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The use of excipients in medicines administered to neonates in Europe

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2. **Nellis G**, Metsvaht T, Varendi H, Toompere K, Lass J, Mesek I, Nunn AJ, Turner MA, Lutsar I, ESNEE consortium. Potentially harmful excipients in neonatal medicines: a pan-European observational study. *Arch Dis Child.* 2015 Jul;100(7):694–699
3. **Nellis G**, Metsvaht T, Varendi H, Lass J, Duncan J, Nunn AJ, Turner MA, Lutsar I. Product substitution as a way forward in avoiding potentially harmful excipients in neonates. *Pediatr Drugs*. 2016; 18:221–230

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In publication 3: participated in the study design, conducted data analyses, and wrote the article.

ABBREVIATIONS

ADME – absorption, distribution, metabolism and elimination
API – active pharmaceutical ingredient
ATC – anatomical therapeutic chemical classification
CI – confidence interval
CNS – central nervous system
GA – gestational age
GCP – good clinical practice
GLP – good laboratory practice
GMP – good manufacturing practice
EDTA – ethylenediaminetetraacetic acid
EEA – the European economic area
EMA – European Medicines Agency
EPA – Environmental Protection Agency
EOI – excipients of interest
ESNEE – European study of neonatal exposure to excipients
EU – European Union
ExcpDG – excipients drafting group
FDA – Food and Drug Administration
ICH – The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.
IQR – interquartile range
IPEC – International Pharmaceutical Excipients Council
NICU – neonatal intensive care unit
NUTS – nomenclature of territorial units for statistics
OR – odd ratio
OTC – over-the-counter drugs
PD – pharmacodynamics
PIL – patient information leaflet
PK – pharmacokinetics
PPS – point prevalence study
RofA – route of administration
SD – standard deviation
SES – service evaluation survey
SmPC – summary of product characteristics
STEP – safety and toxicity of excipients for paediatrics
US – the United States
WHO – World Health Organisation

GLOSSARY OF TERMS

Acceptable Daily Intake (ADI) – The amount of a substance that can be ingested daily for an entire lifetime without causing appreciable adverse effects. It is expressed in mg/kg body weight/day.

Active Pharmaceutical Ingredient (API) – Any substance intended to be used in the manufacture of a medicinal product and that, when so used, becomes an active ingredient of the medicinal product. Such substances are intended to furnish a pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease, or to affect the structure and function of the body.

Adverse Drug Reaction (ADR) – Any noxious or unintended reaction to a drug that is administered in standard doses by the proper route for the purpose of prophylaxis, diagnosis, or treatment.

Bioavailability – The rate and extent at which the API is absorbed from a pharmaceutical dosage form and becomes available at the site(s) of action.

Bioequivalence – Two pharmaceutical products are bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives, and their bioavailabilities, in terms of peak (Cmax and Tmax) and total exposure (area under the curve) after administration of the same molar dose under the same conditions, are similar to such a degree that their effects can be expected to be essentially the same.

Confidence Interval (CI) – A range, calculated from sample data, within which a population parameter, such as the population mean, is expected to lie, with a given level of confidence.

Dosage Form – A dosage form is the physical form in which a drug is produced and dispensed, such as a tablet, a capsule, or an injectable.

Drug – Any substance for human or veterinary use that is intended to modify or explore physiological systems or pathological states for the benefit of the recipient.

Drug Product – A finished dosage form, for example, tablet, capsule, or solution, that contains an active ingredient, generally with excipients, that has been prepared for consumer use and that has undergone all stages of production including packaging and labeling. In this thesis, the term drug product, pharmaceutical product, medicine, and product are used interchangeably.

Excipient (pharmaceutical excipient) – Pharmaceutical excipients are substances that are included in a pharmaceutical dosage form not for their direct therapeutic action, but to aid the manufacturing process, to protect, support or enhance stability, or for bioavailability or patient acceptability.

Functionality – A desirable property of an excipient that aids and/ or improves the manufacture, quality, or performance of the drug product.

Gestational age (GA) – Time from the first day of last normal menstrual period to date of birth, usually expressed in complete weeks. When a pregnancy has been achieved by assisted reproductive technology, GA is calculated from two weeks before the date of conception.

Good Clinical Practice (GCP) – An international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects.

Good Laboratory Practice (GLP) – A code of standards concerning the testing of medicines in laboratories during their development.

Good Manufacturing Practice (GMP) – Minimum requirements for the quality management system methods, and facilities or controls to be used for the manufacture, processing, packing, or holding of a drug product and its ingredients.

Harm – Damage to health, including the damage that can occur from loss of product quality or availability.

Inactive Ingredient Database (IID) – An FDA database containing information on excipients present in FDA-approved drug products.

International Nonproprietary Name (INN) – The shortened scientific name based on the active ingredient. WHO is responsible for assigning INNs to pharmaceutical substances.

Marketing Authorization (Product License, Registration Certificate) – A legal document issued by the competent drug regulatory authority that establishes the detailed composition and formulation of the product and the pharmacopoeial or other recognized specifications of its ingredients and of the final product itself, and includes details of packaging, labelling and shelf-life.

Newborn classification based on gestational age (WHO)

Term – (37 to 42 weeks of gestation)
Late preterm (32 to <37 weeks of gestation)
Very preterm (28 to <32 weeks of gestation)
Extremely preterm (22 to <28 weeks of gestation)

New Excipient – An excipient used for the first time in a drug product or a new route of administration. Equivalent to "Novel Excipient".

No Observed Adverse Effect Level (NOAEL) – The highest dose of a substance that, in a given toxicity test, causes no biologically significant effects in the exposed test animals.

Pharmaceutical Alternatives – Products are pharmaceutical alternative(s) if they contain the same molar amount of the same active pharmaceutical

moiety(s) but differ in dosage form (e.g. tablets versus capsules), and/ or chemical form (e.g. different salts, different esters). Pharmaceutical alternatives deliver the same active moiety by the same route of administration but are otherwise not pharmaceutically equivalent. They may or may not be bio-equivalent or therapeutically equivalent to the comparator product.

Pharmaceutical Equivalence – Products are pharmaceutical equivalents if they contain the same molar amount of the same API in the same dosage form if they meet comparable standards, and if they are intended to be administered by the same route. Pharmaceutical equivalence does not necessarily imply therapeutic equivalence, as differences in the excipients and/ or the manufacturing process and some other variables can lead to differences in product performance.

Risk Assessment – A systematic process of organizing information to support a risk decision to be made within a risk management process. It consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.

1. INTRODUCTION

"Doctors are men who prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing." (Voltaire; 1694–1778)

Medicines contain active pharmaceutical ingredients (API) and a range of "other chemicals" known as pharmaceutical excipients. Hundreds of different excipients are used in manufacturing process to improve the quality, stability, bioavailability, and patient acceptability of medicines.[1] Benefits of medicines will not be possible in the absence of excipients in many cases. They function as diluents, fillers, solvents, emulsifiers, binders, lubricants, glidants, sweeteners, preservatives, and flavouring or colouring agents and make up, on average, about 90% of each product.[2] Therefore, administration of medicines usually entails exposure to pharmaceutical excipients.

Most pharmaceutical excipients are recognized as safe. However, increasing number of adverse reports from single excipients raises concerns particularly for the most vulnerable groups of patients, i.e. children and especially neonates.[3–6] Excipients have undergone exhaustive short- and long-term studies for toxicological endpoints in adult population but not in pediatric subpopulations. It is known that neonates handle some excipients differently from older age groups[7] – excipients have been associated with significant adverse events, including death, when safety data have been extrapolated from adult data.[8–10] Many of the physiological processes governing absorption, distribution, metabolism and elimination (ADME) of drugs are immature/ different in neonates.[11] Furthermore, recent research in paediatrics and developmental toxicology has elaborated the concept of "windows of vulnerability"[12] – critical periods in early development when exposures to even minimal doses of toxic chemicals can disrupt organ formation and cause lifelong functional impairments.[13]

If excipients cause or are likely to cause harm in neonates, age-appropriate formulations without specific excipients should be developed. From an economic point of view, age-appropriate neonatal formulations should be limited to those that are absolutely needed. Here, a full understanding of the presence of specific excipients in drug products administered to neonates and the extent of neonatal exposure to these excipients is a cornerstone. However, there is insufficient information about the risks generated by excipient exposure because systematic surveys have not been performed. Although some excipient exposure data in neonates are available and raise concerns, these are limited to single/ couple of country(s)/ unit(s)/ excipient(s).[14–16] For instance, Lass et al. have shown that almost all neonates have received potentially harmful excipients in two tertiary care hospitals in Estonia.[15] No understanding of the Europe-wide situation exists, yet it is essential/ warranted to recognise the extent as well as the seriousness/ severity of the issue. Targeting large multi-country populations should be preferred to reach market sizes of interest to achieve reformulation

and substitution where needed. Such approach may also provide a basis for suggestions about product substitution, where products free of unwanted excipient(s) are available, without the need for expensive reformulation process.

The unique feature of excipient studies is that the use of excipients varies considerably between formulations of the same API. This means that to assess the exposure to excipients, information about medicines use need to be gathered in a way that captures data about API, manufacturer, dosage form, and trade name of the formulation to identify the specific product used. When the data have to be quantified dosing regimens and individual demographic data should also be collected. Here, different observational cross-sectional study designs can be implemented. However, little is known about the effect of study design in excipient exposure studies. Large international studies are almost entirely missing. Accordingly, the methodological aspects have not been addressed.

Excipient exposure study including neonatal exposure to potentially harmful excipients in Estonia has been conducted in Tartu University by J. Lass.

