PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Determining the exposure of maternal medicines through breastfeeding: the UmbrelLACT study protocol - A contribution from the ConcePTION project
AUTHORS	Van Neste, Martje Nauwelaerts, Nina Ceulemans, Michael Van Calsteren, Kristel Eerdekens, An Annaert, Pieter allegaert, karel smits, anne

VERSION 1 – REVIEW

REVIEWER	Kaytlin Krutsch Texas Tech University Health Sciences Center
REVIEW RETURNED	11-Dec-2023

GENERAL COMMENTS	The manuscript, which shares a protocol for clinical lactation pharmacology research, was a privilege to read. The authors have commendably navigated through the complex landscape of lactation pharmacology, presenting a balanced view of the various methodologies and theories prevalent in the field. It makes a significant contribution to the field and offers a well-reasoned solution to reduce barriers to entry into lactation medication research. Review abstract (methods) for minor errors in English grammar (e.g., once vs one, singular vs plural) Page 6, line 23. Please clarify this sentence with further explanation of the “generic basis” Page 6, line 29. UmbrelLACT study is a prospective, observational, open-label, clinical lactation study. Consider modifying terminology “open-label” it is used for interventional studies. Page 6, line 53 & Table 1. Referring to requests/compounds: How are participants taking multiple medications handled? Particularly if the medications are taken at different times, how will the sampling schedule work? If they are taking an acute and chronic medication,

	are they be asked to complete child questionnaires at 2 weeks and 2 months? Page 6, line 58. Is there an option to pool materials or data from other biobanks? Page 7, line 29. Is there a location parameter for the protocol? You mention in Page 6, line 31 that recruitment could happen at "external health facilities." Will participants be limited to Belgium or the EU? Page 9, line 18. Is home sampling self-directed or supervised? Page 9, line 37. How is maternal or infant blood sampling accomplished (at home, in lab…)? Page 10, line 38. Please clarify how you define a healthy volunteer. For example, is a healthy volunteer a healthy, lactating person who does not take medications, herbal supplements? What about vitamin consumption?

REVIEWER	Catriona Waitt
REVIEW RETURNED	27-Dec-2023

GENERAL COMMENTS	The authors highlight an important and understudied area – providing information on the transfer of drug from mother to breastfed infant. This protocol aims to be quite open to study various different medications in lactating women receiving the drug, and to be able to accurately calculate the true volume of milk produced over at 24 hour period. This aim to be very comprehensive and complete is a strength, but I think also brings some important challenges. I note that the protocol has received ethical approval and is likely already underway, but I wish the authors to consider the following. Introduction 1. Some of the benefits of breastfeeding to both mother and child are cited. However, there can also be quiet considerable debate in the literature, partly because of confounding factors such as socio-economic status. Other important benefits which may deserve a mention relate to immunology/ allergy/ obesity etc – it seems the more we understand about these, the more clear the benefits of breastfeeding are. 2. Lines 36-37 – the authors talk about the unnecessary cessation of breastfeeding. Is there a reference or a figure to support the actual prevalence of premature discontinuation of breastfeeding because of medication harm? 3. Page 6, lines 5-25 – some of the challenges and variation in study design of lactation studies are summarised. But there has been quite clear guidance on how to best do these studies for some time ie FDA draft guidance of 2005, revised in 2019 (but

