N3RO STATISTICAL ANALYSIS PLAN

CONFIDENTIAL

Study name	N3RO
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Authorised and approved by N3RO Trial Management Committee

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TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Definition
BPD	Bronchopulmonary dysplasia
CPAP	Continuous positive airway pressure
CRF	Case report form
DHA	Docosahexaenoic acid
EDD	Estimated date of delivery
GEE	Generalised estimating equation
HFOV	High frequency oscillatory ventilation
IPPV	Intermittent positive pressure
ITT	Intention to treat
IVH	Intra-ventricular haemorrhage
LCPUFA	Long-chain polyunsaturated fatty acids
NEC	Necrotising enterocolitis
NICU	Neonatal intensive care unit
PMA	Post menstrual age
ROP	Retinopathy of prematurity
SAP	Statistical analysis plan

1. PREFACE

This statistical analysis plan (SAP) describes the planned analyses for the N3RO trial:

Docosahexaenoic acid for the reduction of bronchopulmonary dysplasia in preterm infants born at less than 29 weeks gestational age: a randomised controlled trial. The N3RO (N-3 fatty acids for improvement in Respiratory Outcomes) trial.

The following documents were reviewed in preparation of this SAP:

- N3RO Trial Protocol (Version 3.1, 22nd September 2015)
- N3RO Case Report Form (CRF) and other data collection tools
- N3RO Trial Monitoring Committee Charter (Version 1, 7th November 2013)

The SAP does not cover planned analyses for any side studies or follow up studies to N3RO.

2. STUDY OBJECTIVES

N3RO is a phase III randomised controlled trial designed to determine the degree to which dietary docosahexaenoic acid (DHA) given to infants born less than 29 weeks' gestation reduces the incidence of bronchopulmonary dysplasia (BPD) at 36 weeks post menstrual age (PMA).

3. STUDY METHODS

3.1 Overall study design

Randomised controlled, blinded, multicentre trial.

3.2 Selection of study population

3.2.1 Inclusion criteria

1. Born at less than 29 weeks' gestational age
2. Within 3 days of commencing enteral feeds
3. Has a legally acceptable representative capable of understanding the informed consent document and providing consent on the infant's behalf

3.2.2 Exclusion criteria

1. Infants who have a major congenital or chromosomal abnormality
2. Women providing breast milk who are taking supplements providing >250 mg DHA per day and do not wish to stop taking supplements
3. Infants participating in another fatty acid study
4. Infants receiving intravenous lipid emulsions containing fish oil when used as early lipid parenteral nutrition support (e.g. Omegaven, SMOFlipid, Lipoplus)

3.3 Intervention

Infants were randomised to receive one of the following study emulsions:

1. Aqueous emulsion of DHA oil (tuna oil) containing 20% total fat (with 70% of total oil as DHA) that will deliver around 60 mg of DHA for each 0.5 mL of emulsion
2. Control (soy oil) emulsion with no DHA

Infants were given 0.5 mL/kg/day of the study emulsion. The emulsion was given in three divided doses of 0.17 mL/kg/dose. The study emulsion was administered enterally through the naso/oro-gastric tube immediately preceding a scheduled feed.

3.4 Method of treatment assignment

Infants were randomly assigned to receive the DHA or control emulsion according to an independently generated web-based randomisation schedule. Infant sex, gestational age and study centre (as defined in the table below) were used to stratify the randomisation. The randomisation schedule used blocks of size 2, 4 and 6 in the ratio 1:2:1, with the size of the block chosen at random and treatment allocations randomly permuted and balanced within blocks. Infants from a multiple birth were randomised individually.

Stratification Variable	Categories
Sex	Male
	Female
Gestational age at birth	Less than 27 completed weeks
	27 to less than 29 completed weeks
Study centre	
Australia	Women's and Children's Hospital
	Flinders Medical Centre
	King Edward Memorial Hospital
	Royal Women's Hospital
	Mercy Hospital for Women
	Monash Medical Centre
	John Hunter Hospital
	Royal Hospital for Women
	Liverpool Hospital
	Mater Mothers' Hospital
New Zealand	Wellington Hospital
	Waikato Hospital
Singapore	KK Women's and Children's Hospital

3.5 Blinding

Participants and their family, care providers, outcome assessors, investigators and data analysts are blinded to treatment allocation. Treatment and control emulsion are identical in viscosity, colour and packaging. The study emulsions are iso-caloric and differ only in the amount of DHA.