2. REVIEW OF THE LITERATURE

Neonates are the group of children under 28 days of age,[17] divided into term (37 to 42 weeks of gestation), late preterm (32 to <37 weeks of gestation), very preterm (28 to <32 weeks of gestation), and extremely preterm (22 to <28 weeks of gestation) neonates.[18] Neonates at different stages of maturity/ immaturity, particularly very and extremely premature ones, but also late preterm and term newborns may have serious medical problems requiring extensive interventions.[19–21] In preterm neonates age-specific pathologies include surfactant-deficient lung disease, bronchopulmonary dysplasia, patent ductus arteriosus, necrotizing enterocolitis, retinopathy of prematurity, intraventricular haemorrhage, and periventricular leucomalacia. Term neonates may present with hypoxic ischaemic encephalopathy, persistent pulmonary hypertension of the newborn, meconium aspiration or congenital structural or functional anomalies.[22] All neonates have age-specific immune dysfunction which makes them vulnerable to a range of infections.[23]

A huge number of different medicines are used in neonates,[19] that are essential for the treatment of both acute and chronic illnesses. While the need for medicines in neonates is indisputable, up to 90% of medicinal products are used unauthorised or off-label in this population worldwide.[18,24–29] It means that most medications administered to neonates lack convincing data to support their safety and efficacy. These drugs have been developed for adults or older children and contain excipients thought to be safe in these age groups.[10] It has been anticipated, that excipients that have not caused problems in older age groups can be assumed to be safe in neonates unless there is biological evidence to the contrary. Response to pharmaceutical agents is dependent on multiple factors, including but not limited to differences in metabolic capacity and organ system development.[30] Although extrapolation of efficacy from adult to paediatric population is feasible for some medicines, supportive paediatric data like pharmacokinetic (PK) data and safety information are still required.[31]

2.1 Requirements to paediatric/ neonatal medicines

As the choice of formulation depends on the condition to be treated and the clinical status of the newborn, age-appropriate formulations and strengths using appropriate excipients must be developed/ made available.[18] The importance of using age-appropriate formulations has been acknowledged by all stakeholders. As stated in the European Medicines Agency (EMA) “Reflection paper on the formulations of choice for the paediatric population”,[10] in the more recent draft “Guideline on pharmaceutical development of medicines for paediatric use”,[32] as well as in the World Health Organization (WHO) document “Points to consider in pharmaceutical development of pediatric medicines”,[33] an ideal dosage form for paediatric patients of all ages should allow both safe and accurate dose administration in multiple/ various conditions and settings. Even if clinical

efficacy and safety have been sufficiently documented for the paediatric population as a group, clinical studies may not have sufficient power to detect differences in subgroups within the studied age interval.

Developing a medicine for children/ neonates poses a variety of challenges including physiological and biological maturation, swallowing difficulties and low tolerance to unacceptable taste.[34,35] Several requirements have been identified as key in the identification of a preferred paediatric dosage form.[36] Desirable attributes of a paediatric dosage form include:[33,36]

1. Minimal administration frequency
2. Minimal impact on lifestyle
3. Convenient, easy, reliable administration
   a. Acceptable and palatable dosage form (e.g. syrups and suspensions instead of traditional solid forms, e.g. tablets)
   b. Minimal requirement for complex calculations for prescribing, dispensing, and administration
   c. Minimal manipulation by health care professionals, parents or caregivers
4. Dose and dose volume/weight adjusted to the intended age group
   a. Soluble in small volumes
   b. Parenteral preparations should contain small dose volumes, at the same time being administered via small needles or cannulas
5. Transportable and low bulk/weight
6. Easily produced, stable in variety of climates
7. Affordable in terms of costs
8. Commercially viable
9. Minimal, non-toxic excipients

Appropriate choice of excipients is indispensable for the implementation of all the above-mentioned requirements.

2.2 Pharmaceutical excipients

"Excipere" is a latin word meaning "to mix", "to gather"[1] or "other than".[2] In 1957, excipients were defined as substances used as a medium for giving a medicament, that is to say with simply the functions of an inert support of the active principle(s).[37] In 1974 they were described as "any more or less inert substance added to a prescription in order to confer a suitable consistency or form to the drug: a vehicle".[37] Nowadays the International Pharmaceutical Excipients Council (IPEC) defines an excipient "as any substance other than the active drug or pro-drug that is included in the manufacturing process or is contained in a finished pharmaceutical dosage form".[38]

Historically, because of their lack of targeted pharmacological action, excipients have been considered inert agents and their importance has been largely underestimated.[1] In the past, excipients were derived from materials of natural origin and employed in the pharmaceutical field in original form, without further processing to improve their chemical or physical characteristics. Analytical tests conducted by pharmaceutical industry were limited and insufficient to characterise excipients' quality, even less their safety and functionality.[39] However, as early as 1950 T.G.Randolph called attention to the problems of excipients in medicines. He suggested that manufacturers change their excipients to less allergenic substances and state the composition of the "inert ingredients".[40] The traditional concept of excipient has undergone considerable evolution: from simple, chemically and pharmacologically inert vehicle to essential adjuvant, guaranteeing and optimising the performance of a modern medicinal product.[39]

2.2.1 The role of pharmaceutical excipients in medicines

Excipients contribute to the performance of the drug to improve safety, bioavailability, and efficacy of the final formulation.[39] The properties of the final dosage form are, in the most part, highly dependent on excipients.[41] Excipients (1) aid processing of the system during manufacture, (2) protect, support, or enhance stability, bioavailability, or patients acceptability,[35] (3) assist in product identification, and (4) enhance any other attribute of the overall safety and effectiveness of the drug delivery system during storage and use.[42,43]

One of the paradoxes of pharmaceutical formulation science is that, although excipients do not treat the disease, the disease cannot be treated without them.[44] Most bulk APIs are not useful to the patient until they are formulated into a medical product, and that would not be possible without excipients.[44] Excipients are required to overcome the chemical, physical, and microbiological challenges posed by developing a (paediatric/ neonatal) formulation to achieve a predictable therapeutic response. Almost all pharmaceutical design aspects are in direct relation to the excipients (Figure 1).[45]

The concept of "functionality", introduced recently, means adding excipients in order to enhance performance, quality, and safety profile of medicinal product.[39,46] For example, liposomal amphotericin reduces exposure of renal tubular cells and subsequent toxicity.[47] In some cases, excipients are essential to ensure the stability of the API and/ or to optimise the delivery or the kinetics of the API and therefore have a substantial effect on bioavailability.[43]

Accordingly, it alludes to excipients having a purpose, which contrasts with the old terminology of "inactive ingredients" which hints at the property of inertness.[46]

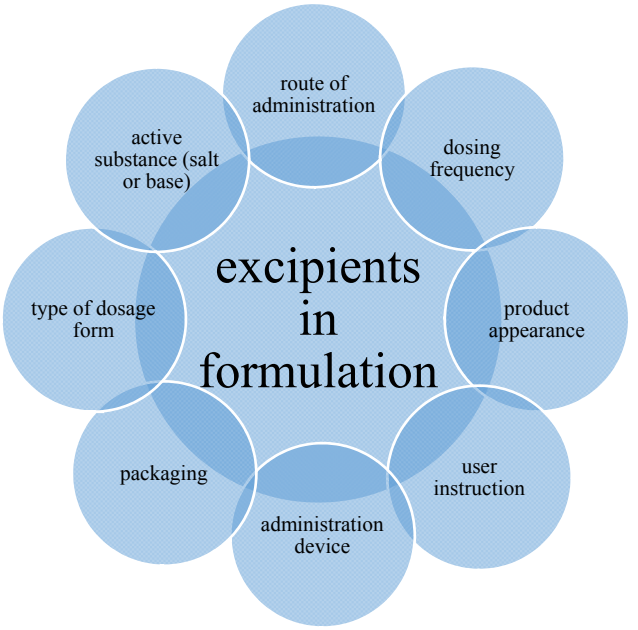


Figure 1. Pharmaceutical design aspects (adapted from Riet-Nales et al., 2016)[45]

2.2.2 Classification of excipients

Excipients can be classified in different manner, e.g. based on type (standard, mixed or co-processed excipients), origin (animal, vegetable, mineral or synthetic sources), chemical class (alcohols, carboxylic acid, carbohydrates, dyes, esters, glycerides, halogenated hydrocarbon derivates, organic mercurial salts, phenolic compounds, proteins or polymers), and functions excipients perform in the formulations.[48,49] The most appropriate from the clinical point of view and therefore often used classification is based on the function – excipients are sub-divided into various functional classes, depending on the role that they are intended to play in the resultant formulation (Table 1).