	notably still in ‘draft’ form). Begg and colleagues called for a unified approach quite some years ago. Why is it then that this hasn’t been happening? Is it because people don’t know about the guidance? Or because it is not mandated or incentivised? Or because it is too difficult to carry out? Or other reasons? Study Design 1. I would like more detail on how the women are identified (is every breastfeeding woman receiving a medication going to be invited, for example – or if there is some sort of prioritisation, this should be stated in the protocol). How is the consenting done? Since the study has ethical approval, the ICFs should also be an appendix. 2. The authors correctly state that there is often almost no data upon which to build a sample size calculation. However, some general principles can be considered, for example with regard to the degree of precision with which we hope to estimate a PK parameter. Whilst it is recognised that a single, well characterised maternal blood, breastmilk and infant blood profile can add much to the literature when there is nothing currently written, it is also better to aim for a small number ie 5-10 participants on each of the target drugs. Is there going to be an attempt to have several on each particular drug? 3. Is there an approximate list of target drugs? It currently comes across extremely vague – I appreciate the spirit of the ‘umbrella’ protocol, but are there some specific areas of immediately recognised priority? 4. 15 lactating women per year ‘according to feasibility’ – this number seems quite low, compared to the 50% of breastfeeding women who are anticipated to require some form of medication. Why? 5. One of my biggest concerns relates to the 24 hour collection. Has this been piloted for feasibility and acceptability? It is extremely difficult to accurately collect 24 hour profiles for other body fluids ie 24 hour urine samples. How can we know if this has been done accurately? How much error might this introduce? 6. How will the mother accurately measure the volume of milk? 7. The paper doesn’t actually state much about the 24 hour collection and how that data will be used. 8. So the infant is not to breastfeed during this period of time? I presume they can be given back the milk in a bottle. But what happens if the baby does not take the bottle? How will we know for sure that the mother hasn’t given covert breastfeeds for comfort (of both her and the baby)? 9. The women are required to self-report dosing time in the 3-days prior to PK sampling. How accurately do you think this can be done? Will there be any other support to the women, for example phonecalls from study nurses etc? Have you thought about using WhatsApp video calls or other methods to ascertain this dosing time with more precision? 10. How will the maternal blood sampling be done if the participant is at home during this time period?

	11. How will you identify the healthy volunteers to donate 'blank' breastmilk? Is there a consent form available for these? 12. The biochemical analysis part is very short and somewhat vague. Which assays does the team have access to? Where there is currently no breastmilk assay, do you plan for a partial validation for this new matrix, as according to FDA guidelines for bioanalysis? Is it cost effective to develop a whole new assay for the analysis of a very small number of women? I am not quite clear how this will actually work out in practice! Or is it that through the imiConception network there is such a wide network of academic and industry partners, that this is not foreseen to be a challenge? 13. Page 13, lines 26-30 – the sample size is likely to be very insufficient to investigate infant health outcomes. Furthermore, this is cross-sectional rather than longitudinal. The information gained from this aspect will be very limited. 14. With regard to the statistics – the formulae for RID etc are fine. But on Page 14 lines 5-9, the paragraph is very short and vague. Are the PK data going to be explored in other ways ie with non compartmental analysis? 15. PPIE – how have the patients and public been specifically involved, particularly with regard to the slightly burdensome sampling schedules? Case Report Form Slight concern about the woman's ability to complete this accurately. Will any additional support be provided? Sampling and Storage instructions This is detailed here, but the protocol itself requires more detail. I am still concerned about the ability of a woman to do this with accuracy, and want to know if the procedure has been piloted and what the feedback of the women is. It does not state how the blood is to be drawn – there is no mention of going to a facility or a visit from a healthcare worker, but neither does it suggest the woman takes it herself.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1
Kaytlin Krutsch, Texas Tech University Health Sciences Center

Dear Reviewer 1, Dear Prof. Kaytlin Krutsch,

We thank you for your time invested to review our manuscript. Please find the detailed responses below and the corresponding revisions highlighted in the re-submitted files.

Comments to the Author
The manuscript, which shares a protocol for clinical lactation pharmacology research, was a privilege to read. The authors have commendably navigated through the complex landscape of lactation

pharmacology, presenting a balanced view of the various methodologies and theories prevalent in the field. It makes a significant contribution to the field and offers a well-reasoned solution to reduce barriers to entry into lactation medication research.

Thank you for your comment and your acknowledgement for the complex landscape and barriers in lactation studies.

Review abstract (methods) for minor errors in English grammar (e.g., once vs one, singular vs plural)

We adapted this grammatical error and revised our abstract for remaining errors.