Unblinding could occur during the conduct of the trial in emergency situations where management of an infant required knowledge of treatment allocation. In the event unblinding was required, the Principal Investigator would be contacted in all these instances but kept blinded to treatment allocation. The number of participants unblinded for this reason will be reported as a trial quality characteristic (see Section 6.3).

3.6 Sample size

The rate of BPD in infants born less than 29 weeks' gestation in the DINO trial was 51.4% (Makrides et. al., 2009). To detect a 10% absolute reduction (19% relative reduction) in the incidence of BPD at 36 weeks PMA from 51.4% in the control group to 41.4% in the high-dose DHA group (with 90% power, two tailed α =0.05), a sample size of 1244 is required (622 per group). This sample size takes into account the clustering (non-independence) of multiple births (variance inflation factor 11%) and deaths (4% as estimated from the DINO trial). The variance inflation factor for multiple births was based on 26% of infants born less than 29 weeks gestational age being from multiple births, an intracluster correlation coefficient for BPD of 0.52 and an average cluster size of 2.07 for these infants (as observed in the DINO trial in which mothers were randomised). While the variance inflation factor may be reduced by randomising infants rather than mothers, the calculated sample size ensures sufficient power even in the unlikely event that all infants from multiple births are randomised to the same treatment arm as their siblings.

4. SEQUENCE OF PLANNED ANALYSES

4.1 Interim analyses

The independent Trial Monitoring Committee in N3RO reviewed general study progress, the BPD event rate and key secondary/safety outcomes. Trial monitoring did not however involve a planned interim analysis or formal stopping rules.

4.2 Final analyses and reporting

Once the study has been completed and all data have been entered, a blinded review of the data will be conducted and final changes will be made to this SAP. No statistical analyses will be performed until the final version of this SAP has been approved.

To facilitate statistical analyses, uninformative treatment group codes will be made available to the trial statistician once the database has been finalised. The analysis of all primary and secondary outcomes will be performed using these uninformative treatment group codes. Results of the statistical analyses will be made available to the Chief Investigators and key Associate Investigators. The blinding will be completely broken after the results have been presented to the Investigators.

Any post-hoc, exploratory analyses which were not identified in this SAP will be clearly identified in

the final report. Any deviations from the planned analyses detailed in this SAP will be documented with reasons in a post-analysis version of the SAP.

5. GENERAL ISSUES FOR STATISTICAL ANALYSIS

5.1 Analysis software

All analyses will be performed using SAS version 9.3 or later, and Stata Release 13 or later.

5.2 Analysis approach

The planned analysis of the two randomised groups will be undertaken using an intention-to-treat (ITT) approach; participants will be analysed according to the treatment they were randomised to receive, irrespective of compliance with the protocol. In estimating the effect of treatment across all randomised participants, all observed outcome data will be included in the primary analysis under a missing at random assumption (see Section 5.3).

Note the study design was consistent with the ITT principle since infants were followed up irrespective of compliance.

5.3 Methods for withdrawals, missing data and outliers

Data collected on infants up until the time of withdrawal will be included in the statistical analysis. Following withdrawal, data will be collected and used where permission has been obtained to do so.

To address missing data, multiple imputation involving 100 imputations will be performed, even if only a small percentage of data are missing. Use of 100 imputations ensures that the loss of power compared to full information maximum likelihood methods is <1%, even when the fraction of missing information is high (Graham et. al., 2007). Imputation will be performed separately by treatment group (to facilitate subgroup comparisons) using chained equations. For each outcome, covariates pre-specified for adjustment in the analysis model or for conducting subgroup analyses will be included in the imputation model. To account for potential differences between singletons and infants from a multiple birth, multiple birth status will also be included in conditional equations for each outcome. Other auxiliary variables useful for improving the prediction of missing outcome values will be added to the imputation model as appropriate. Results will not be imputed for time to event outcomes or where the outcome is undefined due to death. Analyses will be performed on both unimputed and imputed data, with conclusions to be drawn based on the results of the analyses

performed on imputed data.