Certain excipients can have different functional roles in different formulation types and its concentration may vary depending on the purpose of use. For example, propylene glycol can be used as a solvent, antimicrobial preservative, humectant, stabilising agent, or plasticiser.[41]

Table 1. Classification of excipients by function (modified from Chaudhari and Patil, 2012)[48]

Excipient category	Function in formulation	Examples*
Diluents	Fillers, bulking agents	Lactose, **sorbitol**, starch
Binders	Adhesives	Acacia, gelatin, povidone
Lubricants	Reduce inter-particular friction	Talc, surfactants, stearic acid
Glidants	Improve flow characteristics of powder mixture	Colloidal silicon dioxide, corn starch
Disintegrants	Facilitate disintegration after administration	Starches, cellulose, clays
Coating materials	Protect tablet ingredients	Povidone, beeswax, acacia
Solvents	Dissolving solute/ API	Water, **ethanol**, acetone
Co-solvents	Increase the solubility of solute in solvents	**Ethanol**, **sorbitol**, **propylene glycol**, glycerin
Buffers	Maintain pH	Phosphate buffers, acetate buffers
Antimicrobial preservatives	Prevent microbial growth	**Benzyl alcohol**, **parabens**
Antioxidants	Control oxidation	Ascorbic acid, tocopherols
Wetting agents	Aid wetting and dispersion of hydrophobic APIs	Sodium lauryl sulphate, lecithins, **polysorbates**
Antifoaming agents	Discourage formation of foam	Simethicone, alcohols
Thickening agents	Prevent settling/ sedimentation	Methylcellulose, microcrystalline cellulose
Humectants	Retard evaporation of aqueous vehicles	**Propylene glycol**, glycerol
Chelating agents	Protect from catalysis	Disodium EDTA, citric acid
Emulsifying agents	Prevent coalescence	Sodium lauryl sulphate
Flocculating agents	Prevent caking	Starch, sodium alginate
Sweeteners	Impart sweetness	**Sorbitol**, **saccharin**, sucrose
Coloring agents	Aesthetic appearance, product identification	Amaranth, erythrosine, eosin, titanium dioxide, carotene
Flavours	Impart flavour	Aromatic waters, syrup, menthol, orange

*Excipients that is of interest in this thesis are shown in bold
EDTA, ethylenediaminetetraacetic acid

2.2.3 Pharmacological principles of excipients in neonates and adults

PK include parameters of ADME and determine the relationship between the exposure and body concentration.[30] In terms of physiological and anatomical factors the neonate has to be considered as a "unique drug recipient".[1,50,51] For all the specific variables of the drug kinetics (absorption, blood esterase activity, body water and lipid ratios, plasma protein binding, organ perfusion rates, metabolic degradation and elimination), there are clear differences between neonates and older infants and children not to mention adults.[13,50,52,53]

Drug pharmacodynamics (PD), i.e. biochemical and physiologic effects exerted in the body, may also vary in neonates in terms of drug-receptor interactions, receptor number, receptor affinity, and receptor regulation and modulation.[54–56] In combination with altered PK, this may influence the therapeutic and toxic effects of pharmaceutical product significantly.[54] Still, there is a lack of tools to assess PD effects in neonates and little information exists about the effect of human ontogeny on interactions between drugs and receptors and the consequence of these interactions.[55,56]

Singularity of neonatal PK/ PD in relation to excipients. Anatomical, physiological, and biochemical changes that occur from birth as well as any pathologic condition affecting the newborn influence PK/ PD of drugs as well as excipients.[57,58] In developmental pharmacology this immaturity is well documented as influencing the dose of drugs administered to neonates but less well described is the effect on the handling of pharmaceutical excipients.[59]

For enteral medicines, the variability in gastric pH, prolonged rate of gastric emptying, immaturity of the intestinal mucosa, and decreased first-pass metabolic capacity may predispose newborns to higher oral bioavailability and systemic concentrations for some chemicals.[34,51,52,60–62] Decreased levels of serum binding proteins,[63] but also the presence of increased serum fatty acid and bilirubin levels, can increase the unbound fraction of excipients.[52,60]

Higher relative amount in combination with immature metabolic/ elimination pathways[64,65] may lead to the saturation of metabolism and accumulation of an excipient.[66] Hydroxylating activity[63] and conjugation with glucuronic acid (glucuronidation)[65,67] appear to be the two metabolic pathways which are the most defective at birth, while sulphate[68] and glycine conjugation and dealkylation activities are close to the adult pattern.[50,69] Cytochrome P450 content in neonates is 50% that of adult levels. Tran et al. reported estimated alcohol dehydrogenase content to be about 10-fold lower in perinatal period compared to adults.[70] Renal elimination is also reduced in neonates due to immature glomerular filtration, tubular secretion and reabsorption.[71,72] As a result, both renally- and hepatically-cleared pharmaceuticals may exhibit longer half-lives.[73,74]

Early postnatal period includes the primary developmental events of the central nervous system and is extremely susceptible to certain neurotoxins such as **propylene glycol** and **ethanol**.[75] Neonates may have proportionately higher brain levels of circulating chemicals due to a higher brain to body weight

ratio,[76] lower levels of plasma proteins, and the fact, that molecules that enter the cerebrospinal fluid and brain during early development are cleared more slowly and will accumulate to a greater extent than later in development.[77]

Simultaneous administration of multiple excipients metabolised/ eliminated through the same immature pathways may further increase the accumulation of potentially toxic substrates.[9] For example, **propylene glycol** accumulation may occur when administered in combination with another substrate of alcohol dehydrogenase (limiting step of metabolism) such as **ethanol**.[9]

Accumulation of **propylene glycol** in neonates following repeated administration was demonstrated.[78,79] The initial renal elimination of propylene glycol in (pre)term neonates is 15% of total clearance compared to 45% in adults.[80] Potential accumulation/ toxicity of propylene glycol is also affected by the activity and saturation of alcohol- and aldehyde dehydrogenase.[66] The Du et al. study confirmed that significant propylene glycol concentrations may be obtained in the brain (up to ~ 0.456 mg/g tissue) following a single dose of 1 mg/kg in rats.[81] In the Kelner and Bailey study with five patients receiving medications containing propylene glycol, the cerebrospinal fluid concentrations of propylene glycol were as high as 85% of the serum concentrations.[82]

The fact that PK may also have great variations within the neonatal population in relation to the developmental age of the newborn was confirmed by De Cock et al. who showed that birth weight and postnatal age are the most important covariates for clearance of propylene glycol in neonates.[78]

Benzyl alcohol is metabolised to benzoic acid for further detoxification through glycine conjugation to form hippuric acid.[83] The availability of glycine is the rate-limiting factor in the formation of hippuric acid.[84] Although the glycine conjugation pathway is relatively mature in term newborns, preterm neonates are unable to conjugate benzoic acid efficiently.[7,10,85] The main safety concern with **benzoic acid** is its ability to displace bilirubin from albumin.[86] This risk exists with oral, parenteral, and topical formulations. The hazard/ risk of developing kernicterus is also to be considered when benzyl alcohol is used since benzoic acid is one of its metabolites. LeBel et al. showed a smaller amount of hippuric acid in the urine of premature neonates compared with term newborns after exposure to benzyl alcohol, indicating that hippuric acid formation is deficient in given patients.[7]

Para-hydroxybenzoic acid (PHBA), the principal metabolite of **parabens**, may be conjugated with glycine or sulfate (maturity is close to the adult levels) and glucuronic acid (immature in neonates) for further renal elimination. In principle, one more mature metabolic pathway might compensate the immature one. However, higher proportions of free parabens were determined in urinary spot samples from preterm neonates compared to adults, still showing the prevalence of metabolic immaturity.[87]

2.3 Safety of pharmaceutical excipients in children and neonates

"As to diseases, make a habit of two things – to help, or at least, to do no harm." (Hippocrates; ca. 460 BC – ca. 370 BC)

Safety is one of the most important requirements (besides quality and efficacy/ functionality) of the pharmaceutical compound including excipients.[37]

2.3.1 Disasters with pharmaceutical excipients

The inclusion of excipients with inadequately studied safety profile in medicines has resulted in several disasters (Table 2).

Table 2. Paediatric excipient disasters (modified from Choonara and Rieder, 2002)[89]

Year	Drug	Excipient	Deaths	Country
1937	Sulphanilamide elixir	Diethylene glycol used as solvent	105	USA
1970	Bathing foam	Contained hexachlorophene	Significant number of neonates developed neurotoxicity	USA
1972	Talc baby powder	Contained 6.3% hexachlorophene	36	France
1982	Sodium chloride Water	Benzyl alcohol	16	USA
1984	Vitamin E	Polysorbate 80	38	USA
1992	Paracetamol	Diethylene glycol used as solvent	47	Nigeria
1995	Paracetamol	Diethylene glycol used as solvent	51	Bangladesh
1998	Paracetamol	Diethylene glycol used as solvent	85	Haiti
2006	Cough syrup	Glycerine contaminated with diethylene glycol	46	Panama
2008	Teething formula	Glycerine contaminated with diethylene glycol	84	Nigeria

"*…to realize that six human beings, all of them my patients, one of them my best friend, are dead because they took medicine that I prescribed for them innocently, and to realize that that medicine which I had used for years in such*

cases suddenly had become a deadly poison in its newest and most modern form…" (Letter by Dr. A.S. Calhoun, October 22, 1937)[88] Seventy-one adults and 34 children died in 1937 after taking an "Elixir Sulfanilamide"; the United States (US) Food and Drug Administration (FDA) identified diethylene glycol, the excipient used as a solvent, as the killer.[88] Unfortunately, one of the earliest disasters in modern paediatric drug therapy has been the most repeated (Table 2).

Nowadays, it is clear, that common excipients used in a formulation may have an unintended influence on bioavailability/ bioequivalence in children and neonatal population.[90] The historical assumption that excipients are inactive is rapidly fading. Unfortunately, the safety issues of excipients still do not receive a proper attention in modern neonatal pharmaceutical care and are not just historical events.[47]

2.3.2 Types of excipient interactions/ toxicity

Excipients have the potential to harm patients in two ways. First, by introduction of a chemical (e.g. toxicity, physiological effect) or physical hazard (e.g. irritation). Secondly, adversely affecting the API availability or performance (e.g. changes in the bioavailability or modified release).[91] Excipients have been associated with specific safety issues: allergic reactions, intolerances, diminished absorption of API, inhibition of physiological processes, cytotoxicity etc.[1] Today, it is well known that certain excipients may produce incompatibles with the API, another excipient or with intracellular chemicals – excipient-drug, excipient-excipient, and excipient-human body interactions.[49] Some examples of different types of interactions are shown in Table 3.

