alternative treatments will be assessed using Standardized mean differences (SMD), using the Benzodiazepines group as the reference. These comparisons are intended to describe covariate balance across groups and may also inform the feasibility of propensity score-based adjustment in future comparative effectiveness studies.

Characterisation of individuals with pregnancy losses (objective 6): Similar to what is described above (for objective 1), covariates will include age, comorbidities, and prior medication use, based on predefined conditions and drugs (see section 8.6.4 Other covariates), and large-scale characterisation, including all recorded and the 10 most frequent pre-index concept-based characteristics available in the data before 365 before index date, based on code/s, and classified into conditions (medical history), and medicine use.

Drug utilisation

Characterisation of treatment with Benzodiazepines and alternative treatments during pregnancy (Objective 2): The "DrugUtilisation" package will be used to characterise the use of *Benzodiazepines and alternative treatments during pregnancy*. The number and % of individuals receiving each of a pre-specified list of *Benzodiazepines and alternative treatments during pregnancy,* including:

- Frequency and % of indication/s, based on the pre-specified list of diagnoses recorded before therapy initiation (where available).
- Reporting of minimum, p25, median, p75, mean, standard deviation, and maximum initially prescribed or dispensed dose/strength (where available), by ingredient.

- Reporting of minimum, p25, median, p75, mean, standard deviation, and maximum treatment duration.
- % of prescriptions with dispensation during pregnancy period (SIDIAP only).

Trajectories for benzodiazepine prescriptions and switch to alternative treatments (Objective 4) (see 8.6.3. Outcomes/s) will be described throughout the year before pregnancy, pregnancy period, and up to 1 month following pregnancy end date (using "DrugUtilisation" and "*TreatmentPatterns*"). Patterns of drug restart and switching among individuals taking benzodiazepine will be described. The drug era gap will be set at 30 days, as previously specified for objective 2. Specifically, we will investigate whether individuals restart benzodiazepine after discontinuation, switch to other active comparators, try both medications or remain untreated during specific periods starting from the year before pregnancy and throughout the entire pregnancy period. The following proportion will be reported:

- Restarting the same treatment.
- Switching to a different treatment.
- Restarting the same treatment while also switching to another.
- Discontinuing treatment altogether (neither the original treatment nor any potential switch).

Incidence Prevalence

Several objectives will involve incidence or prevalence estimations. For objective 3, the prevalence of drug classes of interest (see 8.6.3. Outcomes/s) will be calculated as the proportion of study participants who were prescribed a drug of interest per pregnancy week during the study period. Prevalence will be reported overall and for each active ingredient separately (grouped as long- and short-acting), if counts allow.

For objective 5, the incidence rates of outcomes of interest (see 8.6.3. Outcome/s) will be calculated as the number of new events per 100,000 person-years of the population at risk during the study period. Rates will be provided using consecutive time-at-risk windows from the pregnancy start date, by consecutive gestational weeks, in which, if no event occurs, those who remain event-free will continue to contribute to the subsequent week-risk intervals (See specifications for incidence rate calculations below). For rates by treatment used during pregnancy, individuals will start contributing to time-at-risk from the date of the first exposure and remain at risk throughout pregnancy, irrespective of discontinuation or switch, following an intention-to-treat approach. Additionally, individuals with exposures to different drugs during pregnancy will be allowed to contribute time-at-risk for multiple exposures, as no washout period relative to other drug classes will be imposed (requirementsAtEntry will be set to false, allowing a dynamic cohort entry).

For objective 7, incidence rates of the potential negative control outcomes of interest (see 8.6.3. Outcome/s) will be calculated as the number of new events per 100,000 person-years of the population at risk during the study period among users of benzodiazepines or alternative treatments during pregnancy.

Stratifications

Objectives 1, 2, and 5 will also include stratifications using age categories and pregnancy period (<=20 weeks, >20 weeks), independently.

Results will be stratified by different time windows (relative to the pregnancy start date when periods exceed the pregnancy period, such as for objective 4). For others, results will be provided by consecutive gestational weeks (when counts allow).

If counts allow, Objectives 2, 3, 4, and 5 will be stratified by each benzodiazepine active ingredient, grouped by benzodiazepines' half-life (short—and long-acting). For objective 4, alternative treatments will include all treatments combined.