Page 6, line 23. Please clarify this sentence with further explanation of the “generic basis”

We thank you for this suggestion. As the predictions of one physiologically-based pharmacokinetic (PBPK) model for breastfeeding women and their breastfed infants is thoroughly evaluated by real-world data from this study, this prediction can function as starting point for future PBPK models for other compounds. This means that the building blocks of population- and medicine-specific parameters are available to build PBPK models in these populations for other compounds, and further improves the certainty confidence in the PBPK model performance. This was further explained in the second to last paragraph of the introduction: ‘These thoroughly evaluated PBPK models will lead to a generic set of breastfeeding-specific population parameters, enabling the construction of structural PBPK frameworks for breastfeeding women and breastfed infants. Such frameworks might subsequently be applied with increasing certainty to similar medicines for which in vivo data are still lacking.’

We hope this additional information clarified this specific sentence.

Page 6, line 29. UmbrelLACT study is a prospective, observational, open-label, clinical lactation study. Consider modifying terminology “open-label” it is used for interventional studies.

We thank you for this remark, and removed the term ‘open-label’ to avoid misunderstanding with interventional studies.

Page 6, line 53 & Table 1. Referring to requests/compounds: How are participants taking multiple medications handled? Particularly if the medications are taken at different times, how will the sampling schedule work? If they are taking an acute and chronic medication, are they be asked to complete child questionnaires at 2 weeks and 2 months?

For each patient, inclusion is based on 1 main compound of interest. The sampling schedule starts at intake of this compound and the timing of the last child questionnaire is based on the acute or chronic character of this compound as well.

Data regarding the intake of other interesting medication during breastfeeding is registered in the medicine questionnaire. The samples can be analyzed for a second compound as well, as we will cover a period of 24h, however, this might not start at the intake of a second interesting compound.

In the unlikely event that the participant would take a combination of an acute and chronic treatment, we would indeed ask to complete the child questionnaires at 2 weeks and 2 months. This way we can focus on the differences in the acute and chronic time frame.

This information was added in the next to last paragraph of the subsection ‘study visits - sampling day’: ‘If the participant is using a second compound of interest, the samples will be analyzed for that

compound as well.' and in the last paragraph: 'If an acute and chronic treatment is combined, the participants will be asked to complete the questionnaire at 2 weeks and 2 months after the last sampling day.'

Page 6, line 58. Is there an option to pool materials or data from other biobanks?

If other groups studying the same compound(s) in breastfeeding women, we are very open to collaborate and pool data with other research groups/biobanks, if the protocol and samples collection is in line with our protocol while respecting all guidelines and regulations in the relevant biobanks. Consequently, data sharing and transfer agreements will be developed per request.

This was added in subsection 'study design' as follows 'Optional, available cases in literature or data from other biobanks with a similar sampling method can be pooled with new data achieved from the current study protocol, while respecting all guidelines and regulations in the relevant biobanks, to generate more insights from the pooled available observations.'

Page 7, line 29. Is there a location parameter for the protocol? You mention in Page 6, line 31 that recruitment could happen at "external health facilities." Will participants be limited to Belgium or the EU?

There is no strict limitation on the location of the patients. However, sample collection in patients outside of Belgium or the EU is less feasible for our group (e.g. study team member to draw the blood samples, transport to Belgium). To be consistent throughout the paper, we also removed 'in Belgium' in the last paragraph of the introduction section.

Page 9, line 18. Is home sampling self-directed or supervised?

The human milk sampling is mainly self-directed. The participants receive a Standard Operating Procedure (SOP) document with the sampling and storage instructions before the start of the sampling day. During the first collection of a human milk sample, a member of the study team is generally present to go over the procedure with the participant. Furthermore, a member of the study team is accessible during the 24h period. However, the SOP document is sufficiently clear to instruct the participant for self-directed sampling.