Table 3. Different types of harmful excipient interactions

Excipient	Type of interaction	Description
Diethylene glycol	Excipient-body	CNS, renal, and hepatic toxicity[75,92]
Benzoic acid/ sodium benzoate	Excipient-drug	Pronounced inhibitory effect on the formation of salicyluric acid from salicylic acid and may result in increased concentration and persistence of salicylic acid in the body[93]
Ethanol	Excipient-excipient	Inhibits the formation of hippuric acid from benzoic acid[94] Competitively inhibits the metabolism of propylene glycol[9,95]
Ethanol	Excipient-body	CNS depressant by binding to the gamma-aminobutyric acid A receptor[96]

CNS, central nervous system

2.3.3 Classification based on safety of excipients

Based on the safety profile excipients were classified by Lass et al. (Table 4) as potentially safe, potentially harmful and known to be harmful, and excipients with no safety data available or with unspecific description.[15]

Table 4. Classification of excipients according to the potential safety/ toxicity (modified from Lass et al., 2012)[15]

Safety status	Description	Examples
Potentially safe	No ADR reported	Citric acid, hydrochloric acid, water, starch, simethicone
Potentially harmful and known to be harmful	ADR reported	Propylparaben, benzyl alcohol, benzoates, propylene glycol, polysorbate 80, ethanol, benzalkonium chloride, sorbitol
No safety data found	No data found in the literature on human exposure and toxicity	Sodium carmellose
Description of the excipient in SmPC or PIL unspecific	Description does not allow a specific literature search	Flavouring agents, coloring agents

ADR, adverse drug reaction; PIL, package insert leaflet; SmPC, summary of product characteristics

2.3.4 Potentially harmful excipients in neonates

Today, we have well established (does not mean exhaustive) safety databases on existing excipients, and new excipients are required to undergo extensive animal safety testing before they can be used in clinical studies.[97] For some excipients, there are data to support "safe" exposure levels in adults. However, the safety profile of some common excipients (Table 4; Table 5) may differ between children and adults as well as across the various paediatric sub-groups. Excipients that have been highlighted as having a potential to cause toxicological problems in neonates are shown in Table 5. These are referred to as excipients of concern or problematic excipients or excipients of interest (EOI) for this thesis.

Table 5. Excipients with reported adverse effects in neonates and older children

Excipient	Reported adverse effects	
	Newborns (<28 days old)	**Children >28 days old**
Propyl-paraben	Hyperbilirubinemia, hypersensitivity reactions, (oestrogenic effects)[41,98]	Allergic reactions, bronchospasm[98]
Polysorbate 80	E-Ferol syndrome – thrombo-cytopenia, renal dysfunction, hepatomegaly, cholestasis, ascites, hypotension, metabolic acidosis[41]	Hypersensitivity following topical and intramuscular use[41]
Propylene glycol	Skin irritation, CNS depression, cardiovascular, hepatic, respiratory adverse events, hyperosmolality; lactic acidosis[1,41,98]	Large volumes associated with adverse effects most commonly on the CNS[41]
Benzyl alcohol	Metabolic acidosis, seizures, gasping, intraventricular haemorrhage, kernicterus, fatal toxic syndrome[1,41,85,98,99]	May cause toxic and allergic reactions in children up to 3 years old[98,100]
Benzoic acid, sodium benzoate	Hypersensitivity, kernicterus[41,86,98]	Skin, eye, and lung irritation, urticaria, angiooedema[98]
Saccharin sodium	Urticaria, photosensitivity reactions[101]	Generally regarded as safe; skin hypersensitivity[41]
Sorbitol	Diarrhoea, nutrient malabsorption, diabetic-like symptoms[49,102]	Mild laxative effect[98]
Ethanol	Lactic acidosis; hypoglycaemia; CNS effects[1,41,103]	Harmful for those with liver disease or epilepsy; skin irritation; may alter the effects of other medicines; hypoglycaemia; CNS effects[96,98,103]
Benzal-konium chloride	Ototoxic when applied to ear, skin irritation and hypersensitivity, eye irritation[1,41,98]	Skin and eye irritation, bronchospasm[98,104]

CNS, central nervous system

2.3.4.1 Safety of EOI and current implications

The amount usually plays a critical role in safety/ toxicity of excipient in formulation. Maximum tolerated doses for excipients, determined by animal safety testing, are usually referenced for use in adults and are not necessarily applicable to their use in children and particularly in neonates. Furthermore, even in adults saturation of metabolic clearance of e.g. propylene glycol occurs at lower doses (0.2 g/kg) than in rats and rabbits (2 g/kg),[66] indicating the imperfection of animal safety studies. While acceptable daily and cumulative intake of excipients is not clearly defined for different paediatric age groups,

some excipients, e.g. benzyl alcohol, should be totally avoided in neonates.[83] Fortunately, existing clinical data and comprehensive set of non-clinical data have allowed estimating the acceptable daily intake (ADI) and no observed adverse effect levels (NOAEL) for some excipients that are of interest in this thesis (Table 6).

Table 6. Acceptable daily intake (ADI) and no observed adverse effect level (NOAEL) of EOI

Excipient	ADI	NOAEL	Comments
Propylparaben[87]	2 mg/kg	100 mg/kg	Applicable to all ages including neonatal period
Polysorbate 80[105,106]	10 mg/kg calculated as total polysorbate esters	1000 mg/kg	No neonatal data E-Ferol syndrome in preterm neonates
Propylene glycol[66,107]	1 mg/kg (neonates) 50 mg/kg (< 5 years) 500 mg/kg (adults)	192 mg/kg (term neonate) 150 mg/kg (1-year child) 126 mg/kg (4-year child)	Co-administration with any substrate of alcohol dehydrogenase such as ethanol may induce serious ADRs in neonates
Benzyl alcohol[41,98,100,108]	Should not be used in neonates 5 mg/kg (adults, WHO) Subchronic 1 mg/kg; chronic 0.3 mg/kg (adults, EPA)	Not specified	No paediatric data "Gasping syndrome" observed in neonates. May cause toxic and allergic reactions in children up to 3 years old
Benzoic acid, sodium benzoate[86]	5 mg/kg (sum of all)	500 mg/kg	No neonatal data Inhibitory effect on the formation of salicyluric acid from salicylic acid Displace bilirubin from albumin
Saccharin sodium[41]	2.5–5 mg/kg	Not specified	No neonatal data
Sorbitol[41]	Not specified (< 20 g/day in adults)	Not specified	No paediatric data
Ethanol[33,103]	Blood ethanol levels should not exceed 25 mg/dL (AAP)/ 1 mg/dL (EMA) after a single dose (or a dose of 6 mg/kg) in children younger than 6 years	Not specified	CNS effects at 10 mg/dL
Benzalkonium chloride	Not specified	Not specified	As residue in food 0.1 mg/kg

AAP, American Academy of Pediatrics; ADR, adverse drug reaction; CNS, central nervous system; EMA, European Medicines Agency; EOI, excipient of interest; EPA, Environmental Protection Agency; WHO, World Health Organisation

Propylene glycol is commonly used as an excipient in a variety of drugs and it is also authorised in food products and cosmetics. While it is generally considered safe as a food additive, concerns have repeatedly been raised about potential toxicity of propylene glycol and its acidic metabolites following pharmacologic exposure.[66] High doses of propylene glycol in neonates may result in both biochemical (e.g. hyperosmolarity, lactic acidosis, plasma creatinine, bilirubin) and clinical (cardiovascular, central nervous system (CNS), renal, respiratory, hepatic, hematologic) toxicity.[95,107] For example, Peleg et al. reported a case of propylene glycol intoxication in a premature infant. The infant went into a state of coma after treatment for burns with antiseptic dressings containing propylene glycol. Cessation of the topical treatment resulted in complete recovery. High peak of propylene glycol was measured in urine.[6] MacDonald et al. showed that neonates receiving 3000 mg/day of propylene glycol as a vehicle in an intravenous multivitamin preparation had a higher incidence of seizures than those receiving 300 mg/day in a different vitamin preparation.[114] Clinical data showed that in children from the age of 5 years and adult patients, up to 500 mg/kg/day of propylene glycol could be considered safe.[107] In MacDonalt et al. study neonates receiving 300 mg/day of propylene glycol had a higher risk of developing hyperosmolality compared with neonates not exposed.[114] Therefore, EMA decreased the safety threshold for propylene glycol to 50 mg/kg/day in children less than five years old, and even to 1 mg/kg/day in preterm and term neonates.[107]

Ethanol has been commonly used for years in paediatric and neonatal liquid formulations as a solvent and preservative despite the lack of safety, PK, and PD data. Ethanol acts as a CNS depressant by binding to the gamma-aminobutyric acid A (GABA-A) receptor and by increasing the inhibitory activity of the neurotransmitter GABA.[96] Other ethanol related toxicities in children include hypoglycemia, acidosis, respiratory depression, seizures, hypothermia, and electrolyte abnormalities.[115]

Newborns and infants are at higher risk of both acute and chronic alcohol-related toxicities, e.g. some ethanol containing furosemide and iron formulations are given to the premature newborns for months.[96] Recently, Svirskis et al. identified 47 paediatric liquid medicines in New Zeland containing ethanol and indicated for both acute and chronic use in patients of all ages including preterm neonates.[116] In 1999 Fiocchi et al. found 103 drug products containing ethanol for prescription in children, each of which was able to deliver a theoretical blood concentration more than 20 mg/dL.[117]

Ethanol may cause lasting defects in cognition and behavior in neonates where neuronal differentiation, myelination, and migration are not fully developed.[76] Toxicity on brain maturation in neonates is also supported by non-clinical data.[33] As well, chronic exposure has been shown to be linked to dependence in adolescents and adults.[33] Additionally, ethanol inhibits the formation of hippuric acid from benzoic acid,[94] slowing the metabolism of another potentially harmful excipient (Table 5). Moreover, when administered concomitantly with propylene

glycol, ethanol has a 10 to 20 times greater affinity for alcohol dehydrogenase and therefore competitively inhibits the metabolism of the former and may lead to elevated/ toxic concentrations of propylene glycol.[9,95] In 2011 the US FDA notified healthcare professionals of serious health problems that have been reported in premature babies receiving Kaletra (lopinavir/ ritonavir) oral solution.[9] This solution contains **ethanol** (42,4% v/v or 356 mg/ml) and **propylene glycol** (15,3% w/v or 152,7 mg/ml) in significant amounts. The consequences of administering such amounts of these excipients can be severe or possibly fatal – toxicity related to Kaletra oral solution included hyperosmolality with or without lactic acidosis, renal toxicity, CNS depression (including stupor, coma, and apnea), seizures, hypotonia, cardiac arrhythmias, electrocardiographic changes, and hemolysis.[9] Given toxicity was linked to excipients.