The imputed analysis for each outcome defined in Section 7 will be performed under a missing at random assumption. For the primary outcome, the sensitivity of results to the missing at random assumption will be investigated by assuming that data are missing not at random in the imputation model. Using pattern mixture models, the odds of BPD will be assumed to be between half and twice as high in children with missing data compared to children with observed data. These differences will be applied to control group children only, treatment group children only and children in both treatment groups.

Outliers will be queried during data collection and the statistical analysis. Unless confirmed as a data entry error, outliers will not be excluded from the primary analysis.

5.4 Protocol violations and deviations

No participants will be excluded from the ITT analyses due to protocol deviations.

5.5 Data transformations

No data transformations are planned. The statistical analyses detailed in Section 7 are based on assumptions about the distribution of the outcomes. Should these assumptions turn out to be invalid, data transformations may be required. Data transformations are not planned to correct for departures from normality, since the sample size is sufficient for the central limit theorem to apply (Lumley et. al., 2002).

5.6 Covariates for adjustment

In order to address each hypothesis, both unadjusted and adjusted analyses will be performed. The adjusted analyses will be used to draw conclusions about the effect of treatment, with unadjusted analyses performed for completeness and to confirm the results of the adjusted analyses.

Kahan and Morris (2012) demonstrated that failure to adjust for stratification variables in the analysis of randomised trials biases standard error estimates for the treatment effect upwards. Since infant sex, gestational age at birth and study centre were used to stratify the randomisation process, all analyses will be adjusted for these variables (with categories defined as in Section 3.4). For binary outcomes with low to moderate prevalence, it is anticipated that adjustment for study centre may be problematic due to the large number of centres (13) participating in N3RO. In these instances, study

country (Australia, New Zealand, Singapore) rather than study centre will be adjusted for in the analysis. If convergence or model instability remains an issue, further covariates may need to be excluded from the adjusted analysis. All deviations from the full adjustment strategy will be clearly identified in the final report.

5.7 Planned subgroup analyses

For the primary and secondary outcomes, secondary analyses will be performed to test for evidence of effect modification by sex and gestational age at birth (less than 27 completed weeks, 27 to less than 29 completed weeks). Effect modification by these two factors will be assessed separately by including interaction effects in the statistical models. Separate estimates of treatment effect will be obtained for males and females and for infants born less than 27 completed weeks and 27 to less than 29 weeks, independent of whether the interaction effect is statistically significant, since this is *a priori* of interest.