Methods to derive parameters of interest

Age

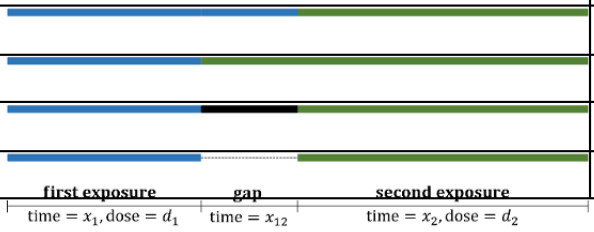
Age at index date will be calculated using January 1st of the birth year as a proxy for the actual birthday. Age categorisations have been defined above (under covariates).

Drug exposure calculation

Treatment duration, representing the assumed period of continuous drug exposure for an individual and drug, will be estimated by measuring the length of the first continuous drug era, allowing a predefined drug era gap of 30 days, as follows:

Exposure starts at the date of the first prescription (or dispensation in the case of NLHR), e.g., the index date the person entered the cohort. For each prescription, the estimated duration of use is retrieved from the drug exposure table in the CDM using the start and end date of the exposure. Subsequent prescriptions will be combined into continuous exposed episodes (drug eras) using the following specifications:

Two drug eras will be merged into one continuous drug era if the distance in days between the end of the first era and the start of the second era is ≤ 30 days. The length in time (days) between the two joined eras will be considered as the period of continuous exposure to the drug during the first drug era, as shown in **Figure 1**, "first" row.

Gap era joint mode	Schematics	Dose in between	Cumulative dose	Cumulative time
"first"		d_1	$d_1 \cdot (x_1 + x_{12}) + d_2 \cdot x_2$	$x_1 + x_{12} + x_2$
"second"		d_2	$d_1 \cdot x_1 + d_2 \cdot (x_2 + x_{12})$	$x_1 + x_{12} + x_2$
"zero"		0	$d_1 \cdot x_1 + d_2 \cdot x_2$	$x_1 + x_{12} + x_2$
"join"		NA	$d_1 \cdot x_1 + d_2 \cdot x_2$	$x_1 + x_2$

The gapEra parameter is used to join exposures into episodes. If the drugEra gap is less than the gapEra parameter, the exposures will be joined into episodes.

Figure 1. Gap-era joint mode.

As previously stated, for each prescription (or dispensation, in the case of NLHR), the estimated duration of use is retrieved from the drug exposure table in the CDM, using the start and end date of the exposure. It will be calculated as the duration of the first treatment era of the benzodiazepine during the study period. Treatment duration will be summarised, providing the minimum, p25, median, p75, and maximum treatment duration. Treatment duration will not be provided for databases where duration cannot be calculated.

Prescription fills trajectories

To describe the prescription-fill trajectories within a benzodiazepine and alternative treatment cohorts following the first exposure to a specific drug, the prescription trajectories will be categorised into four distinct groups, as previously mentioned, and illustrated in **Figure 2**:

- Restarting the same treatment.
- Switching to a different treatment.
- Restarting the same treatment while also switching to another.
- Discontinuing treatment altogether (neither the original treatment nor any potential switch).

The analysed period will include the 365 days before the pregnancy start date and continue until up to one month following the pregnancy end date. Switching to a different treatment will include the use of any of the alternative treatments (combined). Categorisation of trajectories will be assessed for the following periods:

Pre-pregnancy

- 9-12 months before pregnancy
- 5-8 months before pregnancy
- 0-4 months before pregnancy

Pregnancy

- First 20 weeks of pregnancy (<= 20 weeks)
- Second 20 weeks of pregnancy (>20 weeks)

Post-pregnancy

- First month following pregnancy end date.

The figure illustrates the analysis, focusing on the outcomes after the initial exposure to a particular drug (in blue), with consideration of a specific switch drug (in orange). This study examines what occurs within 100, 180, and 365 days following first treatment discontinuation in the cohort.

Figure 2. Trajectories analysis example.