It remains unclear what ethanol exposure is safe for neonates.[118] Chronic exposure to ethanol (> 1 week), even in small doses, through pharmaceutical product is in principle contraindicated below six years of age and limited to two weeks above six years.[33] Adverse CNS effects are already reported with blood ethanol concentrations of 10 mg/dL in children.[33] Therefore the EMA recommendation[103] regarding blood ethanol level after a single dose in children younger than six years (Table 6) seems to be more expedient compared to the American Academy of Pediatrics (AAP) Committee on Drugs.[119]

Bactericidal preservatives like **benzalkonium chloride** are frequently found in beclomethasone and ipratropium bromide nebuliser solutions and can cause paradoxical bronchoconstriction in asthmatic children.[3,104] Padnos et al. described a 3-month old infant hospitalised for croup and underwent placement of tracheostomy tube, which could not be removed for three months because of the occurrence of difficulties in breathing, coughing spasms and development of severe cyanosis upon repeated attempts of extubation. The cause of respiratory difficulty was a severe haemorrhagic dermatitis of tracheal mucosa at the tracheostomy site as a reaction to the benzalkonium chloride solution used for disinfection.[120] Benzalkonium chloride has also been associated with ototoxicity and ophthalmological problems (conjunctivitis, corneal injury).[40] There are no data on ADI for benzalkonium chloride available.

The use of sweeteners and flavouring agents is particularly important in paediatrics to improve palatability.[1] The use of carbohydrates with the potential to raise plasma glucose such as fructose, glucose, or sucrose should be limited or avoided in diabetic children. **Sorbitol** can be used as a sweetener, humectant, and vehicle for oral and topical pharmaceutical liquids and therapeutically as an osmotic laxative/ cathartic. Including sorbitol in formulation as an excipient (sweetener) may entail such adverse drug reactions (ADR) as diarrhoea and malabsorption particularly in neonates.[121] The accumulation of sorbitol in the body has been implicated in diabetic-like symptoms like retinopathy in neonates.[49] The recommendation for ADI of sorbitol in children is not available.

Some allergic reactions have been associated with flavouring agents, the problem being the lack of data on exact composition of these complex mixtures, which may complicate the safety assessment.[122] Napke and Stevens described a few cases of severe abdominal pain, nausea, and vomiting following administration of erythromycin containing tincture of orange; the youngest patient was a 6-week old infant.[123] Substitution of given formulation with another formulated with cherry syrup resolved the problem immediately and completely. At that time (1984) the pharmacist responded as have many practitioners in the past: "The active ingredient is the same; the colouring and flavouring do not matter."[123]

Fortunately, in recent years some new data have appeared providing the safe use of e.g. **methyl hydroxybenzoate (methylparaben, MPB)** in every age group including neonates. Parabens belong to a family of antimicrobial preservatives that are widely used in cosmetic, pharmaceutical, and food industries. During the last decade parabens have been extensively studied to evaluate male reproductive toxicity[124] because of the reports of weak *in vitro* estrogenic activity being between 10,000 to 100,000 fold less potent than that of oestradiol.[125] Oishi suggested that **propyl hydroxybenzoate (propylparaben, PPB)** adversely affects the hormonal secretion and the male reproductive function in rats – decreased testosterone concentration, sperm production and efficiency;[126] at the same time MPB and ethylparabens did not show any adverse effect on the secretion of sex hormones or the male reproductive function.[127] Recent toxicity study conducted according to Good Laboratory Practice in an appropriate and statistically robust manner failed to reproduce the effects of PPB on reproductive function observed by Oishi.[128] This study showed an absence of toxic effects on the maturation of the male reproductive system, up to the highest dose of 1000 mg/kg/d of PPB, thus not indicating any endocrine disrupting potential.[128] These results were confirmed in another juvenile toxicity study conducted by Pouliot et al. and using rats treated from the neonatal period. (unpublished, referenced from EMA reflection paper[87]) Thus, in a recent reflection paper, EMA concluded that the use of MPB in oral formulations up to 0.2% of the product (as within the recommended effective concentrations as a preservative) is not a concern for humans including the paediatric population whatever the age group. Regarding PPB, based on the results from recent studies, a conservative "no observed effect level" of 100 mg/kg has been determined with ADI of 2 mg/kg/day for the use in adults and paediatric patients.[87,129] However, it was considered that the lack of estrogenic effect could not be ascertained at the high dose level in females.

Co-administration of different excipients. The number of different medications administered to a child is the most significant statistical association with the risk of ADR.[130] Published data show that the average number of drugs administered per infant in the neonatal intensive care units (NICU) has progressively increased over the years.[131] Polypharmacy may lead to multiple sources

of EOI, resulting in additive exposure and/ or interactions in metabolism. Ethanol is known to inhibit the formation of hippuric acid from benzoic acid (Table 3).[94] Benzyl alcohol, in turn, in rats inhibits noncompetitively activity of hepatic alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase.[132,133] As excipient toxicity is dose dependent, cumulative exposure is one of the several factors determining the likelihood of toxic effects.[98] Shehab et al. observed a wide range in the cumulative dose of benzyl alcohol and propylene glycol from multiple formulations received by neonates with potentially toxic doses registered during routine care.[134]

2.4 Exposure to EOI in neonates

"Excipients in neonatal formulations are never prescribed, but commonly administered." (Karel Allegaert)

According to available studies, several medicines frequently used in neonates may contain EOI (Table 7).[15]

Table 7. EOI in frequently used medicines in neonates[15,16,41,116,134,135]

Excipient	Function in formulation	Drug
Methyl- and propylparabens	Antimicrobial preservative	Gentamicin inj solution Heparin inj solution Iron oral solution
Propylene glycol	Antimicrobial preservative, humectant, plasticizer, solvent, stabilizing agent, water-miscible cosolvent	Salbutamol nebulisation solution Phenobarbital inj solution Lorazepam inj solution Diazepam oral solution
Polysorbate 80	Dispersing, emulsifying, solubilizing, suspending, and wetting agent, non-ionic surfactant	Epoetin alfa inj solution Phenobarbital inj solution Budesonide nebilisation solution Chloramphenicol opthalmic solution Miconazole ointment
Ethanol	Antimicrobial preservative, solvent, skin penetrant	Heparin ointment Miconazole ointment Alprostadil inj solution Iron syrup OTC products
Benzyl alcohol	Antimicrobial preservative, solvent	Heparin inj solution Phenobarbital inj solution Midazolam inj solution Hydrocortisone inj solution

Table 7. Continuation

Excipient	Function in formulation	Drug
Sodium benzoate	Antimicrobial preservative, lubricant	Simethicone oral suspension Caffeine solution Zidovudine oral solution
Saccharin sodium	Sweetener	Simethicone oral suspension Iron oral solution Nystatin oral suspension
Sorbitol	Sweetener	Iron oral solution
Benzalkonium chloride	Antimicrobial preservative, solubilizing agent, wetting agent	Salbutamol nebulisation solution Chloramphenicol opthalmic solution

EOI, excipient of interest; inj, injection; OTC, over-the-counter drugs

Neonates admitted to the NICU may be exposed to more than 60 parenteral and more than 40 enteral medicines, each of which contains excipients.[136] Especially preterm neonates may be chronically exposed as a result of being treated with several medicines for extended periods. To date, only a few studies of estimated neonatal exposure to excipients have been conducted, one of the reasons being probably methodology issues.

2.4.1 Methods to assess the exposure to excipients

When planning medicine/ excipient exposure studies the research question to be answered needs to be balanced against the implications of study design. In principle, the use of medicines has to be studied first, and methods available in food safety and pharmacoepidemiology can be applied.[137–139] However, information required in excipient studies may not be available in multi-country databases suitable for drug studies.[140] While these data may be available on sales level, individual exposures are of relevance.

2.4.1.1 Qualitative excipient exposure assessment

Different observational study designs can be used in pharmacoepidemiology and particularly in drug utilisation studies,[57,139] not all of them are "perfect" for the assessment of excipient exposure. Case-control studies allow rich data collection for a limited number of medicines/ excipients/ patients and may be used to describe some e.g. ADR in an individual patient(s); data collection is limited to a few centers and therefore is not suitable for comprehensive exposure assessment. Cohort studies have the advantage of allowing data collection over prolonged time periods[24] and therefore can be of particular interest to study rare exposures/ outcomes and drug utilisation patterns over time.[139] However, the expense and duration make it hard to implement in multinational settings.[24]

Economic evaluation plays a role in the choice of a specific study design. There is considerable overlap between acceptable methodologies and those sanctioned by health economists.[141] Here, cross-sectional studies, also known as prevalence studies, are attractive for assessment of medicines use[137,142–147] and can be used for estimating the exposure to excipients as well. Some advantages and disadvantages of different study designs when targeting to estimate excipient exposure are summarised in Table 8.