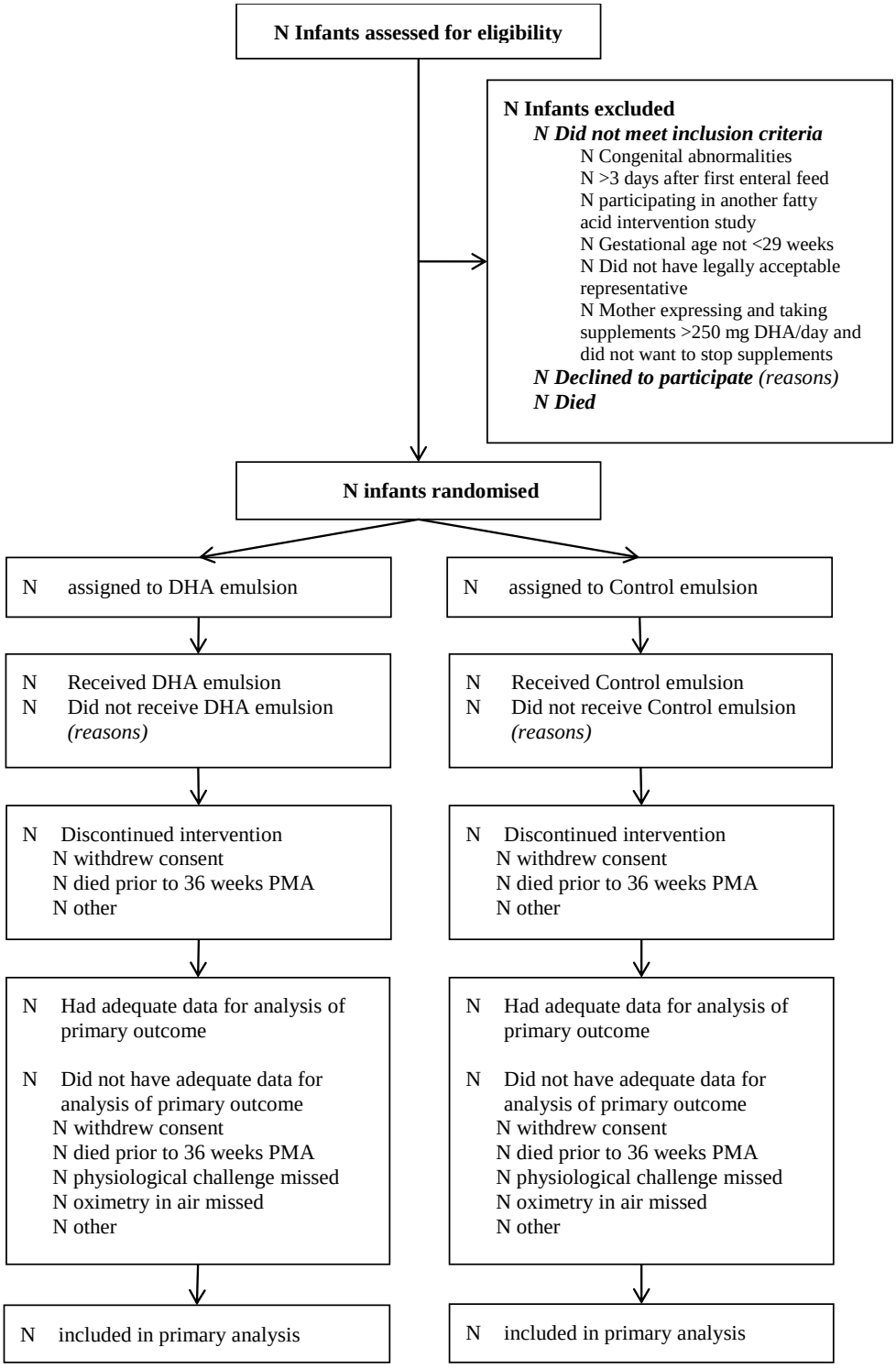
Any unplanned treatment by covariate interactions are to be considered exploratory and will be clearly identified in the final report.

5.8 Multiple comparisons and multiplicity

Multiple hypothesis tests will be performed to assess the effectiveness of high-dose DHA due to multiple outcomes, planned subgroup analyses, unadjusted and adjusted analyses, and analyses based on raw and imputed data. No adjustment will be made for multiple outcomes, as there is only a single primary outcome and the multiple secondary outcomes are considered to be less important. Similarly no correction will be made for multiple comparisons due to subgroup analyses, as less emphasis will be placed on the results of these analyses. Since conclusions will be formed based on imputed results with covariate adjustment, no adjustment will be made for multiple testing of each outcome using adjusted and unadjusted models on raw and imputed data.

6. DESCRIPTIVE STATISTICS

6.1 Participant flow



6.2 Baseline characteristics

A descriptive comparison of the randomised groups will be conducted on the baseline characteristics presented in the following table.

Baseline characteristic	CRF Reference	Categories
Mother		
Age - years	D.2	-
Race	D.6.2	African Caucasian Aboriginal or Torres Strait Islander Maori Other Indigenous Pacific Islander North East Asian South East Asian Southern and Central Asian Other
Education	D.7	
Completed secondary school	D.7.1	Yes/No
Completed any further study	D.7.2	Yes/No
Highest level completed	D.7.3	Certificate/Diploma Bachelor Degree Higher/Post graduate Degree Other
Years in full time education	D.7.4	-

Baseline characteristic	CRF Reference	Categories
Marine oil LCPUFA containing dietary supplements during pregnancy	E.2	Yes/No
Smoked cigarettes during pregnancy	E.3	Yes/No
Birth mode	E.15	Vaginal Instrumental Caesarean section in labour Caesarean section, no labour
Antenatal corticosteroids	E.16	No antenatal steroids First dose <24 hours before birth of baby Complete course Given at more than 7 days before baby's birth
Chorioamnionitis (histopathological evidence)	E.17	Yes/No
Infant at birth		
Sex	A.7	Female Male
Gestational age - weeks	A.8 (plus cross check with E.24)	- <27 weeks 27 to <29 weeks
Plurality	A.9	Singleton Twins Triplets or higher order
Birth weight - g	E.23.1	-
Birth length - cm	E.23.2	-

