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)	An interventional cohort study of prolonged use (>72 hours) of paracetamol in neonates: protocol of the PARASHUTE study
AUTHORS	Haslund-Krog, Sissel; Hertel, Steen; Dalhoff, Kim; Poulsen, Susanne; Christensen, Ulla; Wilkins, Diana; Van Den Anker, John; Brink Henriksen, Tine; Holst, Helle

VERSION 1 – REVIEW

REVIEWER	Reviewer name: Mikko Hallman Institution and Country: University of Oulu Competing interests: Applying funding for a trial on Acetaminophen. Present Reviewer does not feel that PARASHUTE is an actual competitor
REVIEW RETURNED	18-Jan-2019

GENERAL COMMENTS	This manuscript describes the protocol of an ongoing study. It is informative for neonatologist and particularly for those interested in pain therapy. No new data is presented. In this manuscript, the authors describe the protocol of an open-label phase IV multicenter, Denmark-based trial on long-term use of paracetamol in neonates (PARASHUTE trial). The aim is to investigate the safety and effectiveness of prolonged (>3d) paracetamol exposure in neonates by measuring hepatic biomarkers, plasma concentrations of paracetamol, its metabolites and the pain scores. In addition, the aim is to study the interaction between ethanol, a carrier of the phenobarbital drug, and paracetamol. Neonates up to 44 weeks of postmenstrual age, who are treated with oral or intravenous paracetamol, can be included. The plan is to measure ALT, bilirubin and a sophisticated analysis of paracetamol metabolites under a set protocol. Ethanol is measured among the recipients of phenobarbital. Only patients with a consent are included; standard, not strictly-defined dosing strategies are used. The intention is to make the inclusion process less stressful to parents and therefore the inclusion can be postponed 24 hours after the first paracetamol dose. This study deals with significant problem in neonatal medicine: acetaminophen is widely used in many centers, yet no adequate safety studies have been presented. The manuscript, particularly the pronged introduction is informative and well written. The study will likely provide some new and extend the available information on the paracetamol metabolites and the potential toxicity of the drug in this population. However, the protocol has several limitations. These include the evaluation of the efficacy. There is no control group, without treatment as the patients serve as their own controls. The influence of paracetamol on the opioid use remains open. In addition, a follow-up evaluation (at least parent-assessment) would have been desirable.

	Specific comments. 1. Title is complex and tries to explain the actual plan (perhaps a more simple title, “Phase IV study investigating prolonged use of paracetamol in neonates: the protocol of the PARASHUTE trial”. This study is named as phase IV; it mostly deals with pharmacology (see below). Indeed this is necessary as the present drug was adopted in management of newborn without much systematic studies, particularly on infants with different degrees of prematurity. 2. Abstract. The stated aim is to investigate the safety and effectiveness of paracetamol. The investigators state that they have an electronic data capture tool and that each participating center routinely record COMFORT neo-pain scores before and after the drug. It seems that without a comparison group and lack of data on the use of other drugs (such as opioids) this trial may not provide accurate evaluation of the pain, discomfort and of other potential potentially beneficial effects. Correlating pain scores with serum levels of paracetamol is certainly useful. 3. Introduction is well written and informative. In page 6, 2nd paragraph states that only three studies that examine repeated iv paracetamol administration in neonates. This is old data need to be updated. In addition, the basis for recording specific coagulation factors should be briefly explained. 4. Objectives (page 3, first sentence). “Long-term” is misleading. Use another expression here. 5. Page 7, last paragraph. The compulsory and conditional withdrawal of blood specimens is shown. The volume requirements was given later in the text. According to protocol there was no lower GW or BW limit. Does the consent allow withdrawal of extra blood from extremely tiny infants? 6. Page 8, 3rd paragraph. We understand the difficulty of determining the sample size in this case. In general, phase IV studies involve a large population sample. However, according to present stopping rule there is no lower limit of the number of patients. The statement may need to be rephrased. 7. Page 9, last paragraph. Potential adverse long-term effects is not is not pointed out. This is unfortunate and it may depend on budget restrictions although a self-reported questionnaire or permission from the parents to utilize follow-up register data would have been affordable options.