Table 8. Advantages and disadvantages of different study designs in excipient exposure assessment using pharmacoepidemiological methods[57,139]

Study design	Advantages	Disadvantages
Case-control	Allows rich data collection Allows investigation of rare exposures	Data collection is limited to a few centres Drug exposure data collected retrospectively
Cohort design	Data can be collected pro- or retrospectively Allows data collection over prolonged time periods Allows rich data collection Allows estimation of risks Allows estimation of rare exposures	May not be feasible in multicentre/ multinational settings Resource and time-consuming In retrospective study data quality is questionable
Cross-sectional	Allows multinational studies Allows multiple data collection Time and resource saving Depending on design allows individualised approach or data collection over prolonged period	Under- or overestimates exposure of rarely used agents Depending on design limited amount of data or covers very short time periods
Databases analysis	Allows multinational studies Allows long-term collection Allows individualised approach	Do not contain data on formulation and/ or tradenames and/or manufacturers details Resource consuming

Cross-sectional studies, often described as “taking a snapshot”, seem to be the most appropriate for estimating excipient exposure in neonates because they require a relatively shorter time commitment and fewer resources to conduct. These studies can explore the role of factors associated with exposure,[139] data can be collected at the unit or individual level. An example of a unit level cross-sectional observation is a service evaluation survey (SES). Unit level data indicate which medicines are used (including distinct products, APIs, and excipients). In combination with demographic data about the units this method can provide indicative estimates of market size, that may justify the requirement of product reformulation or substitution. Unit level studies can have a longer duration but may not be manageable in a multinational setting.[24] With large volumes of data reported like in excipient studies and especially prolonged recall periods decreasing compliance and underestimation may occur.[57] Individual level data allow for stratification according to important clinical

variables such as the age of the newborn, but this approach requires an extra step of data collection. Point prevalence studies (PPS) have been used in antibiotic consumption studies.[145,146,148] Their extremely short duration may underestimate exposure to less frequently used medicines.

2.4.2 Excipient exposure studies in practice – extent of neonatal exposure to EOI

There are few data available about the extent to which neonates are exposed to excipients (Table 9). Recent observational studies suggest that neonates are exposed to significant amounts of excipients including EOI, like ethanol, propylene glycol, benzyl alcohol, and sorbitol, with the doses occasionally exceeding internationally recommended limits of exposure.[14,134]

Table 9. Review of excipient exposure studies in neonates

Reference	Study population	Study duration	Results	Country
Shehab et al. 2009[134]	Randomly selected sample of neonates from one tertiary care hospital exposed to BA (n=88) or PG (n=82) through parenteral medicines	12 months	Median (range) cumulative dose was 106.3 mg/kg/day (47.5–319.5) for BA[a], and 4554.5 mg/kg/day (869.5–9472.6) for PG[b] in neonates who received medicines via continuous infusion	USA
Whittaker et al. 2009[14]	38 preterm neonates (< 30 weeks gestation and < 1500g birth weight) from one neonatal unit	12 months	20 excipients identified Exposure to ethanol ranged from 0.2 to 1.8 ml/week Exposure to sorbitol ranged from 0.1 to 3.5 g/kg/week[c] All neonates given dexamethasone were exposed to PG with a dose above 25mg/kg/day[b]	UK
Lass et al. 2012[15]	838 neonates from two tertiary care hospitals	12 months	123 excipients identified 88% of neonates exposed to EOI	Estonia
Souza et al. 2014[135]	79 neonates from one neonatal unit	3 months	86 excipients identified 87% of neonates exposed to EOI	Brazil
Fister et al. 2014[16]	48 neonates from one neonatal unit	1 month	60 different excipients identified All neonates exposed to EOI	Slovenia

EOI, excipients of interest; BA, benzyl alcohol; PG, propylene glycol; [a]BA should not be used in neonates; in adults, recommended maximum daily intake is 1–5 mg/kg/day; [b]the safety threshold for PG in neonates is 1 mg/kg/day; [c]using the weight of an average adult male (70 kg) as the denominator, the adult recommendations (20 g/day) equate to 2 g. of sorbitol/kg/week

Despite the European Union (EU) and the US recommendations and incentives, medicines used in neonates still contain excipients with known potential for toxicity.[32,43,149] Neonates in NICU may be exposed to over 20 different excipients per day through only commonly used drugs.[14] In Fister et al. study from Slovenian neonatal unit every neonate was exposed to the two most common excipients known to cause harm to this age group – ethanol and propylene glycol – as these excipients are present in pharmaceutical products recommended for daily antirachitic prophylaxis (vitamin D formulation). Positively, neonates in this study were not exposed to benzyl alcohol. In contrast to this, Garcia-Palop et al. reported benzyl alcohol to be one of the most common EOI in parenteral medicines prescribed to neonates in Spain.[150]

2.4.2.1 Quantitative exposure to EOI in neonates

Only few studies have tried to quantify exposure of neonates to EOI (Table 9). Whittaker et al. reported premature neonates exposed among other excipients to ethanol through iron supplements and furosemide, resulting in chronic exposure to low doses of ethanol (iron long-term therapy) and acute exposure to high doses (management of patent ductus arteriosus).[14] Neonates with chronic lung disease were concominantly exposed to other EOI contained in dexamethasone – benzoic acid, propylene glycol, and sorbitol. All infants receiving dexamethasone exceeded the safe limit of propylene glycol[14] accepted by WHO[41] and EMA.[66]

Shehab et al. reported the median cumulative doses of propylene glycol 180 times the ADI according to the WHO criteria.[134] Amount of propylene glycol administered to neonates during routine care in NICU exceeded the doses above which toxicity has been reported in infants. In the same study critically ill neonates on continuous infusions received a median cumulative dose of benzyl alcohol 21 times the adult ADI of 5 mg/kg/day.[134]

Cordell et al. developed a highly sensitive quantitative method capable of detecting ethanol and its metabolites in micro-volume (≤ 100 microlitre) neonatal plasma samples left over from hospital testing.[151] Further, Pandya et al. reported low blood ethanol concentrations in neonates administered iron and/ or furosemide but markedly elevated blood acetaldehyde, a potentially toxic metabolite of ethanol, concentrations in some preterm infants receiving these medicines.[152]

2.5 Regulatory aspects and initiatives in paediatrics in terms of excipients

"He who studies medicine without books sails an uncharted sea, but he who studies medicine without patients does not go to sea at all." (William Osler; 1849–1919)

Until recently paediatric drug development was not considered an integral part of drug development, some of the reasons being lack of legal requirement to perform paediatric studies and ethical dilemma regarding patient recruitment.[29] Nowadays it is realised that children have the right to properly researched and regulated medicines, and therefore incentives have been proposed by the EU and US regulatory bodies to stimulate paediatric product development.[97,153] The Regulation on medicinal products for paediatric use (Regulation (EC) 1901/2006) creates obligations with regards to conducting clinical trials in paediatric patients including neonates.[154] The EMA "Guideline on the investigation of medicinal products in the term and the preterm neonate" is addressing the impact of immaturity of different organ systems and should be relevant to all investigations of medicinal products that include participation of the neonatal population.[18] Lately, an increase in paediatric studies was notable resulting in new paediatric information in product labeling. In a review of the US FDA databases, Laughon et al. identified 28 drug products studied in neonates and 24 resulted in labeling changes between 1997 and 2010; only for 11 products changes established safety and effectiveness.[155]

In the past, the attention of the pharmaceutical industry and the Regulatory Authorities was directed mainly to controlling the quality of the API rather than that of the excipients; with the approval of drug product came the acceptance of the excipient with their inertness and innocuity taken for granted.[156] Nowadays, according to regulatory requirements from EMA and the US FDA, excipients have to be appropriately evaluated for safety.[39,43,157–159] However, although the US FDA provides guidance for industry on non-clinical studies for the safety evaluation of excipients, and the Joint Expert Committee on Food Additives evaluates excipients taking into consideration the results of long-term toxicological studies with the publication of an ADI, safe levels of excipients in the paediatric and particularly neonatal population are not defined for the majority of excipients.[160,161]

The presence of each excipient in pharmaceutical formulation must be justified both in qualitative (functional) and quantitative terms (optimal amount); an explanation of the choice of the excipient should be provided.[162] Juvenile animal toxicity data should be considered when previous animal and human safety data are judged to be insufficient to support paediatric use. Warning statements relating to the presence of certain excipients in medicinal products have been published by EMA in the guideline "Excipients in the label and package leaflet of medicinal products for human use", intended for use by competent authorities, applicants for a Marketing Authorisation and Marketing Authorisation Holders.[98]

It provides, that excipients known to have a recognised action or effect, e.g. those listed in the Annex to given guideline,[98] need to be declared on the labelling of all products under a separate subheading qualitatively and quantitatively; any adverse reaction specific to excipients should be included.[163] Thus, since 2010 new medicines have to stipulate the quantitative data regarding excipients, listed in the abovementioned Annex, in the summary of product characteristics (SmPC). However, there is no such obligation for the medicines put on the market before 2010. Additionally, since the last revision of the guideline in July 2003, several safety concerns regarding excipients not currently addressed in the guideline, have been identified. For example, the current guideline does not include polysorbate 80 and saccharin sodium. Following the European Commission decision, the objective of the Excipients drafting group (ExcpDG) is to update the labelling of selected excipients listed in the Annex of the guideline mentioned above to encompass pregnant women and children, as well as to add new excipients to the list.[164] The ExcpDG prepares a questions-and-answers document for each excipient under review.[86,100,103,107,165]

IPEC is an international industry association formed in 1991 by manufacturers and users of excipients.[38,166] IPEC's objective is to contribute to the development and harmonization of international excipient standards. According to the IPEC guide "Qualification of Excipients for Pharmaceutical Use" pharmaceutical formulators should consider the proper use of the excipient – the key principle is that of protecting the patient. Although this guide does not address children and neonates as a special population, it states that the intended end use (e.g. neonatal population) of the excipient should be identified and considered in determining appropriate regulatory and Good Manufacturing Practice (GMP) requirements for the excipient;[166] safety, quality and functionality are the main requirements.[167] Recently, IPEC-America submitted suggestion that including information on paediatric use of excipients would improve the quality of data in the US FDA's inactive ingredients database (IID).[168]