Baseline characteristic	**CRF Reference**	**Categories**
Birth head circumference - cm	E.23.3	-
Birth weight <10th percentile for gestational age (calculated according to Beeby, 1996, using sex, gestational age and birthweight)	A.7, A.8, E.23.1	Yes/No
Place of birth	E.14	Tertiary perinatal centre with NICU Non-tertiary hospital Born before arrival at a hospital
Apgar score at 5 minutes	E.18	- <7 ≥7
Resuscitation in the delivery room Intubation Continuous Positive Airway Pressure (CPAP)	E.19	 Yes/No Yes/No
Temperature on admission to NICU	E.21	- <36.5 36.5 to 37.2 >37.2
CRIB II score (calculated according to Parry et. al., 2003, using sex, birthweight, gestation, admission temperature, admission base excess)	A.7, A.8, E.21, E.22, E.23.1	-
Infant at randomisation		
Age - days (any part of a day counts as 1 day)	A.1, A.4, A.5,	-

Baseline characteristic	**CRF Reference**	**Categories**
Days respiratory support between birth and randomisation	A.16	
Endotracheal support (IPPV, HFOV)	A.16.1, A.16.2	-
Non-invasive positive pressure support (CPAP, High flow nasal cannulae)	A.16.3, A.16.4	-
Supplemental oxygen	A.16.5 A.16.6	- -
Therapies between birth and randomisation	A.17	
Surfactant	A.17.2	Yes/No
Nitric oxide	A.17.1	Yes/No
Air leak requiring drainage	A.17.3	Yes/No
Medical treatment for patent ductus arteriosus	A.17.6, A.17.7	Yes/No
Blood products for pulmonary hemorrhage	A17.9	Yes/No
First enteral feed - days (any part of a day counts as 1 day)	A.10	-
Type of first enteral feed	A.10.3	Mother's own milk Donor milk Formula
Fatty acid levels	A.13 and lab results	
Docosahexaenoic acid		-
Eicosapentaenoic acid		-

Baseline characteristic	CRF Reference	Categories
Linoleic acid Arachidonic acid		- -
Age first dose of study emulsion - days (any part of a day counts as 1 day)	C.1	-

Means and standard deviations, or medians and interquartile ranges, will be reported for continuous variables. Frequencies and percentages will be reported for categorical variables. Due to the inclusion of infants from a multiple birth, some characteristics will apply at the infant level (e.g. infant sex) while others will apply at the mother or family level (e.g. mother completed secondary education). For the purpose of summarising baseline and post-randomisation characteristics (see Section 6.3), all variables will be considered to apply at the infant level.

6.3 Measures of compliance and trial quality

A descriptive comparison of the randomised groups will be conducted on the compliance and trial quality characteristics presented in the following table.

Compliance / trial quality characteristic	CRF Reference	Categories
Percentage doses taken, overall and by week following randomisation	C.1	-
Unblinded, overall and before primary outcome	-	Yes/No
Fatty acid levels at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first: Docosahexaenoic acid Eicosapentaenoic acid Linoleic acid Arachidonic acid	F.10 and lab results	

Means and standard deviations, or medians and interquartile ranges, will be reported for continuous variables. Frequencies and percentages will be reported for categorical variables.

6.4 Missing data

Missing data will be summarised descriptively by treatment group for each outcome variable, (Section 7) and baseline characteristic (Section 6.2).

6.5 Randomisation errors

Information will be presented by treatment group on the number of infants randomised in the wrong stratum, the number of infants given the wrong treatment and the number of infants discovered to be ineligible after randomisation.

7. STATISTICAL ANALYSES

Throughout this section the following details are provided for each outcome variable:

- Outcome: A detailed description of the outcome variable, including the type of variable, the relevant assessments and/or sections(s) of the CRF and how it will be calculated (if applicable).
- Effect: The measure of treatment effect to be reported.
- Analysis: The type of statistical analysis to be performed.
- Adjustment: The baseline covariates to adjust for in the adjusted analysis of the randomised groups.