REVIEWER	Reviewer name: Paola Mian Institution and Country: Erasmus MC Sophia's Children Hospital, Rotterdam, The Netherlands Competing interests: No
REVIEW RETURNED	22-Jan-2019

GENERAL COMMENTS	The authors have written a trial protocol to characterize the PK/PD of paracetamol and its metabolites in neonates. It is indeed an important problem. However there are some gaps that needs to be filled. Below are some in no particular order. However, stratified as major and minor. Major 1. The toxicity of paracetamol has been narrowed to hepatic, while some authors also suggest potential nephrotoxicity during repeated administation, while nothing is mentioned on the association between perinatal paracetamol exposure and potential risks for atopy, fertility and neuro-cognition. Please clarify this more throughout the manuscript.

	2. Indications and co-medication (e.g. opioid coadministration) are missing 3. Are nurses and/or care-provides trained on the NEO-COMFORT score and are there any data on kappa values? 4. There will be much more value in this paper if the authors would add some data on the retrospective analysis mentioned in the paper (indication, clinical characteristics, duration, etc) 5. The power calculation reads very 'pragmatic' in my assessment. 6. The abstract method part does not say anything related to analyzing the data (PK, PD or safety) 7. A) The analysis part is very short (" PKPD-modelling will be performed"), how do you analyze the safety parameters? And efficacy parameters? B) I have some concerns related to the analysis of the efficacy (PD) data, as I understood from the protocol that every medication is allowed to be given (including opioids and sedation). This will definitely have some effect on the PD. How do you take this into account? How do you make sure you can say something related to only the PD of paracetamol? Please add this in the manuscript. 8. Is there a specific reason why the authors decided to only focus on ALAT and not on ASAT, PT, creatinine and gamma_GT? Minor 1. There is also a minor (about 10 %) primary renal elimination route 2. The idea to link this also to ethanol exposure is valuable, although it is not clear how the clinical research team will screen for any ethanol exposure, or – since interaction has been described – propylene glycol (commonly present in paracetamol syrups). What specific 'market' products will be used ? 3. I miss the recently published paper of Flint et al on paracetamol pk and its metabolites in the youngest age category of preterms (Pediatr Research) 4. Line 30-33 in the manuscript reference 9 and 10 have been using the same data, but according to your manuscript the results are different. How is that possible as the same data has been used for both publications? 5. Is there any reason why there is no focus in the manuscript on metabolomics (as according to me a delay in measurement of elevated safety parameters -12-24h- can occur), such as mRNA-122, HMGB1, k18?

REVIEWER	Reviewer name: Outi Aikio Institution and Country: Oulu University Hospital, Oulu; Finland Competing interests: None, if my own paracetamol research among preterm infants can be excluded
REVIEW RETURNED	24-Jan-2019

GENERAL COMMENTS	This publication is a research plan about drug metabolites and their interactions as well as the pain relieving effect of paracetamol among newborn infants. This is an interesting plan about a subject that has not been well studied yet. It is well written and clear English text. It is very detailed, and I think that many parts could be shortened. The introduction part of the trial contains most of the relevant studies on the field.

	However, a cohort study on the paracetamol morphine-sparing effect in premature infants should have been cited as well, as present study does not limit the gestational age of the study infants (Härmä et al, J Pediatr 2016). The paracetamol metabolite studies are indeed lacking among newborn infants. In present research plan, I failed to find the study drug dose used. However, I hope no toxic levels are expected. The setting is somewhat troublesome: the authors say that no previous studies exist, but yet they claim their study to be an open, phase 4 trial? As no randomisation, blinding or control population is planned, this study should be defined as a prospective cohort study. The missing power analysis also points to this. Furthermore, the sample size calculation is quite possible even for cohort studies and without previous trials. The question is about defining the primary outcome and the size of the clinical difference sought. When laboratory values are set as the primary outcome, its clinical relevance for the patients should be considered. The exclusion criteria need clarification - they do not tell the reader enough about the generalisability of the results. Another problem is the missing placebo when evaluating the efficacy of a pain medication. It is well known that the interpretation of the pain symptoms of newborn infants is challenging. Unfortunately the outcomes of all the pain scales are deviated if the appraisal knowns that the pain-relieving drug has been given. I am also worried about the use of ethanol-containing drugs in newborn infants in general. Was the ethics committee ok with it? I found one error on page 7, paragraph 4, line 48: missing full stop?