The last EU risk assessment guidelines for excipients (2015/C95/02)[169] requires appropriate GMP for all excipients in medicinal products for human use to be ascertained by a formalised risk assessment in accordance with ICH guideline Q9 on Quality Risk Management (ICH Q9).[170]

2.5.1 Including EOI into medicines during manufacturing process (selection of excipients)

"Coming together is a beginning. Keeping together is progress. Working together is success." (Henry Ford; 1863–1947)

EMA guidelines state that marketing authorisation applicants and holders should ensure that inclusion of excipients into the paediatric/ neonatal formulation is justified and the smallest amount is used to achieve the desired function.[18]

The selection and use of excipients in paediatric formulations should take into consideration the following:[32,33,171]

1. The functionality and safety/ toxicity profile across proposed population
   a. Efficiency/ function of excipient in the formulation and potential alternatives
   b. Metabolism and elimination pathways of an excipient
   c. Toxicity/ safety in the specific age group
2. Therapeutic indication, criticality of the condition to be treated and the therapeutic alternatives
3. Dosage regimen i.e. exposure
   a. Acceptable daily intake (acute toxicity)
   b. The frequency of dosing
   c. The duration of treatment (chronic toxicity)
   d. Polypharmacotherapy (additive toxicity)
4. Route of administration

It is not acceptable to include in a medicinal product an ingredient (excipient) that is not genuinely needed, e.g. preservative agents should not be included in a sterile single-dose product.[172] The feasibility of this approach is well proven by the adoption of the AAP recommendation on removal of ethanol from over 700 liquid preparations for children by industry.[59]

As stated by EMA, any risk identified for an excipient would be acceptable only on condition that this excipient cannot be substituted with a safer available alternative or where the overall benefit/ risk balance for the product outweighs the safety concerns.[173] If this would not be possible and replacement may raise other issues, sufficient development data demonstrating that the lowest concentration of excipient is used, should be established.[33,173] For example, a single-dose injection product should not contain benzyl alcohol as a preservative. However, if it is justified with scientific data, the use of benzyl alcohol would be allowed in a role of co-solvent for a poorly soluble API. The use of such product should be contraindicated in neonates and children up to 3 years old because of adverse reaction concerns.[46]

2.6 Avoiding EOI in neonates

As discussed above excipients are necessary components of medicines and thus hardly can be totally avoided. Multiple situations have been seen over past 20 years, where contaminated products have caused deaths and serious injuries,[174] indicating that medicines without some specified excipients may not pose the gold standard. Most of these tragedies were caused by medicines produced by compounding pharmacies,[174] pointing again to the urgent need of age-appropriate dosage forms and strengths of APIs for the paediatric and particularly neonatal population to minimize the manipulations and therefore the risk of contamination and medication errors.

Errors in drug administration is potentially preventable issue that may result in toxic concentration of some excipients.[33] Errors with potential for harm are

three times more likely to occur in paediatric patients and particularly in neonates than in other age groups[175] due to the use of inappropriate formulations requiring complex calculations and measurement of tiny volumes or multiple dilutions.[18] As a result, neonates are at risk of unpredictable dosing and side effects from potentially harmful ingredients, including excipients.[176,177] For example, in 2007 a Dear Healthcare Provider Letter informed healthcare professionals about lethal overdose of Kaletra oral solution in a preterm neonate, given about ten times the calculated volume.[178] Another case was reported by Masi et al., where after receiving a high loading dose of amiodarone, the newborn rapidly developed cardiogenic shock and multiple organ failures.[5] The hypothesis that the two excipients, benzyl alcohol and polysorbate 80, precipitated the occurrence of the ADRs seems plausible, because the plasma concentration of amiodarone and desethylamiodarone never reached toxic level.[5]

One way to overcome the use of some specified excipients may be to find novel excipients with better safety profile. However, for excipient suppliers developing a novel excipient is not very attractive because of the long development times (from polymer class selection to the launch to commercial sales ~ 12 years), high costs (~ $5–10 million) and risk of failure (54% probability of success).[179] Also, pharma companies are often reluctant to use novel excipients.[180] A novel excipient needs to be toxicologically tested similarly to a new API.[166,173] This requires full evaluation – acute toxicity, subchronical, chronical, genotoxicity and reproductive toxicity studies, and ADME – which would be enormously expensive.[46,181] Despite that, it must be realized that safety issues may only become apparent when the product is used on a larger scale. Therefore, the added value of the novel excipient in a specific paediatric medicinal product must be well balanced against the use of other excipients with an established safety profile, other dosage forms or other routes of administration.[32]

The most attractive way to reduce the exposure to problematic excipients may be to use medicines available on the market which do not contain harmful excipients instead of those with given excipients. As stated in the WHO report, the frequency and severity of ADRs varies among countries not only because of factors such as the spectrum of disease, comorbidities, and different genetic composition but also owing to variations in medicine production, pharmaceutical quality, composition (excipients) of locally-produced pharmaceutical products, and differences in medicine use.[112] As was shown by Brion et al., for 75% of products prepared extemporaneously in one country there are suitable licensed alternatives available in other countries.[182]

Two animal studies have compared the “classic” parenteral amiodarone formulation containing polysorbate 80 and benzyl alcohol with the aqueous formulation, free of these excipients. It was concluded that aqueous formulation is a safer alternative to “classic” amiodarone due to the lack of hypotensive and cardiotoxic effects related to the excipients in the standard product.[183,184] According to the study in humans by Gallik et al. aqueous amiodarone possesses PD effects that have been attributed to amiodarone, whereas it lacks the hypotensive effect of the standard intravenous amiodarone formulation.[185]

van Riet-Nales et al. identified in Dutch medicines database 3542 drug products authorized for one or more paediatric age groups. Of all oral liquid products 52% (208 out of 400) contained at least one excipient of concern; for 22% an alternative liquid was available with the same API but not the given excipients.[186] However, this study was looking at paediatric products in general and within one country. From neonatal point of view, it means that the availability of EOI-free products for substitution is probably lower as different products may not be interchangeable when needed for a specific age category.[186] Contrariwise, these results give a hint that product substitution is possible as a matter of principle.

2.7 Summary of literature

Besides the desired API medicines also contain pharmaceutical excipients that are intended to improve quality and patient acceptability of medicines. Most drug products cannot be made without the use of excipients. Although excipients should be pharmacologically inactive, they may indeed cause significant adverse effects or even death, particularly in neonates. Here, the different physiology and metabolism/ elimination capacity of neonates compared to adults and older children as well as into-group variations in different GA groups should be considered.

Some excipients, i.e. parabens, propylene glycol, polysorbate 80, benzoates, benzyl alcohol, ethanol, saccharin sodium, sorbitol, and benzalkonium chloride have received considerable attention due to the possible adverse effects, particularly in neonates. Risks have been emphasized in several publications and recognized by the regulatory agencies. Still, there are very little systematic data about excipient exposure which is a cornerstone for a safety assessment. Only a few studies have looked at excipients exposure in children including neonates, most limited to a single country or small number of NICUs or of variable methodology. Europe-wide picture of excipients use in neonates is still missing. Moreover, there is a lack of information regarding the preferable/ optimal study methodology that should be used in the assessment of excipients exposure. We are not aware of any head to head comparison of different methods and thus the question how to navigate between different methods in excipient exposure research remains unanswered.

Preventing the administration of potentially harmful excipients to neonates is complex. Neonates are routinely given medicines with no specific paediatric information and therefore age-appropriate formulations may, among other things, solve some excipient's issues. Also, not all formulations with similar indications and APIs unconditionally contain the same excipients. The only study looking at product substitution as the opportunity to reduce exposure to EOI among paediatric medicines was performed within one country and found safer alternative products for one-fifth of oral liquid products with potentially harmful excipients.[186] To our best knowledge there are no studies on a European scale looking at substitution possibilities in neonates.

3. AIMS OF THE RESEARCH

The general aim of this thesis was to access the Europe-wide scale of neonatal excipient exposure with the emphasis to EOIs and to explore the opportunity for reduction of exposure to EOI through product substitution.

The research had the following specific objectives:

1. To compare two different study designs in medicine/ excipient exposure assessment to identify the impact of study design on obtained results and enable implementation of optimal study designs in relation to research question in future studies
2. To compose a comprehensive list of medicines/ excipients used in European NICUs to identify the scale of the problem with overall number of different products used in neonates and proportion of products containing EOI
3. To describe individual EOI administration to European neonates and identify factors associated with exposure to EOI to detect the highest risk situations and subpopulations
4. To identify regional and unit-wide variations in neonatal exposure to EOI in European NICUs
5. To determine opportunities for product substitution among those available on the European market in order to reduce the administration of EOI on product and individual level

4. MATERIALS AND METHODS

This thesis is based on the two multicentre observational cross-sectional studies of routine clinical practice conducted in European NICUs as part of a European Study of Neonatal Exposure to Excipients (ESNEE) as presented in Table 10.

Table 10. Description of studies and analyses of the thesis

Study characteristics	Timing	Population/ prescriptions	Primary aim	Publication
Service evaluation survey	30.05.2011 – 30.09.2011	115 NICUs from 20 countries	To describe general exposure to EOI To explore opportunities for product substitution	III
Point prevalence study	01.01.2012 – 30.06.2012	726 newborns from 89 NICUs from 21 countries	To describe administration of EOI and identify risk factors for exposure	II
Comparative methodology study	Analysis of the SES vs. PPS	APIs used in more than one unit in the SES	To compare the effect of two cross-sectional study designs on estimates of medicines/ excipient use	I

API, active pharmaceutical ingredient; EOI, excipients of interest; NICU, neonatal intensive care unit; SES, service evaluation survey; PPS, point prevalence study

4.1 Ethics

All studies were conducted in compliance with the Guidelines of Good Clinical Practice (GCP)[187] and the Declaration of Helsinki.[188] The SES did not collect any personal data and so did not require ethics committee approval. For participation in the PPS, Ethics Committee approval was obtained in compliance with the respective national guidelines (list available as supplementary material to Metsvaht et al.[189]). No consent for individual patients was sought, as all data were collected in routine clinical practice and anonymised before leaving study sites.