For each outcome variable, statistical significance will be assessed at the 0.05 level using a two-sided comparative test of treatment effect. In addition to treatment effect estimates and p-values, 95% confidence intervals will be reported to express uncertainty about the effect of treatment.

For binary outcomes, if the number of infants experiencing the outcome is considered too small for full adjusted analyses to be sensible, study country rather than study centre will be considered for adjustment. For binary outcomes with low prevalence, further covariates may need to be excluded from the adjusted analysis as appropriate. As described in Section 5.6, any deviation from the full adjustment strategy will be clearly identified in the final report. Following a decision on adjustment, binary outcomes assessed using log binomial generalised estimating equations (GEEs), where the model fails to converge, will be assessed using log Poisson GEEs instead. For time to event outcomes, if the proportionality assumption of the Fine and Gray model is not met, a time-varying

model will be used where time to event will be split into two or more periods in which the proportionality assumption is met. For count outcomes, if the fit of a negative binomial GEE is poor due to an excess of zero values, a zero-inflated negative binomial GEE will be used instead.

7.1 Primary outcome variable

7.1.1 BPD

Outcome: Binary outcome based on the requirement for supplemental oxygen and/or assisted ventilation at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first. More specifically, a BPD diagnosis is made for:

a. Infants receiving mechanical ventilation, CPAP or air or supplemental oxygen delivered by a high flow device at ≥ 2 litres per minute (CRF references F.4.1, F.4.2, F.4.3).
b. Infants receiving supplemental oxygen concentrations ≥ 30% with oxygen saturations between 90% and 96% inclusive (CRF reference F.4.4).
c. Infants in supplemental oxygen ≥ 30% at rest with saturations >96%, or oxygen < 30% at rest with oxygen saturations ≥ 90%, or sub-nasal air at <2 litres, that fail either the reduction phase or room air observation phase of a physiological challenge (CRF references F.4.5, F.4.6, F.4.7, F.5.2, F.5.3).
d. Infants in room air who fail a 15 minute oximetry test (CRF references F.2, F.6.1).

Effect: Relative risk of BPD (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2 Secondary Outcome Variables

7.2.1 Composite of death before 36 weeks PMA or BPD

Outcome:	Binary outcome based on death before 36 weeks PMA or BPD (as defined in 7.1.1).
Effect:	Relative risk of death before 36 weeks or BPD (DHA vs. control).
Analysis:	Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.
Adjustment:	Sex, gestational age at birth, study centre.

7.2.2 Respiratory support to primary outcome

Outcome:	Count outcome based on the number of days any respiratory support was required from birth to the day of primary outcome testing at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first (CRF reference F.7.1).
Effect:	Ratio of mean number of days of respiratory support (DHA vs. control).
Analysis:	Test for a treatment effect using a negative binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.
Adjustment:	Sex, gestational age at birth, study centre.

7.2.3 Discontinuation of endotracheal support

Outcome: Time to event outcome based on the number of days from birth to final intermittent positive pressure and high frequency ventilation (CRF references G.4.1 and G.4.2). For infants continuing endotracheal support, the outcome will be right censored according to the date of discharge home or estimated date of delivery (EDD) (whichever occurs first). Death prior to discontinuation will be treated as a competing risk in the analysis, since death prevents discontinuation from occurring.

Effect: Sub-distribution hazard ratio of discontinuing endotracheal support (DHA vs. control).

Analysis: Test for a treatment effect using a Fine and Gray competing risks regression model, with death treated as a competing risk. A cluster adjusted variance estimator will be used to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.4 Discontinuation of non-invasive positive pressure support

Outcome: Time to event outcome based on the number of days from birth to final continuous positive airways pressure and high flow support (CRF references G.4.3 and G.4.4). For infants continuing non-invasive positive pressure support, the outcome will be right censored according to the date of discharge home or EDD (whichever occurs first). Death prior to discontinuation will be treated as a competing risk in the analysis, since death prevents discontinuation from occurring.