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer 1, Prof. Mikko Hallman, Oulu University Hospital, Oulu; Finland:

Thank you for your review of our paper. We have answered each of your points below.

1. Title is complex and tries to explain the actual plan (perhaps a more simple title, “Phase IV study investigating prolonged use of paracetamol in neonates: the protocol of the PARASHUTE trial”. This study is named as phase IV; it mostly deals with pharmacology (see below). Indeed this is necessary as the present drug was adopted in management of newborn without much systematic studies, particularly on infants with different degrees of prematurity.

Response: The title has been rephrased to: “An interventional cohort study of prolonged use of paracetamol in neonates: protocol of the PARASHUTE study”. See Editors comment and reviewer 3, point 3.

2. Abstract. The stated aim is to investigate the safety and effectiveness of paracetamol. The investigators state that they have an electronic data capture tool and that each participating center routinely record COMFORT neo-pain scores before and after the drug. It seems that without a comparison group and lack of data on the use of other drugs (such as opioids) this trial may not provide accurate evaluation of the pain, discomfort and of other potential potentially beneficial effects. Correlating pain scores with serum levels of paracetamol is certainly useful.

Response: COMFORT neo-pain scores in relation to paracetamol concentration is a secondary endpoint. The study is not powered to evaluate if add on of paracetamol will have a morphine sparing effect. However, all comedication including morphine and other analgesics are registered electronically together with time of administration and dose. Depending on the size of the subgroup receiving monotherapy of paracetamol a correlation between pain scores and serum levels of paracetamol will be investigated. Additionally, we will have numerous and continuous COMFORTneo scores, which will hold interesting information on the pain history over time for neonatal patients.

(p. 9, line 24).

3. Introduction is well written and informative. In page 6, 2nd paragraph states that only three studies that examine repeated iv paracetamol administration in neonates. This is old data need to be updated. In addition, the basis for recording specific coagulation factors should be briefly explained.

Response: To our knowledge there are no other studies that simultaneously have reported repeated i.v. paracetamol administration and measured both pharmacokinetic and hepatic biomarkers. Before the study was initiated we also searched clinical trial.gov and eudra trial website, to avoid any duplicating of studies. The only other studies we have been able to find is related to ductus closure, which is not the scope of the current trial. If other studies exist, please provide us with these references?

The measurement of coagulation factors is briefly described in page 8, line 3. These measures are included as standard measurements in the NICUs.

4. Objectives (page 3, first sentence). “Long-term” is misleading. Use another expression here.

Response: Please refer to the explanation under the first point.

5. Page 7, last paragraph. The compulsory and conditional withdrawal of blood specimens is shown. The volume requirements was given later in the text. According to protocol there was no lower GW or BW limit. Does the consent allow withdrawal of extra blood from extremely tiny infants?

Response: The limits of withdrawal of extra blood is in line with the recommendation made in the ‘Guideline on the investigation of medicinal products in the term and preterm neonate.’ (https://www.ema.europa.eu/en/investigation-medicinal-products-term-preterm-neonate)

Which is 2,4-2,7 ml per kg (page 18, section 9.6 last paragraph in the guideline). For each patient, this is calculated with an upper limit. This limit is never exceeded.

6. Page 8, 3rd paragraph. We understand the difficulty of determining the sample size in this case. In general, phase IV studies involve a large population sample. However, according to present stopping rule there is no lower limit of the number of patients. The statement may need to be rephrased.