We have clarified this in the section 'study visits – sampling day': 'Human milk sampling is performed at the patient's home or during hospitalization for medical reasons, with self-directed sampling instructions provided (see supplementary file 3). A member of the study team is generally present during the first human milk sample collection to assist the procedure and is afterwards available during the 24h collection period. Blood sampling is performed at the patient's home or outpatient clinic at the University Hospitals Leuven, by a member of the study team.'

Page 9, line 37. How is maternal or infant blood sampling accomplished (at home, in lab…)?

The blood samples (maternal and infant) are collected at the patient's home or at the outpatient clinic of the University Hospital Leuven by a trained person from the study team. This is added to the manuscript (section 'sampling day').

Page 10, line 38. Please clarify how you define a healthy volunteer. For example, is a healthy volunteer a healthy, lactating person who does not take medications, herbal supplements? What about vitamin consumption?

We thank the reviewer for this question. The healthy volunteers in our study are healthy, breastfeeding women without pharmacotherapy for an underlying medical condition. Exposure of these volunteers to commonly used products (e.g. vitamins) is collected in a questionnaire (added as supplementary material 7). This was clarified in the section 'healthy volunteers': 'Milk (100-300 mL) and plasma samples (15-20 mL) of healthy volunteers, breastfeeding women without pharmacotherapy for an underlying medical condition, are required for method development of new milk and plasma assays, validation of the analytical methods and development of improved PBPK models.'

Reviewer 2
Catriona Waitt

Dear Reviewer 2, Dear Prof. Catriona Waitt,

We thank you for your time reviewing our manuscript. Please find the detailed responses below and the corresponding revisions highlighted in the re-submitted files.

Comments to the Author

The authors highlight an important and understudied area – providing information on the transfer of drug from mother to breastfed infant. This protocol aims to be quite open to study various different medications in lactating women receiving the drug, and to be able to accurately calculate the true volume of milk produced over at 24 hour period. This aim to be very comprehensive and complete is a strength, but I think also brings some important challenges. I note that the protocol has received ethical approval and is likely already underway, but I wish the authors to consider the following.

Introduction

1. Some of the benefits of breastfeeding to both mother and child are cited. However, there can also be quiet considerable debate in the literature, partly because of confounding factors such as socio-economic status. Other important benefits which may deserve a mention relate to immunology/ allergy/ obesity etc – it seems the more we understand about these, the more clear the benefits of breastfeeding are.

We agree that there are many more benefits of breastfeeding that could be described in our introduction. Therefore, we highlighted the benefits related to immunology and obesity as well in the introduction, as follows 'Breastfed children are less likely to suffer from childhood infections, at least in part due to antibodies in human milk, and have a lower risk of being overweight.'

2. Lines 36-37 – the authors talk about the unnecessary cessation of breastfeeding. Is there a reference or a figure to support the actual prevalence of premature discontinuation of breastfeeding because of medication harm?

A recent study, conducted by the teratology information service Lareb in The Netherlands, showed that 12% of their participants did not start breastfeeding and in 11% of these women, the cause was medication (Passier et al., 2023; https://doi.org/10.1016/j.ntt.2023.107209). Furthermore, out of women who did not meet their desired breastfeeding duration, 17% reported having stopped breastfeeding because of a medical condition or intake of medicine (Odom et al., 2013; doi: 10.1542/peds.2012-1295). A review from 2015 confirms the lower initiation and/or duration of breastfeeding because of maternal pharmacotherapy (Saha et al., 2015; doi: 10.1186/s13006-015-0053-6). These last 2 references were added to the manuscript.

3. Page 6, lines 5-25 – some of the challenges and variation in study design of lactation studies are summarised. But there has been quite clear guidance on how to best do these studies for some time ie FDA draft guidance of 2005, revised in 2019 (but notably still in 'draft' form). Begg and colleagues called for a unified approach quite some years ago. Why is it then that this hasn't been happening? Is it because people don't know about the guidance? Or because it is not mandated or incentivised? Or because it is too difficult to carry out? Or other reasons?