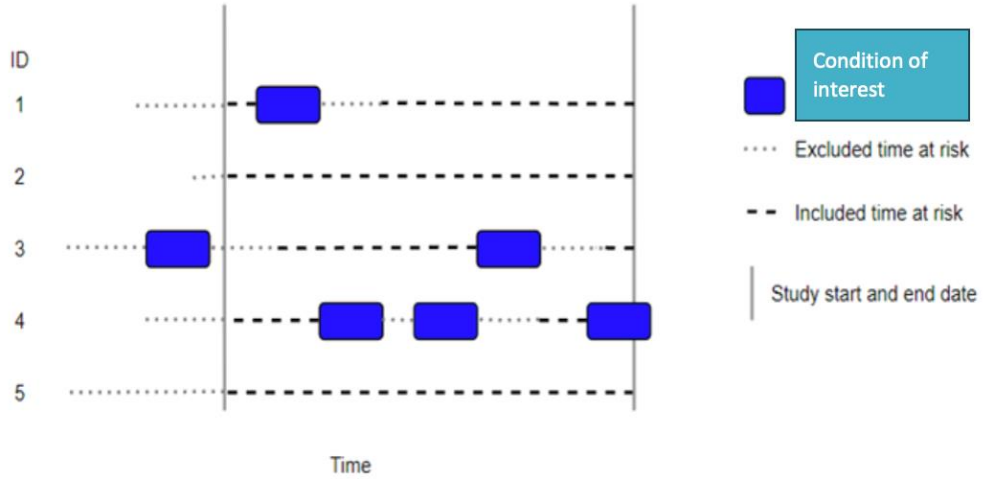
Population-level incidence calculation

Incidence rates of the outcomes of interest for the overall pregnancy population, will be calculated as the number of newly diagnosed with the outcome of interest divided by the person-years as contributed by the population at risk of the outcome during the period for each gestational week. Follow-up is censored upon the end of the observation period, the outcome of interest, the pregnancy end date, or upon death, whichever comes first. Incidence rates will be given together with 95% Poisson confidence intervals.

Patient ID 1 and 4 contribute time at risk between the study start until they have an incident outcome of interest. Patient ID 2

and 5 contribute time at risk between the study start and end date as no outcome of interest is observed between this period nor before the study start date. Patient ID 3 was excluded from the analysis and does not contribute time at risk since a previous diagnosis was observed before the study start date.

Figure 3 represents an example of incidence rate estimation.



Patient ID 1 and 4 contribute time at risk between the study start until they have an incident outcome of interest. Patient ID 2 and 5 contribute time at risk between the study start and end date as no outcome of interest is observed between this period nor before the study start date. Patient ID 3 was excluded from the analysis and does not contribute time at risk since a previous diagnosis was observed before the study start date.

Figure 3. Example of incidence rate estimation.

For the calculation of incidence rates by exposure to benzodiazepines and alternative treatments during pregnancy (objective 5), the numerator will consist of individuals with the outcome of interest occurring within the pregnancy period (i.e. date of first prescription of treatment of interest) and the denominator will consist of person time from index date until the outcome of interest, end of pregnancy period, or loss of follow up, whichever comes first. Individuals might contribute to multiple exposure cohorts as individuals might be exposed to different classes of prophylactic drugs. Incidence rates will be estimated at consecutive gestational week time window post-index date (objective 5), if counts allow. Individuals will be censored at the end of each time window if they do not experience the event of interest, reflecting distinct periods at risk.

Population-level prevalence calculation

The prevalence of use will be calculated as the proportion of study participants who were prescribed a benzodiazepine and alternative treatments during pregnancy on a gestational-week basis (when counts allow). Prevalence will be calculated as period prevalence (the proportion of people in the denominator population also present in the outcome cohort over the given time interval).

Table 11. Primary, secondary, and subgroup analysis specification.

A. Primary analysis 1

Hypothesis:	n/a

Exposure contrast:	n/a
Outcome:	Prevalence of benzodiazepine and alternative treatment use during pregnancy, characterisation of users, treatments, and trajectories of use.
Analytic software:	IncidencePrevalence, DrugUtilisation, TreatmentPatterns, CohortCharacteristics, PatientProfiles
Model(s):	n/a
Confounding adjustment method	***Name method and provide relevant details, e.g. bivariate, multivariable, propensity score matching (specify matching algorithm ratio and calliper), propensity score weighting (specify weight formula, trimming, truncation), propensity score stratification (specify strata definition), other.***
	n/a
Missing data methods	***Name method and provide relevant details, e.g. missing indicators, complete case, last value carried forward, multiple imputation (specify model/variables), other.***
	No imputation will be done.
Subgroup Analyses	***List all subgroups***
	n/a