Responses: In general, neonatal studies, do not include very large sample size (https://ec.europa.eu/health/sites/health/files/files/paediatrics/docs/paediatrics_10_years_ema_technical_report.pdf, page 34-36). In the manuscript page 8, last paragraph it is stated: “It was therefore decided to include a minimum of 60 patients and a maximum of 120 patients”. The lower limit is 60 patients. The largest trial that has been performed previously in a similar group was 50 (Palmer et al).

7. Page 9, last paragraph. Potential adverse long-term effects is not pointed out. This is unfortunate and it may depend on budget restrictions although a self-reported questionnaire or permission from the parents to utilize follow-up register data would have been affordable options.

Response: Due to the nature of the design, which allows co medication, it will not be possible to assesses the causality using WHOUMC (https://www.who.int/medicines/areas/quality_safety/safety_efficacy/WHOcausality_assessment.pdf)

or Naranjo scales (https://livertox.nih.gov/Narajo.html) .First line in the last paragraph page 9, has been changed to: “At present, few hepatic safety data exist on prolonged paracetamol treatment”.

Response to Reviewer 2, Paola Mian, Erasmus MC Sophia's Children Hospital, Rotterdam, The Netherlands:

Thank you for your comments. Our answers to your points are as follows.

Major

1.The toxicity of paracetamol has been narrowed to hepatic, while some authors also suggest potential nephrotoxicity during repeated administation, while nothing is mentioned on the association between perinatal paracetamol exposure and potential risks for atopy, fertility and neuro-cognition. Please clarify this more throughout the manuscript.

Responses: Due to the very limited amount of blood that can be drawn for each patient the authors decided to primarily to evaluate the hepatic toxicity due to the nature of the parent compound metabolic pathway, a bracket has been to the abstract after safety “(hepatic tolerance)” to be align with the objective page 7, line 15 emphasising on hepatic toxicity. We do collect all laboratory results that are analysed because of other test taken from each patient, herein nephrotic parameters to see if we can find a trend. We have no information about the mothers’ potential use of analgesics during pregnancy. A paragraph has been added page 8, line 7: “This study does not include data on perinatal exposure of paracetamol, since paracetamol is an over the counter drug in Denmark and is used quite frequently. The nature of the study will therefore not reliably be able to backtrack during which part of the perinatal period the neonate has been exposed and to which extend.”

2. Indications and co-medication (e.g. opioid coadministration) are missing

Response: We are collecting data on all other co-medications given including diagnosis, weight, length, head circumference, apgar score, birth and all doses of paracetamol which is now added page 8, line 6.

3.Are nurses and/or care-provides trained on the NEO-COMFORT score and are there any data on kappa values?

Response: All nurses in all NICUs in Denmark who use the COMFORTneo score is certified with a kappa value > 0,65 (internal documents in Danish: ttp://vip.regionh.dk/VIP/Admin/GUI.nsf/Desktop.html?open&openlink=http://vip.regionh.dk/VIP/Slutbruger/Portal.nsf/Main.html?open&unid=XC54B627F183C500DC1257B830026A1F8&dbpath=/VIP/Redaktoer/130147.nsf/&windowwidth=1100&windowheight=600&windowtitle=S%F8g) Now added to the manuscript page 8, line 11.

4.There will be much more value in this paper if the authors would add some data on the retrospective analysis mentioned in the paper (indication, clinical characteristics, duration, etc).

Response: This was only done as a preliminary examination, but we are happy to submit this as a supplementary file. See Table S1.

5.The power calculation reads very ‘pragmatic’ in my assessment.

Response: We were not able to do a power calculation due to the nature of the study. The included number is based on a thorough research of previous study.

Please also refer to point 6, 1 reviewer.

6. The abstract method part does not say anything related to analyzing the data (PK, PD or safety)

Response: A paragraph has been added page 4, line 17 : COMFORT neo pain scores and population pharmacokinetic analysis of paracetamol samples will be analysed simultaneously using non-linear mixed effects models. One and two compartment models with first order elimination will be tested for disposition. An E max model with lag time will be tested for pain.