We thank the reviewer for this reflection. The FDA has indeed published their draft of considerations for the study design of clinical lactation studies, with a focus on regulatory approval. However, this draft guidance still has a lot of room for individual decisions and is not a clear guideline on the study design of clinical lactation studies. We are aware that this guidance is currently under revision, and so is the EMA guidelines (pharmacovigilance). This at least suggests that this is a dynamic field, with changing practices, so that there is value to publish this protocol.

Furthermore, as mentioned by Begg et al., human milk is a complex matrix with a variable composition, even in a single feeding moment. Therefore, for each compound and/or study (site), different study designs and collection methods can be considered. Additionally, this protocol captures the potential added value of PBPK models in lactation cases, which has not been described by the FDA guidance. However, our focus is not the cause of different clinical lactation study methods or why the FDA guidance has not been implemented yet.

By sharing our UmbrelLACT protocol in this manuscript, we aim to share our methods for different compounds as a starting point for standardized clinical lactation studies, in which the information needed for future PBPK models are collected as well.

Study Design

1. I would like more detail on how the women are identified (is every breastfeeding woman receiving a medication going to be invited, for example – or if there is some sort of prioritisation, this should be stated in the protocol). How is the consenting done? Since the study has ethical approval, the ICFs should also be an appendix.

Based on the recruitment strategy approved by the Ethics Committee Research UZ/KU Leuven, the study information is shared through multiple ways, such as physicians at the University Hospitals Leuven, on the BELpREG project website and social media channels and other external health facilities. If a woman is interested in the study, she has to contact the study team who will a) provide detailed information on the study, and b) plan a screening visit. This is explained in the subsection 'screening visit' of study design.

We have also added some more clarity to the section 'study design' regarding our recruitment methods, as follows: 'Distribution of the study awareness and information may occur via different channels, i.e., the University Hospitals Leuven, the BELpREG pregnancy registry in Belgium (www.belpreg.be) or external health facilities, so that interested breastfeeding women are able to contact the study team.'

After the screening visit, we ask the interested woman to send the original signed informed consent form back to our study team. We agree that the ICF should be included as supplementary materials. We have added this in the resubmission.

2. The authors correctly state that there is often almost no data upon which to build a sample size calculation. However, some general principles can be considered, for example with regard to the degree of precision with which we hope to estimate a PK parameter. Whilst it is recognised that a single, well characterised maternal blood, breastmilk and infant blood profile can add much to the literature when

there is nothing currently written, it is also better to aim for a small number ie 5-10 participants on each of the target drugs. Is there going to be an attempt to have several on each particular drug?

We agree that it is interesting to group participants who are taking the same drug, if possible. At present, we can announce that for 2 different compounds, observations of 2 patients for each compound are included in the UmbrelLACT study. This illustrates that we indeed aim to collect several cases per compound, if feasible. Furthermore, as the data will also be used to adequately evaluate the predictive performance of the PBPK models, only a small number of cases is needed for each compound.

We clarified this in the subsection 'outcomes' as follows 'If feasible, we aim to collect several cases per compound.'

3. Is there an approximate list of target drugs? It currently comes across extremely vague – I appreciate the spirit of the 'umbrella' protocol, but are there some specific areas of immediately recognised priority?

We understand this question of the reviewer. A list of targeted drugs was not determined for our umbrelLACT protocol. Our priority are compounds of which only limited or no data are available and of which the risks through exposure in human milk are unknown. Examples of these drugs can be for example cardiovascular compounds (e.g. atorvastatin, simvastatin, lisinopril, enalapril), immunomodulatory drugs (e.g., dimethyl fumarate) and anti-epileptics (e.g. topiramate, pregabalin). To keep possibilities for inclusion as broad as possible, no predefined restrictions on compounds were made. This allows flexibility within the umbrella approach, compared to a compound specific study protocol. We hope this sufficiently clarifies our methodology.