B. Primary Analysis 2

Hypothesis:	n/a
Exposure contrast:	n/a
Outcome:	Incidence of pregnancy loss overall and stratified by benzodiazepine and alternative treatments users during pregnancy
Analytic software:	IncidencePrevalence, CohortCharacteristics, PatientProfiles
Model(s): ***(provide details or code)***	n/a
Confounding adjustment method	***Name method and provide relevant details, e.g. bivariate, multivariable, propensity score matching (specify matching algorithm ratio and calliper), propensity score weighting (specify weight formula, trimming, truncation), propensity score stratification (specify strata definition), other.***
	n/a
Missing data methods	***Name method and provide relevant details, e.g. missing indicators, complete case, last value carried forward, multiple imputation (specify model/variables), other.***
	No imputation will be done.
Subgroup Analyses	***List all subgroups***
	n/a

Sensitivity Analysis

For objective 3, in SIDIAP, a sensitivity analysis will be conducted, in which individuals with a prescription record during pregnancy will also be required to have at least one dispensation record during pregnancy, as prescriptions may be affected by auto-renewal for chronic medications.

For objective 5, two sensitivity analyses are planned:
a. Sensitivity analysis will be conducted excluding individuals with a pregnancy within the year prior.

b. Sensitivity analysis using a complete-case approach, excluding pregnancies with unknown outcomes from the analysis.

Patient privacy protection

All analyses will be conducted separately for each database and carried out in a federated manner, allowing analyses to be run locally without sharing patient-level data. Cell counts <5 will be suppressed to comply with the database's privacy protection regulations.

8.9 Evidence synthesis

Results will be presented separately for each database, and no meta-analysis of results will be conducted. A meta-analysis using only two CDM data sources may be subject to bias due to limited ability to assess heterogeneity and the potential for unmeasured differences in population characteristics or data capture across sites, and can lead to unstable estimates or misleading results.(30)

9. DATA MANAGEMENT

9.1 Data management

All databases are mapped to the OMOP CDM. This enables the use of standardised analytics and tools across the network since the structure of the data and the terminology system is harmonised. The OMOP CDM is developed and maintained by the Observational Health Data Sciences and Informatics (OHDSI) initiative and is described in detail on the wiki page of the CDM: https://ohdsi.github.io/CommonDataModel and in The Book of OHDSI: http://book.ohdsi.org.

The analytic code for this study will be written in R. Each data partner will execute the study code against their database containing patient-level data and will then return the results set which will only contain aggregated data. The results from each of the contributing data sites will then be combined in tables and figures for the study report.

9.2 Data storage and protection

For this study, participants from various European Union (EU) member states will process personal data from individuals which is collected in national/regional electronic health record databases. Due to the sensitive nature of this personal medical data, it is important to be fully aware of ethical and regulatory aspects and to strive to take all reasonable measures to ensure compliance with ethical and regulatory issues on privacy.

All databases used in this study are already used for pharmaco-epidemiological research and have a well-developed mechanism to ensure that European and local regulations dealing with ethical use of the data

and adequate privacy control are adhered to. In agreement with these regulations, rather than combining person-level data and performing only a central analysis, local analyses will be run, which generate non-identifiable aggregate summary results.

The output files are stored in the DARWIN Digital Research Environment. These output files do not contain any data that allow identification of subjects included in the study. The DRE implements further security measures to ensure a high level of stored data protection to comply with the local implementation of the General Data Protection Regulation (GDPR) (EU) 679/20161 in the various member states.

10. QUALITY CONTROL

General database quality control

Several open-source quality control mechanisms for the OMOP CDM have been developed (see Chapter 15 of The Book of OHDSI http://book.ohdsi.org/DataQuality.html). In particular, data partners are expected to run the OHDSI Data Quality Dashboard tool (https://github.com/OHDSI/DataQualityDashboard). This tool provides numerous checks relating to the conformance, completeness and plausibility of the mapped data. Conformance focuses on checks that describe the compliance of the representation of data against internal or external formatting, relational, or computational definitions, completeness in the sense of data quality is solely focused on quantifying missingness, or the absence of data, while plausibility seeks to determine the believability or truthfulness of data values. Each of these categories has one or more subcategories and are evaluated in two contexts: validation and verification. Validation relates to how well data aligns with external benchmarks with expectations derived from known true standards, while verification relates to how well data conforms to local knowledge, metadata descriptions, and system assumptions.