7. A) The analysis part is very short (“ PKPD-modelling will be performed”), how do you analyze the safety parameters? And efficacy parameters? A paragraph has been added page 9, from line 9: Pharmacokinetic and pharmacodynamics (PK/PD) modelling

Structural and Stochastic model development

During the data compilation, the paracetamol plasma concentrations will be logarithmically-transformed prior to modelling, and concentration-time data will be fitted to both one and two compartment model with first-order elimination for the disposition. Different models to characterize the absorption will be tested for the oral administrated paracetamol. Inter-individual variability and Inter-occasion variability will be tested in relationship to the PK parameters. Both exponential, proportional and combined residual error models will be tested.

Covariate model

Once the base model (structural and stochastic model combined) have been identified, the influence of the different covariates (e.g. weight, length, BMI) will be tested on the PK parameters, possible correlation will be identified through visual inspection. If possible relevant covariates will be tested on relevant parameters, as judged by visual inspection, in stepwise forward and backward deletion using the stepwise covariate model tool (PsN).

Simulation

COMFORT neo pain scores and population pharmacokinetic analysis of paracetamol samples will be analysed simultaneously. An E max model with lag time will be tested for pain. The PD relationship with the paracetamol concentration and COMFORT neo scores, will only be assessed in the periods where the patients receives paracetamol as the only analgesic. However, the longitudinally COMFORT neo scores might hold important data on the pain history over time.

Model evaluation

Selection criteria for final model will be evaluated using prediction corrected visual predictive checks and bootstrap analysis, a statistic significant level of $p<0.05$ improvement of fit will be used.

B)I have some concerns related to the analysis of the efficacy (PD) data, as I understood from the protocol that every medication is allowed to be given (including opioids and sedation). This will definitely have some effect on the PD. How do you take this into account? How do you make sure you can say something related to only the PD of paracetamol? Please add this in the manuscript.

Response : In alignment with previous studies we will only report PD data on the patients that receives paracetamol as monotherapy , e.g. nineteen patients in the PARANEO study (Allegaert et al 2013). We have added the following paragraph to page 9, line 25: The PD relationship with the paracetamol concentration and COMFORT neo scores, can only be assessed in the periods where the patients receive paracetamol as monotherapy. However, the longitudinal COMFORT neo scores might hold important data on the pain history over time.

8.Is there a specific reason why the authors decided to only focus on ALAT and not on ASAT, PT, creatinine and gamma_GT?

Response: In the previous literature (described in the introduction) different hepatic biomarkers have been measured. We chose the most common one measured in the two NICUs. Creatinine measurements will be available as a part of the clinical care from most patients and will be added to the dataset.

Minor

1.There is also a minor (about 10 %) primary renal elimination route

Response: this is described in page 5: “Less than 4% is excreted unchanged in the urine in all ages [7,11,16]”.

2. The idea to link this also to ethanol exposure is valuable, although it is not clear how the clinical research team will screen for any ethanol exposure, or – since interaction has been described – propylene glycol (commonly present in paracetamol syrups). What specific ‘market’ products will be used ?

Response: All phenobarbital products used in the two NICUs contain ethanol (except one oral solution). If the included patient for some reason has received one of the ethanol containing phenobarbital products, p-ethanol is measured. Phenobarbital was chosen since the products contains the most ethanol. They also contain some propylene glycol. We have a full description of the contents in the protocol since the products are extemporaneously prepared.

3.I miss the recently published paper of Flint et al on paracetamol pk and its metabolites in the youngest age category of preterms (Pediatr Research)

Response: A paragraph has now been added to the introduction, page 5, line 19.

4. Line 30-33 in the manuscript reference 9 and 10 have been using the same data, but according to your manuscript the results are different. How is that possible as the same data has been used for both publications?