4. 15 lactating women per year 'according to feasibility' – this number seems quite low, compared to the 50% of breastfeeding women who are anticipated to require some form of medication. Why?

The majority of women taking medication during breastfeeding are using a compound that is known to be safe during lactation, such as paracetamol, ibuprofen or amoxicillin. When only limited knowledge is available, women often decide to not combine breastfeeding with medicine intake. This is a limitation on the number of possible participants of our study.

In addition, as the sample collections occur at the patient's home, a member of the study team needs to be available and sometimes to travel to collect the samples. At present, our team has 2 experienced PhD students involved in the UmbrelLACT study. Therefore, 'according to feasibility' refers to the (current) limitations of the availability of the study team as well. Furthermore, the bioanalysis and PBPK analysis is also resource taking. This means we have to decide on a case-by-case basis and prefer to be and remain conservative in our predefined estimations.

5. One of my biggest concerns relates to the 24 hour collection. Has this been piloted for feasibility and acceptability? It is extremely difficult to accurately collect 24 hour profiles for other body fluids ie 24 hour urine samples. How can we know if this has been done accurately? How much error might this introduce?

The 24 h collection is also mentioned in the previously discussed FDA guidance, so is not new. This method reflects the daily human milk intake of the child, which is used to estimate the child's exposure.

Furthermore, the feasibility of human milk collection over 24 hours was (pilot-) tested before writing this protocol. Our group studied the exposure of bosentan and sildenafil in human milk in a case report (Nauwelaerts et al., 2022; DOI: 10.3389/fphar.2022.881084). This patient successfully collected human milk samples over a 24-hour period at two different days.

We standardize the human milk collection procedure by using a SOP document providing clear instructions for self-directed sampling to the patients. During the first collection of the human milk samples, a member of the study team is generally present to go over the procedure of human milk sample collection. Furthermore, if there are problems or questions during the 24 hour collection period, a member of the study team can be contacted and is accessible to assist. This was added in the subsection 'study visits – sampling day': 'A member of the study team is generally present during the first human milk sample collection to assist the procedure and is afterwards available during the 24h collection period.'

Furthermore, the human milk samples are collected opportunistically. This means that the women are asked to express their milk each time they would normally breastfeed their child, to minimally disturb the child's day schedule. These samples, combined with the prediction of the PBPK model, will provide reliable information regarding the 24h time concentration profile.

6. How will the mother accurately measure the volume of milk?

A measuring cup is provided for the participants during the collection of samples at home. This was clarified as follows 'For each sampling moment, the total volume of milk (determined using a measurement cup) and sampling time are noted, to simulate the child's milk intake of a feeding session.'

7. The paper doesn't actually state much about the 24 hour collection and how that data will be used.

We agree that this was not clearly stated in the manuscript. As we want to minimize the burden for participants, we ask the women to express their milk each time they would normally breastfeed their child. We have clarified this in the subsection 'Study visits-Sampling day-Human milk' as follows: 'The total available milk volume of 1 feed is collected from both breasts by using an electric pump each time the woman would normally breastfeed her child.'

The volumes of these expression sessions are noted by the participants, so that these can be used to accurately reflect the intake of the infant over a 24h period. Only 5 to 10 mL is collected from each expression session, and used for analysis.

The samples will be analyzed to determine the concentration of medicine in that matrix, as clarified in the section 'biochemical analysis' and further used to calculate multiple PK and exposure determinants (as listed in 'outcomes').

8. So the infant is not to breastfeed during this period of time? I presume they can be given back the milk in a bottle. But what happens if the baby does not take the bottle? How will we know for sure that the mother hasn't given covert breastfeeds for comfort (of both her and the baby)?

We understand this remark of the reviewer. The aim of this study is to collect all the human milk through expression, which indeed means that the child cannot breastfeed during this sampling collection period. After collection of 5-10 mL of an expression session, the participant can decide how the remainder of the collected milk is used, which means that they can indeed give the milk in a bottle to the child. We do explain this method thoroughly to the women before they have to decide to participate to this study and this method. At each time, participants are free to decide to prematurely withdraw from the study if they prefer to not continue.

