European Study of Neonatal Exposure to Excipients (ESNEE)

Excipients affect the safety, efficacy and quality of medicines particularly in neonates who are a highly vulnerable group of patients. At present there is a lack of data about the impact of excipients in this age group, and reports have indicated that some may be toxic for neonates, highlighting the need for an urgent assessment of neonatal excipients. A group of researchers from across Europe (ESNEE) have come together in order to use their combined expertise to address this urgent issue and provide an evidence base for discussion.

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Keywords

excipient; adverse effect; formulation; ethanol; propylene glycol; sorbitol; polysorbate 80

Key points

Graham S., Turner M. European Study of Neonatal Exposure to Excipients (ESNEE). *Infant* 2011; 7(6): 196-99.

1. There is little data on exposure among neonates in Europe to potentially toxic excipients contained in medicines.
2. A European-based questionnaire and point prevalence study as well as a systematic literature review and clinical studies of excipient exposure are being initiated.
3. The data will enable decisions to be made regarding reduction of neonatal exposure to excipients through product substitution and priorities for reformulation.

Neonates, newborn babies up to the age of 28 days after their expected date of delivery, are a highly vulnerable group of patients who may become seriously ill as they adapt to life outside of the uterus. This is particularly the case in premature neonates whose bodies have yet to fully develop and start functioning effectively, but may also be observed in full-term newborns who may also be seriously unwell. Consequently, neonates are frequently treated with a variety of different medicines as part of their care.

Excipients

A medicine is comprised of an active pharmaceutical ingredient (API), which is more commonly referred to as the "drug," along with a range of other chemicals (known as "excipients"). These excipients are necessary to overcome chemical, physical and microbiological challenges posed by developing formulations which are of sufficient quality to be used in clinical practice. For example some drugs do not dissolve in water and need other chemicals to do this; tablets, capsules and other solid dosage forms can be fragile and need chemicals to make them easy to use. Medicines also need to have an appropriate shelf life to make them economic, in which case preservatives are often added. Manufacturers overcome these problems in different ways, which means that each brand of medicine will contain the same active drug, but may differ from other brands of the same medicine with respect to their excipients.

Although pharmaceutical companies have carried out extensive work to ensure that excipients are safe when used in adults, there has been limited work to examine the exposure and safety of excipients in neonates. This poses a major concern for neonatal healthcare professionals, as coupled with the fact that excipients are not inert, neonates handle them very differently to older age groups. For some excipients, use in neonates has led to significant harm and even death[1]. Two recent observational studies of medicines administered to neonates suggest that this age group are exposed to significant amounts of excipients, some of which can exceed internationally recommended limits for exposure[2,3].

This lack of testing surrounding the safety of both individual and combined excipients in neonates calls for an urgent assessment of key excipients. An assessment would allow pharmacists, when developing new medicines for use in neonates, to ensure that only the excipients that are necessary for the formulations are included and that those posing a high risk are substituted where appropriate.

European Study of Neonatal Exposure to Excipients

Neonatal and pharmaceutical experts from across Europe (Liverpool, Leicester, Belfast (UK); Paris (France) and Tartu (Estonia)) came together at the end of 2009 in order to address these concerns. Their main aim is to provide an evidence-base for discussions about excipients. With funding secured from ERA-NET "Priority Medicines for Children"

(PRIMEDCHILD), the consortium was formalised and the European Study of Neonatal Exposure to Excipients (ESNEE) was born. ERA-NET is a network of funding organisations from European member states with the ambition to bring coherence and cooperation to national research programmes. The programme, which is jointly sponsored by the University of Liverpool and Liverpool Women's NHS Foundation Trust, will run for a two-year period.

ESNEE is a research programme which aims to develop a range of methodologies that will provide an integrated assessment of exposure among neonates in Europe to potentially toxic excipients contained in medicines. The project will develop novel techniques and will contribute information required for the use and development of formulations.

The ESNEE programme comprises the following six work packages;

1. To undertake a comprehensive European-based questionnaire and a point prevalence survey of excipient exposure in neonates, in order to highlight opportunities for product substitution and priorities for reformulation.
2. To conduct a systematic review in order to identify existing information about impact of excipients on the development in human neonates and juveniles in other species.
3. To develop techniques that allow small volume blood samples to be used in population excipient kinetic (EK) models of systemic excipient exposure in human neonates.
4. To conduct a cohort study of neonates exposed to selected excipients, including blood sample for EK assays.
5. To develop EK models for selected excipients.
6. To integrate the results of the objectives in work packages one to five in order to identify formulation challenges relating to excipient usage in neonates.