Study-specific quality control

Concepts and phenotypes of interest will be developed and assessed using the following R packages: “CodelistGenerator”, "CohortDiagnostics”, and “DrugExposureDiagnostics”. The study code will be based on the following R packages: to estimate incidence rates and prevalence (“IncidencePrevalence”), to characterise individuals (“PatientProfiles” and “CohortCharacteristics”), to describe characteristics of drug use (“DrugUtilisation” and “TreatmentPatterns”). These packages will include numerous automated unit tests to ensure the validity of the codes, alongside software peer review and user testing.

11. LIMITATIONS OF THE RESEARCH METHODS

The following limitations need to be considered:

Pregnancy study and PET use in DARWIN EU® Data Network:

This study will require using the PET, on which DARWIN EU® tools and analytical pipelines have not been tested. While the PET has been developed and used in the data sources proposed for this study, this is the first instance of using the PET within the DARWIN EU® data network. Therefore, a secondary objective of the study includes assessing that the table and tools adapt well to fulfil the study objectives.

Pregnancy outcomes:

Data sources can under-record spontaneous or induced pregnancy losses that do not end up with a health encounter within the system. This is most likely to affect early pregnancy losses before gestational week 8. For NLHR, because pregnancies and outcomes are mandatory for notification from week 12 on, in addition to being a nationally representative sample, we expect little under-recording from this point on. For SIDIAP, while this data source is regional, its coverage is estimated at around 75% of the Catalonian-resident population. Pregnancies under care in settings outside the public sector (e.g., private care settings,

homebirths) are likely to be underrepresented or missing in the data. In this sense, 1.7% of pregnancies in SIDIAP have an unknown pregnancy outcome,(20) despite having gestational length available — in most cases ≥40 weeks — suggesting that these may correspond to births occurring in settings not captured by the data source. Still, some individuals may appear in the system for administrative purposes, such as leave certification. Incomplete recording of outcomes will be addressed through a sensitivity analysis restricted to complete cases, as described previously.

Early pregnancy losses (<12 weeks) may introduce selection bias. Individuals whose losses are recorded may differ systematically from those not recorded —for reasons such as more frequent healthcare use, underlying comorbidities, or complications prompting medical follow-up. In contrast, women experiencing early, uncomplicated losses who do not seek care would remain unregistered, potentially biasing estimates if the underlying risk profiles differ between these groups. While restricting the analysis to pregnancies with a first encounter beyond ≥12 weeks, as a sensitivity analysis, help assess the extent of this potential bias by focusing on pregnancies consistently captured in the data, this is not possible within the PET.

Elective termination of pregnancy represents a competing risk for pregnancy loss, as pregnancies that end in termination are at risk of later loss. This dynamic may influence the observed rates of pregnancy loss, hypothesising that unplanned pregnancies could be more frequent among benzodiazepine users, who may also have different patterns of termination. This could introduce selection bias in the observed rates. Therefore, caution is warranted when interpreting them.

Relevantly, in SIDIAP, a potential limitation is that voluntary pregnancy terminations are lower than expected. While this does not introduce misclassification within the identified cohort, it implies that the study population may not fully reflect all conceptions.

Exposures, indications, comorbidities, and lifestyle factors:

The recording of events used for characterisation may vary across databases and be captured in another care setting than the included database. For example, prescriptions of the drugs of interest, as well as diagnoses relative to this study arising from private care settings, could lead to misclassification.

Lifestyle factors (e.g., smoking, alcohol use) are of interest, although these may not be systematically recorded and thus may be unavailable in databases. We will explore their availability from the PET. While this study will be descriptive in nature, unmeasured factors such as drug use, poor diet, or stress may contribute to adverse outcomes, including pregnancy loss.

The use of the drugs of interest, benzodiazepines and alternative treatments, will be derived from prescription data for SIDIAP, while from dispensation data in NLHR. Data does not reflect treatment compliance (a recording of a prescription or dispensation does not mean that the individuals actually took the drug). In addition, assumptions around the duration of drug use will be unavoidable and might lead to overestimating treatment duration (considering a 30-day drug gap era). For databases where duration cannot be calculated due to, e.g. missing information on quantity, dosing or end date, e.g., treatment duration will not be provided. Additionally, in SIDIAP, some chronic medications hold automatic prescription renewal for some time period, which could lead to overestimating the length of exposure. For this reason, among prescriptions, at least one dispensation during pregnancy will be required in SIDIAP, to reduce misclassification.

12. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

Adverse events/adverse reactions will not be collected or analysed as part of this evaluation. The nature of this non-interventional evaluation, through the use of secondary data, does not fulfil the criteria for reporting adverse events, according to module VI, VI.C.1.2.1.2 of the Good Pharmacovigilance Practices (https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/guideline-good-pharmacovigilance-practices-gvp-module-vi-collection-management-submission-reports_en.pdf).

13. GOVERNANCE BOARD ASPECTS

IRB approval will be requested for all databases participating in the study.

14. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

A study report, including an executive summary and the specified tables and/or figures, will be submitted to EMA by the DARWIN EU® coordinating centre upon completion of the study. An interactive dashboard incorporating all the results (tables and figures) will be provided alongside the study report. The full set of underlying aggregated data used in the dashboard will also be made available if requested. Additionally, a manuscript reporting the study results is planned to be drafted and submitted to a peer-reviewed journal, upon completion of the study report.

15. OTHER ASPECTS

This study will require using the PET on which DARWIN EU® tools and analytical pipelines have not been tested. Therefore, the first month of the study will require assessing that the table and tools adapt well to fulfil the study objectives. Following the initial assessment, we will follow up on potential difficulties that may challenge the project's viability.

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17. ANNEXES

Concept/code list/s and/or algorithms used for the identification of exposure/s and/or outcome/s (where relevant and available).

Appendix I. Concept/code list/s and/or algorithms used for the identification of exposure/s and/or outcome/s.

Appendix I. Table 1. List of preliminary codes of drug exposures

Long acting benzodiazepines (half-life >24 h)	**Concept codes**
bromazepam	19030353
brotizolam	19039262
chlordiazepoxide	990678
clobazam	19050832
clonazepam	798874
cloxazolam	19051096
diazepam	723013
flunitrazepam	19055224
medazepam	19125106
nitrazepam	19020021
nordazepam	19080959
Oxazolam	40799014
prazepam	19050461
Short-acting benzodiazepines (half-life <=24 h)	
alprazolam	781039
clorazepate	790253
estazolam	748010
fludiazepam	35198139
flurazepam	756349
lorazepam	791967
lormetazepam	19007977
midazolam	708298
nimetazepam	35197946
oxazepam	724816
temazepam	836715
triazolam	704599
SSRI's	
paroxetine	722031
fluoxetine	755695
citalopram	797617

sertraline	739138
escitalopram	715939
fluvoxamine	751412
SNRI's	
venlafaxine	743670
milnacipran	19080226
levomilnacipran	43560354
duloxetine	715259
desvenlafaxine	717607
Z-hypnotics	
zolpidem	744740
zopiclone	19044883
zaleplon	720727
eszopiclone	757352
Melatonin and melatonin receptor agonists	
melatonin	1301152
ramelteon	781182
tasimelteon	44814600

Concept IDs include all descendants unless otherwise specified.

Appendix I. Table 2. List of Preliminary codes for Indications

	Concept codes
Anxiety disorders	
Anxiety	441542
Panic disorders	436074
Obsessive compulsive disorder (OCD)	440374
Post traumatic stress disorders	436676
Sleep disorders including insomnia	
Insomnia	436962
restless legs syndrome	73754
breathing-related sleep disorders	4009650
History of mental illness (excluding anxiety and insomnia/sleep disorders)	
Depression	440383
Schizophrenia and psychotic disorders	436073
Bipolar disorder	436665

Concept IDs include all descendants unless otherwise specified.

Appendix I. Table 3. List of Preliminary codes of comorbidities

Neurological disorders	**Concept codes**

Seizures and epilepsy	4029498
Gynaecological/obstetric history and disorders	
Pregnancy history before current pregnancy episode (LMP):	
Live birth	From PET
Spontaneous abortion	From PET
Induced abortion	From PET
Stillbirth	From PET
Cardiovascular disorders	
Hypertension	316866
Endocrine disorders	
BMI (at pregnancy start)	From PET
Overweight (at pregnancy start)	From PET
Obesity (at pregnancy start)	From PET
Diabetes	201820
Thyroid disorders	141253
Rheumatological disorders	
Antiphospholipid syndrome	4098292
Systemic lupus erythematosus	255891
Haematological disorders	
Sickle cell anaemia	22281
Prescriptions	
Anticoagulating agents	1145735 1592988 45892847 45775372 43013024 40241331 1315865 1322207 19084670 19026343 19092139 1301025 1301065 1367571 19001014 1308473 1310149
CNS drugs (SNRIs, antipsychotics, others)	21604687 21604719 21604709 21604729 21604726
Hydroxychloroquine	1777087
Lifestyle factors	

Smoking	From PET
Alcohol use	From PET
Healthcare use	
Number of visits before pregnancy (categorical)	

LMP=Last menstrual period. PET=Pregnancy extension table. CNS=Central nervous system. Concept IDs include all descendants unless otherwise specified.