However, we cannot be absolutely certain that covert breastfeeds do not happen, but we do believe in the honesty of our very motivated participants when reporting back to the study team.

9. The women are required to self-report dosing time in the 3-days prior to PK sampling. How accurately do you think this can be done? Will there be any other support to the women, for example phonecalls from study nurses etc? Have you thought about using WhatsApp video calls or other methods to ascertain this dosing time with more precision?

The prospective self-reporting of dosing time prior to the sampling day is explained before the start of the study. No additional active support (e.g. phone or video calls) is installed. We do believe that the participants can do this accurately themselves at home, in line with other prospective studies for which diaries or medical information has to be reported. The main goal of this medicines list is to know if a dose has been forgotten by the participant, or if interactions between medicines have to be anticipated. The prospectively collected medicine list is part of the supplementary file 2.

10. How will the maternal blood sampling be done if the participant is at home during this time period?

The blood sampling will occur at the participant's home or at the outpatient clinic at the university hospitals Leuven by a trained member of the study team. This is added to the manuscript (section 'study visits – sampling day').

11. How will you identify the healthy volunteers to donate 'blank' breastmilk? Is there a consent form available for these?

The healthy volunteers in our study are healthy, breastfeeding women without pharmacotherapy for an underlying medical condition. Exposure of these volunteers to commonly used products (e.g. vitamins) is collected in a questionnaire. This was added in the section 'healthy volunteers': 'Milk (100-300 mL) and plasma samples (15-20 mL) of healthy volunteers, breastfeeding women without pharmacotherapy for an underlying medical condition, are required for method development of new milk and plasma assays, validation of the analytical methods and development of improved PBPK models.'

The informed consent form and questionnaire for healthy volunteers was translated to English and added as supplementary materials 6 and 7.

12. The biochemical analysis part is very short and somewhat vague. Which assays does the team have access to? Where there is currently no breastmilk assay, do you plan for a partial validation for this new matrix, as according to FDA guidelines for bioanalysis? Is it cost effective to develop a whole new assay for the analysis of a very small number of women? I am not quite clear how this will actually work out in practice! Or is it that through the imiConception network there is such a wide network of academic and industry partners, that this is not foreseen to be a challenge?

Thank you for this relevant question. As we want to study different compounds opportunistically, it is difficult to know the needed assays beforehand. While working on this study, we want to further broaden our existing IMI ConcePTION network to a wider network of academic and industry partners that may have assays available. Furthermore, we do have contact with several (international) research institutes that can develop different assays. These new developed assays should be validated according to the EMA guidelines on bioanalytical method validation in the relevant institution. For each compound for which samples might be collected, we ask beforehand in and outside the ConcePTION network if an assay is or may easily become available in human milk or another matrix. This was clarified in the section 'biochemical analysis': 'For the quantification of medicines concentration in the milk and blood samples, Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) will mainly be used, or a different method depending on the compound(s), at a facility where the requested assay(s) is/are or will easily become available for human milk or another matrix.'

13. Page 13, lines 26-30 – the sample size is likely to be very insufficient to investigate infant health outcomes. Furthermore, this is cross-sectional rather than longitudinal. The information gained from this aspect will be very limited.

We agree that the information gained on the health outcome of the child will be limited, but this information is essential in this type of case reports / case series, and can be a starting point for further investigation. Each participant will be asked to fill out the questionnaire regarding the general health outcome of the child at least 2 times, thereby collecting some longitudinal data. Clarification was added in the subsection 'secondary outcomes': 'Third, maternal questionnaires will be used as an instrument to investigate the health outcome of the exposed children up to a maximum of 2 months after the last sampling day and will provide valuable, although limited, information on potential side effects of maternal medicines' use in the breastfed child.' We expect that any medical journal considering one of the UmbrelLACT papers for publication will request this type of information, even being aware of the limitations.