Response: The reference has now been corrected and the sentence rephrased page 5, line 22. In reference 9 they state: “Studies by our own group and by others, in both adult and paediatric populations have previously been interpreted to suggest that the glucuronidation clearance of paracetamol is up-regulated upon multiple dosing “. Since the number of allowed references is limited we have cited this article alone instead of the original adult studies.

5. Is there any reason why there is no focus in the manuscript on metabolomics (as according to me a delay in measurement of elevated safety parameters -12-24h- can occur), such as mRNA-122, HMGB1, k18?

Response: Initially we planned on measuring mRNA-122 together with another PhD-student in relation to her project. However, the sample needs to be analysed very shortly after it has been drawn from the patient and this was not feasible. We did not have opportunity to measure the other safety parameters mentioned.

Reviewer: 3, Dr. Outi Aikio, Institution and Country: Oulu University Hospital, Oulu; Finland

Thank you for your comments. Our answers to your points are as follows.

1. The introduction part of the trial contains most of the relevant studies on the field. However, a cohort study on the paracetamol morphine-sparing effect in premature infants should have been cited as well, as present study does not limit the gestational age of the study infants (Härmä et al, J Pediatr 2016).

Response: Both Ceelie et al (page 5, line 7 reference 5) and Härmä et al showed significant morphine sparring effect of paracetamol. This has been added, see page 6, last paragraph.

2.The paracetamol metabolite studies are indeed lacking among newborn infants. In present research plan, I failed to find the study drug dose used. However, I hope no toxic levels are expected.

Response: The included patients will receive the dose prescribed by the responsible clinician, typically the doses are 30-40 mg/kg/day depending on gestational age. All this is registered. We do not expect toxic levels.

3.The setting is somewhat troublesome: the authors say that no previous studies exist, but yet they claim their study to be an open, phase 4 trial? As no randomisation, blinding or control population is planned, this study should be defined as a prospective cohort study. The missing power analysis also points to this.

Response: The title has been changed accordingly to “An interventional cohort study of prolonged use of paracetamol in neonates: protocol of the PARASHUTE study”. The method section has also been rephrased to accommodate this as well. See reviewer 1, point 1 and Editors comment as well.

Furthermore, the sample size calculation is quite possible even for cohort studies and without previous trials. The question is about defining the primary outcome and the size of the clinical difference sought. When laboratory values are set as the primary outcome, its clinical relevance for the patients should be considered.

Response: No sample size calculation is available from similar studies described in the manuscript (reference 2-4). This study was designed as an exploratory study to examine if and when the hepatic biomarkers increases during the treatment.

The exclusion criteria need clarification - they do not tell the reader enough about the generalisability of the results.

Response: All new-borns less than 44 weeks post menstrual age with expected paracetamol treatment of 3 or more days are included (limited to the indications mentioned in the inclusion criteria). Accordingly, the results will be generalized to this group of new-borns which is expected to primarily consist of mature new-borns with pain following gastrointestinal surgery, birth traumas and chest tubes insertion. Subgroup analyses within the larger disease categories will be conducted to enhance generalizability within certain groups of neonates.

4. Another problem is the missing placebo when evaluating the efficacy of a pain medication. It is well known that the interpretation of the pain symptoms of newborn infants is challenging. Unfortunately the outcomes of all the pain scales are deviated if the appraisal knowns that the pain-relieving drug has been given.

Response : It is a very difficult population to give placebo. We chose the PD as a secondary aim, precisely because it is so difficult to isolate the effect of paracetamol. All nurses are certified in the COMFORTneo pain scale, with a kappa value > 0,65.

This is the best tool we have, though limited.

5.I am also worried about the use of ethanol-containing drugs in newborn infants in general. Was the ethics committee ok with it?

Response: We are not exposing neonates to ethanol-containing drugs as per protocol. They only receive what they would normally get. All phenobarbital products used in the two NICUs (except one oral solution) contain alcohol. Many other medicinal products used in NICUs contain ethanol, we chose phenobarbital because these products contain the most.

I found one error on page 7, paragraph 4, line 48: missing full stop?

Response : The paragraph has been corrected.