Appendix II: ENCePP checklist for study protocols

ENCePP checklist for study protocols

Study title: DARWIN EU® – Utilisation of commonly used benzodiazepines during pregnancy and the incidence of pregnancy losses

EU PAS Register® number: EUPAS1000000536
Study reference number (if applicable): P3-C3-009

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	5, 8.2
1.1.2 End of data collection[2]	☒	☐	☐	
1.1.3 Progress report(s)	☐	☐	☒	
1.1.4 Interim report(s)	☐	☐	☒	
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	
1.1.6 Final report of study results.	☒	☐	☐	

Comments:

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7, 8.4
2.1.2 The objective(s) of the study?	☒	☐	☐	
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☒	☐	

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.
[2] Date from which the analytical dataset is completely available.

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	8.1.
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	8.2.
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	8.7.
3.4 Does the protocol specify measure(s) of association? (e.g. risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☐	☐	☒	
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☐	☐	☒	

Comments:

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	8.4
4.2 Is the planned study population defined in terms of:				8.3, 8.4, 8.5
4.2.1 Study time period	☒	☐	☐	
4.2.2 Age and sex	☒	☐	☐	
4.2.3 Country of origin	☒	☐	☐	
4.2.4 Disease/indication	☒	☐	☐	
4.2.5 Duration of follow-up	☒	☐	☐	
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	8.5

Comments:

Section 5: Exposure definition and measurement		**Yes**	**No**	**N/A**	**Section Number**
5.1	Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☒	☐	☐	8.5.2
5.2	Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☐	☐	☒	
5.3	Is exposure categorised according to time windows?	☐	☐	☒	
5.4	Is intensity of exposure addressed? (e.g. dose, duration)	☐	☐	☒	
5.5	Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	
5.6	Is (are) (an) appropriate comparator(s) identified?	☐	☐	☒	

Comments:

Section 6: Outcome definition and measurement		**Yes**	**No**	**N/A**	**Section Number**
6.1	Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	8.5.3
6.2	Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	8.5.3
6.3	Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☐	☒	☐	
6.4	Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☐	☒	

Comments:

Section 7: Bias		**Yes**	**No**	**N/A**	**Section Number**
7.1	Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☐	☐	☒	

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☐	☐	☒	
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☐	☐	☒	

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☐	☐	☒	

Comments:

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	8.5
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	8.5
9.1.3 Covariates and other characteristics?	☒	☐	☐	8.5
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☐	☐	☒	
9.2.2 Outcomes? (e.g. date of occurrence, multiple events, severity measures related to event)	☒	☐	☐	8.2
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☐	☒	☐	
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	8.5.2
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	8.5.3

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.3.3 Covariates and other characteristics?	☒	☐	☐	8.5.4
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☒	☐	☐	8.2

Comments:

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	8.7
10.2 Is study size and/or statistical precision estimated?	☐	☐	☒	8.7
10.3 Are descriptive analyses included?	☒	☐	☐	8.7
10.4 Are stratified analyses included?	☒	☐	☐	8.7
10.5 Does the plan describe methods for analytic control of confounding?	☐	☐	☒	
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☒	☐	
10.7 Does the plan describe methods for handling missing data?	☐	☒	☐	
10.8 Are relevant sensitivity analyses described?	☐	☒	☐	

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	9
11.2 Are methods of quality assurance described?	☒	☐	☐	9
11.3 Is there a system in place for independent review of study results?	☐	☐	☒	

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1.1 Selection bias?	☐	☒	☐	
12.1.2 Information bias?	☒	☐	☐	11
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☐	☒	☐	
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☐	☐	☐	8.2, 11

Comments:

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	13
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	9

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	4

Comments:

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	14
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	14

Comments:

Name of the main author of the protocol: Julieta Politi

Date: 03/03/2025

Signature: JP