European-based questionnaire and point prevalence study

Work package one, led by the University of Tartu, Estonia, has seen the development of a European questionnaire and a Point Prevalence Study (PPS), which will be distributed to multiple neonatal units across 27 EU countries, along with Switzerland, Norway and Iceland. The collective aim will be to gather information about drugs used in neonates (eg brand/manufacture, doses, methods of administration). Those centres providing more in-depth information, through the ESNEE PSS, will allow the research group to maximise the precision of estimates of exposure to excipients in neonates and provide the basis for the detailed excipient kinetic study (EK), which in turn will lead to highlighting the needs and opportunities for product substitution and priorities for reformulation. It will also allow the researchers to gain insight into the variations of prescribed drugs, along with excipient exposure rates among the different European countries and regions.

Systematic literature review

Robert Debré Hospital, France, has commenced working on a systematic literature review (work package two) in order to identify data relevant to the EK modelling of blood excipient levels in neonates. They will also identify potential toxicity and biological markers for toxicity, and examine the issues faced in order to minimise the risks arising from excipients used in neonates, and/or produce excipient-free medicines for neonates.

A generic search, using pre-specified keywords has been the method of choice in order to identify research papers which have been carried out on formulations and/or excipients, along with more specific keywords for particular molecules (eg sodium metabisulphate). The search strategies have been repeatedly reviewed before being applied to scientific literature databases. The primary unit of analysis upon completion will be the chemical entity of each excipient.

Development and validation of assays

Work package three is being led by the PK/PD Laboratory at Queen's University, Belfast (QUB), with close collaboration from researchers in Leicester. The aim here will be to use dedicated assay platforms in order to determine which excipients are found in neonatal dry blood spot (DBS) samples. DBS are collected by applying a few drops of blood onto specially manufactured absorbent filter paper. The blood thoroughly saturates the paper and is air dried for several hours. Once in the laboratory, a small disc of saturated paper can be obtained using a manual hole punch, which is then dropped into a flat microtitre plate. The blood is eluted out and dilutions can then be made for subsequent testing. The process of sample extraction from DBS will consist of using bespoke aqueous or organic phase extraction, followed by sample clean-up (eg using solid phase extraction) and finally analysis by HPLC. HPLC is an analytical chemistry technique which combines the physical separation capabilities of liquid chromatography, with the mass analysis capabilities of mass spectrometry. It is a powerful technique which can be used for the specific detection and potential identification of chemicals in complex mixtures such as drugs.

ESNEE clinical study (including blood sample for EK assays)

The ESNEE clinical study (led by Liverpool) will be a preliminary investigation of exposure to selected excipients in neonates and a pilot study about how neonates deal with excipients from the priority list. Detailed information will be collected on all medicines administered (hence excipient dose) and demographic details (eg gestational age, weight), with particular attention being paid to the accurate recording of brand, dose and timing of medicines containing excipients that the neonates are exposed to during routine clinical care.

Clinical data will be supplemented by information from blood samples taken opportunistically or during research-specific sampling periods (whichever the neonate's parents/guardians consent to) for this multicentre observational study. The majority of trial sampling will take place at the same time as clinical sampling episodes and will not require any changes to treatment. It is anticipated that throughout Europe a total sample size of 1,300 will be met, with 500 participants being recruited

ESNEE: Priority list of excipients

- Propylene glycol
- Ethanol and metabolites (fatty acid ethyl esters and non-oxidative ethanol metabolites)
- Propylhydroxybenzoate and other parabens
- Sodium benzoate/benzoic acid/ benzyl alcohol
- Polysorbate 80
- Sorbitol

TABLE 1 The priority list of excipients for the European Study of Neonatal Excipient Exposure.

from at least 20 UK neonatal units. Up to 100 neonates, contributing between 1 and 10 samples each (no more than 1mL in total), will be used to study each excipient, with each neonate contributing to the study of at least one excipient.

The blood samples will be collected and stored in tubes ("wet" samples) or on filter paper ("DBS"[4]).

The priority list of excipients (see **TABLE 1**) for investigation was agreed upon using surveys conducted by UK and Estonian partners. The UK survey was conducted in partnership with MCRN and gathered data from 43 neonatal units. A total of 149 excipients was found in 64 products.

Sodium benzoate is highly toxic to premature babies but was found in 10 medicines administered to neonates. Preservatives such as parabens (and their sodium salts) and propyl parahydroxybenzoate were found in 24, and ethanol in eight. It was decided that polysorbate 80 would also be included in the priority list on the advice of the European Medicines Agency. The known safety concerns of the priority list are detailed in **TABLE 2**.