Effect: Sub-distribution hazard ratio of discontinuing non-invasive positive pressure support (DHA vs. control).

Analysis:	Test for a treatment effect using a Fine and Gray competing risks regression model, with death treated as a competing risk. A cluster adjusted variance estimator will be used to account for the clustering of infants within mother.
Adjustment:	Sex, gestational age at birth, study centre.

7.2.5 Discontinuation of low flow or supplemental oxygen

Outcome:	Time to event outcome based on the number of days from birth to final low flow or supplemental oxygen (CRF references G.4.5 and G.4.6). For infants continuing low flow or supplemental oxygen, the outcome will be right censored according to the date of discharge home or EDD (whichever occurs first). Death prior to discontinuation will be treated as a competing risk in the analysis, since death prevents discontinuation from occurring.
Effect:	Sub-distribution hazard ratio of discontinuing low flow or supplemental oxygen (DHA vs. control).
Analysis:	Test for a treatment effect using a Fine and Gray competing risks regression model, with death treated as a competing risk. A cluster adjusted variance estimator will be used to account for the clustering of infants within mother.
Adjustment:	Sex, gestational age at birth, study centre.

7.2.6 Severity of BPD

Categorisation of the severity of BPD as mild, moderate or severe according to the diagnostic criteria recommended by the USA National Institute of Child Health and Human Development, National Heart, Lung and Blood Institute and Office of Rare Diseases Workshop (Jobe, 2001).

7.2.6.1 Mild BPD

Outcome: Binary outcome based on treatment with supplemental oxygen for ≥28 days (CRF reference F.7.1) up to 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or discharge home, whichever occurs first, and in air (CRF reference F.6.1) at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first.

Effect: Relative risk of mild BPD (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.6.2 Moderate BPD

Outcome: Binary outcome based on treatment with supplemental oxygen for ≥28 days (CRF reference F.7.1) up to 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or discharge home, whichever occurs first, and need for <30% oxygen or subnasal air at <2 litres per minute, i.e. failed either the reduction phase or room air observation phase of a physiological challenge (CRF reference F.5) at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first.

Effect: Relative risk of moderate BPD (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.6.3 Severe BPD

Outcome: Binary outcome based on treatment with supplemental oxygen for ≥28 days (CRF reference F.7.1) up to 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or discharge home, whichever occurs first, and the need for ≥ 30% oxygen and/or positive pressure (CRF reference F.4.1 to F.4.4) at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first.

Effect: Relative risk of severe BPD (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.7 Clinical BPD

Outcome: Binary outcome based on supplemental oxygen or any respiratory support at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first, according to clinical management at that time (CRF reference F.2)

Effect: Relative risk of clinical BPD (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account

for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.8 Surfactant

Outcome: Binary outcome based on surfactant administration by discharge home or EDD (whichever occurs first) (CRF reference G.3).

Effect: Relative risk of surfactant administration (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.9 Nitric Oxide

Outcome: Binary outcome based on nitric oxide administration by discharge home or EDD (whichever occurs first) (CRF reference G.5.1).

Effect: Relative risk of nitric oxide administration (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.10 Air leak requiring drainage

Outcome: Binary outcome based on air leak requiring drainage by discharge home or EDD (whichever occurs first) (CRF reference G.8).

Effect: Relative risk of air leak requiring drainage (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.11 Treated Ductus Arteriosus

Outcome: Binary outcomes based on:

(1) Medically treated ductus arteriosus (CRF reference G.10.1).

(2) Surgically treated ductus arteriosus (CRF reference G.10.1).

Effect: Relative risk of treated ductus arteriosus (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.12 Postnatal steroids for lung disease

Outcome: Binary outcome based on postnatal steroids taken for lung disease to discharge home or EDD, whichever occurs first (CRF reference G.5.2.1).

Effect: Relative risk of postnatal steroid use (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.13 Total days of caffeine

Outcome: Count outcome based on the number of days received caffeine to discharge home or EDD, whichever occurs first (CRF reference G.5.3.1).

Effect: Ratio of mean number of days received caffeine (DHA vs. control).

Analysis: Test for a treatment effect using a negative binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.14 Total days of diuretics for lung disease

Outcome: Count outcome based on the number of days received diuretics for lung disease to discharge home or EDD, whichever occurs first (CRF reference G.5.4.1).