14. With regard to the statistics – the formulae for RID etc are fine. But on Page 14 lines 5-9, the paragraph is very short and vague. Are the PK data going to be explored in other ways ie with non compartmental analysis?

We agree that the paragraph regarding statistics was short and vague. Therefore, we have added this information to the 'outcomes' subsection to clarify how the outcomes can be interpreted.

As the samples are collected opportunistically, these will always be reported using descriptive statistics. It will not always be possible to do non-compartmental analysis. If possible, however, non-compartmental analysis will be explored, such as determining the area under the milk concentration-time curve, the average concentrations and mean milk concentrations, as mentioned in the subsection 'primary outcomes'.

15. PPIE – how have the patients and public been specifically involved, particularly with regard to the slightly burdensome sampling schedules?
A pilot case report was conducted to test the feasibility of the 24 hour human milk sample collection method from this study (Nauwelaerts et al., 2022; DOI: 10.3389/fphar.2022.881084). The participant of this case report executed the 24h collection period twice and did not mention the sampling methods as highly burdensome. We clarified this in the PPI section as follows 'Furthermore, the feasibility of the 24h human milk collection period was successful executed in a recently published case report of our group.' As the reviewer has likely noticed, we also intend to report on this patient and public perspective/experiences once we have a 'case series' within this protocol.

<u>Case Report Form</u>
Slight concern about the woman's ability to complete this accurately. Will any additional support be provided?
Prior to the start of the study, the CRF is explained in detail to the patient. If the patient has however specific questions concerning the CRF, a member of the study team is available at any time to be contacted and for further support and explanation.

Sampling and Storage instructions
This is detailed here, but the protocol itself requires more detail.

The sampling and storage instructions are described in more detail in the original protocol itself. Due to the word count, we have summarized the relevant details.

I am still concerned about the ability of a woman to do this with accuracy, and want to know if the procedure has been piloted and what the feedback of the women is.

We thank the reviewer for this question. As mentioned above, this procedure has been successfully piloted showing to be feasible in practice. Furthermore, the participants receive detailed information, as well as a SOP document with the sampling and storage instructions before the start of the sampling day and a member of the study team is accessible during the collection period, as well as prior to completing the CRF. We hope this clarifies the feasibility of the study procedures as described in the manuscript.

It does not state how the blood is to be drawn – there is no mention of going to a facility or a visit from a healthcare worker, but neither does it suggest the woman takes it herself.

The blood samples are indeed collected at home or at the outpatient clinic at the university hospitals Leuven, by a member of the study team. This was added in the subsection 'Sampling Day'.

VERSION 2 – REVIEW

REVIEWER	Catriona Waitt
REVIEW RETURNED	09-Feb-2024

GENERAL COMMENTS	Thank you for your detailed responses to my concerns. All of the questions have been satisfactorily answered, and any limitations are clearly understood by the investigators. The project is important and ambitious, and sharing the protocol is an important step in sharing knowledge. I will be very interested to know how the project goes, and it would be interesting to get some kind of further feedback from the mothers about the 24 hour milk collection. I know it is mentioned in FDA guidelines and the seminar Begg paper, but from my experience both as a researcher in lactation pharmacology and as a former breastfeeding mother, I know that for some it will be difficult! You give a single example of having tested it out - which is good, but during the course of this protocol you will have good opportunity to further assess how the women find the process, and then report back on this to the wider research community.

REVIEWER	Kaytlin Krutsch Texas Tech University Health Sciences Center
REVIEW RETURNED	19-Feb-2024

GENERAL COMMENTS	Thank you for the comprehensive clarifications provided in the revised manuscript. My concerns have been addressed. I appreciate the practicality and scalability of the protocol's design. The feasibility of the approach enhances the potential impact of the study, which is particularly significant given the minimal gains made overall in this topic over the past decades.

VERSION 2 – AUTHOR RESPONSE

None