Development of EK models

Drug and excipient level measurements from different neonates will be combined to construct powerful models which will indicate how much of the drug or excipient is in the blood at any given time following administration of the medicine. These models will be built on blood samples taken either opportunistically or at pre-specified time points and will be led by the University of Leicester.

The models developed by this study will indicate how much of each excipient can be expected in the bloodstream of neonates given medicines containing those excipients. This data and data from parallel studies may allow us to extrapolate whether the modelled blood excipient concentrations pose a potential risk to neonates.

Integration of results and dissemination

Work package six will see the results from work packages one to five being drawn together in order to provide information about excipient exposure across the EU. It is envisaged that this will promote reduction of neonatal exposure to excipients by highlighting opportunities for product substitution (ie with excipient-free or excipient-low medicines currently used in some countries but not others) and priorities for reformulation. Reformulation may lead to the medicines becoming more expensive – any increased costs will be passed on to the NHS. Recommendations for reformulation will need to be based on a benefit-risk analysis.

It will be important to disseminate the results from ESNEE in order to provide information and advice to industry, clinicians, pharmacists and regulators. The European Medicines Agency (EMA), Paediatric Committee, European Paediatric Formulations Initiative (EuPFI) and the World Health Organisation (WHO) are just some of the routes which will be used for the dissemination of results.

Conclusion

This project gives staff on neonatal units the chance to shape the future of medicines that are given to the babies in their care. Ethical approval is in place for the point prevalence survey and the clinical studies of excipient exposure. All data will be used in an integrated way to give babies the best possible medicines. We are looking for colleagues willing to contribute to this exciting initiative by collecting data for the point prevalence survey. If you are interested, please contact **esnee@lwh.nhs.uk**

Known safety concerns of excipients included in the study

Excipient	Biochemical/other effects	Safety concerns	Pharmaceutical issues in neonates
Sodium benzoate/ benzoic acid		Neonates appear to lack the capacity to conjugate with glycine. This leads to the build up of benzoic acid which can cause metabolic acidosis and neurotoxicity	In the UK all formulations of topical antifungal agents on the market contain sodium benzoate or benzoic acid
Propylparaben (propyl hydroxy-benzoate; propyl parahydroxybenzoate)	May affect protein binding by bilirubin. Suggestion of oestrogenic activity with potential reproductive effects that requires further work	Suggestion that long-term accumulation can occur in some tissues	Widely used in medicines given to healthy babies
Ethanol		Intoxication Effects on neurones	Widely used in medicines given to healthy babies
Polysorbate 80 (polyoxyethylene sorbitan fatty acid ester)		Serious adverse reactions, including some deaths, in low-birthweight infants administered an IV vitamin E preparation containing a mixture of polysorbates 20 and 80	Widely used in medicines given to healthy babies
Propylene glycol	Can intoxicate in same way as ethanol but one third as potent in this regard	Ototoxicity; cardiovascular effects; CNS toxicity; seizures; hyperosmolarity; lactic acidosis	Median exposure of 34mg/kg/24hr for 48hr in preterm neonates did not affect short-term adaptation to birth
Sorbitol		May cause problems in people with congenital fructose intolerance (c. 1:20,000 live births); osmotic laxative effect	

TABLE 2 Summary of available/known toxicity data of the excipients to be studied in the ESNEE project.

Acknowledgements

The ESNEE consortium members are:

- Mark Turner (University of Liverpool and Liverpool Women's NHS Foundation Trust)
- Tony Nunn, Utpal Shah (Alder Hey Children's NHS Foundation Trust)
- Hussain Mulla, Hitesh Pandya (University of Leicester)
- James McElnay, Jeff Millership, Shirish Yakkundi (Queen's University Belfast)
- Andre Rieutord (Hôpital Antoine Béclère, France)
- Thomas Storme, Pascal Vaconsin (Hôpital Robert Debré, France)
- Irja Lutsar, Tuuli Metsvaht, Heili Varendi, Georgi Nellis (University of Tartu, Estonia).

The ESNEE programme is funded by ERA-NET PRIOMEDCHILD, co-ordinated by ZonMW in the Netherlands. In the UK this is supported by MRC grant number G1100158.

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Erratum

In the article entitled 'Late preterm babies – their problems and care' written by Allistair Jennings and published in the July 2011 issue of *Infant*, pages 126-30, references 17-22 were inadvertently omitted from the printed version. We would like to apologise for this omission.

The complete article is available on the website (www.infantgrapevine.co.uk) and the missing references are reproduced below:

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