Effect: Ratio of mean number of days received diuretics for lung disease (DHA vs. control).

Analysis: Test for a treatment effect using a negative binomial GEE. The model will use an independence working correlation matrix to

account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.15 Serious adverse event

Outcome: Binary outcomes based on:

(1) Deaths to 36 weeks PMA.

(2) Total deaths.

Effect: Relative risk of death (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.16 Length of hospital stay

Outcome: Time to event outcome based on the number of days from birth to first discharge home from hospital (CRF reference H.1). For infants yet to be discharged home at the completion of the trial, length of hospital stay will be right censored according to the date the study database is finalised. Death prior to discharge will be treated as a competing risk in the analysis, since death prevents the event of interest (discharge home) from occurring.

Effect: Sub-distribution hazard ratio of discharge home (DHA vs. control).

Analysis: Test for a treatment effect using a Fine and Gray competing risks regression model, with death before discharge treated as a competing risk. A cluster adjusted variance estimator will be used to account for the clustering of infants within mother.

Note: a cumulative incidence plot will produced to descriptively compare treatment groups.

Adjustment: Sex, gestational age at birth, study centre.

7.2.17 Infant size (weight, length and head circumference)

Outcome: Continuous outcomes based on weight, length and head circumference at:

(1) 36 weeks PMA or discharge home, whichever occurs first (CRF reference F.9).

(2) Discharge home or EDD, whichever occurs first (CRF reference G.22).

The measures will be converted to z-scores according to growth standards by Beeby (1996). Standardisation will take into account the exact age of the child at the relevant assessment time-point through the linear interpolation of weekly mean anthropometric values. The z-scores are assumed to be approximately normally distributed.

Effect: Difference in mean z-score (DHA vs. control).

Analysis: Test for a treatment effect using a linear GEE. The model will use an independence working correlation matrix to account for the

clustering of infants within mother.

Adjustment Study centre, z-score at randomisation.

7.2.18 Feeding tolerance - days to reach full enteral feeds

Outcome: Count outcome based on the number of days from birth to reaching full enteral feeds (CRF reference G.19).

Effect: Ratio of mean number of days to reaching full enteral feeds (DHA vs. control).

Analysis: Test for a treatment effect using a negative binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.19 Feeding tolerance - days feeds interrupted to primary outcome

Outcome: Count outcome based on the number of days on which one or more feeds were interrupted from randomisation to the day of primary outcome testing at 36 weeks PMA (36 weeks and 0 days to 36 weeks and 6 days inclusive) or day of discharge home, whichever occurs first (CRF reference C.1).

Effect: Ratio of mean number of days feeds interrupted (DHA vs. control).

Analysis: Test for a treatment effect using a negative binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.20 Retinopathy of prematurity (ROP)

Outcome: Binary outcomes, defined for unilateral and bilateral eye disease, based on:

(1) Stage ≥3 ROP (CRF reference G.14).

(2) ROP requiring therapy (CRF reference G.14).

Effect: Relative risk of ROP (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.21 Intra-ventricular haemorrhage (IVH)

Outcome: Binary outcomes based on:

(1) Any IVH (CRF reference G.13.2).

(2) Grade 3 or 4 IVH (CRF reference G.13.2).

Effect: Relative risk of IVH (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account

for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.22 Cerebral cystic formation

Outcome: Binary outcomes based on:

(1) Periventricular leukomalacia (CRF reference G.13.3).

(2) Porencelaphic cysts (CRF reference G.13.3).

Effect: Relative risk of a cerebral cystic formation (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.23 Confirmed sepsis

Outcome: Binary outcome based on a confirmed sepsis episode (CRF reference G.12).

Effect: Relative risk of confirmed sepsis (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

7.2.24 Confirmed necrotising enterocolitis (NEC)

Outcome: Binary outcome based on confirmed NEC (CRF reference G.11).

Effect: Relative risk of confirmed NEC (DHA vs. control).

Analysis: Test for a treatment effect using a log binomial GEE. The model will use an independence working correlation matrix to account for the clustering of infants within mother.

Adjustment: Sex, gestational age at birth, study centre.

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