4.2 Study design and data collection

We invited to participate all 27 EU countries plus Iceland, Norway, Switzerland, and Serbia. A network of national contact persons (Lead Contacts) was built by the ESNEE consortium.[190] Search for national Lead Contacts was carried out

through national professional/ scientific societies of neonatology, paediatrics and/ or perinatology as well as through personal contacts of the ESNEE consortium members or other FP-7-funded consortia (NeoMero, TINN).[191,192]

Sample size calculation

In the SES study, the Lead Contacts were asked to recruit as many hospitals and units providing neonatal care in the country as possible and no formal sample size calculation was made.

In the PPS, sample size estimation was intended to be based on cluster sampling analysis stratified by country, assuming conservatively a 15% neonatal admission rate of all live births[21,193–195] and a response rate of 50–70% of invited units as described in previous neonatal surveys.[196–199] Based on the Eurostat Nomenclature of territorial units for statistics (NUTS) (or equivalent if NUTS classification is not available for the country) regional distribution of the population and reported nation-wide birth rates, the number of potentially available neonatal admissions was calculated and the representative sample size estimated for each country and region.[200] A complete list of institutions involved in neonatal care was to be created for each randomly chosen NUTS2 region with subsequent involvement of all units to cover a whole region with proportional representation of different unit levels. As a full list of neonatal units was not available and the response rate in the SES did suggest insufficient recruitment, similarly to the SES, all contacts provided by Lead Contacts were invited to participate in the PPS.

Participating units

All general neonatal, intermediate and neonatal intensive care, as well as mixed paediatric and neonatal intensive care units with more than 50% of admissions consisting of neonates, were eligible. Units were stratified according to the level of care as follows: level 1 providing neonatal special care; level 2 providing high dependency care, short-term intensive care and low birth weight (< 2500 g.) care, and level 3 providing comprehensive intensive care including extremely low birth weight (< 1000 g.) infants.[201,202] Units offering different levels of care were classified according to the highest level they provided.

Data collection

In both studies all eligible neonates (≤28 days of postnatal age) in the unit at 8 a.m. on the study day(s) were included. Each participating unit was free to choose the most appropriate day(s) for data collection during a prespecified period – from May 30th to September 30th, 2011 in the SES, and one of three fixed two-week periods from January 1st to June 30th, 2012 in the PPS.

In the SES all medicines prescribed to neonates were recorded on pre-formulated Excel spreadsheets over three consecutive days as detailed in Table 11.

In the PPS data collection was performed in a pre-populated web-based database within one day. Individual prescriptions and demographic data were recorded for all neonates receiving any prescriptions active on the study morning at 8 a.m as detailed in Table 11.

In both studies, printable data collection forms were available as an alternative to electronic data insertion.

Table 11. Data collected during the SES and PPS

Hospital form (only PPS)	Hospital name, population coverage, sub-type of hospital[a], name/ email/ post address of the ESNEE administrator/ contact person, names of neonatal units, names/ emails of local investigators
Department form (SES and PPS)	Date of survey, name of department, type of department, annual neonatal admissions/ admissions <32 weeks of GA, number of neonates during survey, number of patients receiving any drug
Neonatal demographic data (only PPS)	Patient identifier, date of birth, gender, APGAR score, GA, birth/ current weight, organ failures
Drug form (SES and PPS)	Trade name, manufacturer, active pharmaceutical ingredient, strength, strength unit, dosage form, route of administration
Prescription form (only PPS)	Single dose/ times a day, start of treatment

[a]teaching or non-teaching hospital
GA, gestational age; SES, service evaluation survey; PPS, point prevalence study

All prescriptions, including micronutrients, iron, vitamins, parenteral nutrition solutions, and topical agents were recorded. Blood products, glucose and electrolyte solutions, vaccines, nursery care topical agents, herbal medicines, and food including breast milk fortifiers were excluded. Extemporaneous/ compounded forms of medicines were not included in described analysis.

In both studies, the data were pooled for analysis without any comment on treatment strategies of individual participating units.

4.3 Data management

SES data collection forms were checked for legibility with two rounds of queries regarding missing data. Each drug product was classified according to trade name (i.e. brand name), manufacturer, pharmaceutical dosage form, and strength of API. The list of medicines obtained in the SES was used to prepopulate choices in the PPS database. To minimize the risk of data entry errors all "newly appearing" drug products in the PPS were added to the database by the ESNEE team.

4.4 Identification of excipients

Excipient content for each medicine was identified from the national SmPC which were first accessed through national medicines agencies and then through medicines agencies of other European countries if the drug product was not registered in the specified country. If needed, further searches from the homepages of manufacturers as well as from credible public databases (e.g. www.diagnosia.com), were performed. Finally, local hospital contacts were asked to provide information regarding excipient composition for medicines with no SmPC available.

Due to the significant amount of synonyms used in SmPCs the list of identified excipients was reviewed for possible overlaps using non-proprietary-, trade-, and chemical names, and Chemical Abstracts Service (CAS) registry numbers. Excipients having different names but a same chemical base were consolidated, e.g. dibasic sodium phosphate includes anhydrous, dihydrate and dodecahydrate forms.

For more detailed analyses only EOI we included as detailed in Table 4 and Table 5. An excipient was considered as EOI if it was known to be harmful in neonates. The priority list of EOI for investigation (Table 5), was agreed upon using surveys conducted by the UK and Estonian partners.[15,190] Polysorbate 80 was included in the priority list on the advice of EMA.[190]

Finally, EOI were categorised in a non-exhaustive way into three groups based on main function: antimicrobial preservatives, i.e. parabens (propyl-, ethyl-, and methylparaben), benzoates (benzyl alcohol, benzoic acid, and sodium benzoate), and benzalkonium chloride; solvents and solubilising agents, i.e. propylene glycol, polysorbate 80, and ethanol; and sweetening agents, i.e. sorbitol and saccharin sodium. Although benzyl alcohol does not belong to benzoates chemically, in this study, it was included into benzoates group as a precursor of benzoic acid in humans.

4.5 Data analysis

Descriptive statistics were used as appropriate to describe the main features of collected data:

- Characterisation of participating units
  - Number of units from each country
  - Proportion of annual live births and neonatal admissions covered by study units in participating countries
  - Prevalence of different levels of care and teaching hospitals
- Consumption of medicines
  - Number of different formulations according to API, manufacturer, dosage form, route of administration, and strength of API
  - Comparison of estimated frequency of medicine use between the SES and PPS

- Demographic characteristics and prescription data of patients
  - Number/ proportion of neonates with at least one drug prescription
  - Number of prescriptions per neonate in relation to the GA in the PPS
- The general extent of EOI administration
  - Number/ proportion of products/ prescriptions containing at least one or more EOI in relation to the route of administration and geographical region
  - Number/ proportion of neonates exposed to EOI

Characteristics of a variable's distribution are presented in tabular format and histograms; measures of central tendency (the mean, median) and variability (SD, IQR) were used where appropriate. To compare variables between each other and between the two study contingency tables were used to find odds ratios and confidence intervals.

Logistic regression analyses were performed to find the effect of study design in medicine use assessment and identify factors associated with the administration of EOI. Analyses were performed with Stata Software (ver. 12.1).

Product substitution analysis was performed with Microsoft® Excel 2013 (ver. 15.0). Simple logistic regression was used to determine the reduction in the odds of neonatal exposure to EOI through substitution.[203]

4.5.1 Exposure to EOI: extent and risk factors

The PPS data were used to estimate the influence of covariates on the administration of each EOI.

First, **univariate logistic regression analysis** was used to study the effect of potential covariates on the administration of each EOI (Table 13).

Further, a three-step **multivariable logistic regression analysis** was applied. All covariates with $p < 0.05$ from the univariate analysis were fitted into mixed effects models (Table 13). Model 1 aimed to identify variables associated with EOI administration to individual neonates. East region and extremely preterm neonates were used as the reference. Model 2 aimed to describe the associations between the above-mentioned covariates and the presence of a particular excipient in each prescription. In addition to covariates explored in model 1, API was included to adjust for variations in EOI administration by API. APIs were classified according to the 1st level of Anatomical Therapeutic Chemical (ATC) classification; the 3rd level grouping was used for antibiotics. ATC group with the largest number of prescriptions with respective excipient was used as a reference. Finally, the route of administration was added into model 2 (Model 3) to inform on formulation-related variance and to estimate the potential for product substitution. Topical prescriptions were excluded from the latter analysis due to small numbers of prescriptions.

Table 13. Statistical analyses in risk factor analysis

Model	Tested variables	Outcome variable
Univariate logistic regression analysis	Geographical region[a] GA category[b] Hospital teaching status Level of care[c]	Administration of a specified excipient to a neonate, yes/no
Three-step multivariable logistical regression analysis		
Model 1	Geographical region[a] GA category[b] Level of care[c]	Administration of a specified excipient to a neonate, yes/no
Model 2	API in addition to Model 1 variables	Presence of a specified excipient in a prescription, yes/no
Model 3	RofA in addition to Model 2	

API, active pharmaceutical ingredient; GA, gestational age; RofA, route of administration
[a]Northern, Southern, Western, and Eastern Europe according to the United Nations Statistics Department)[204]
[b]<28 weeks, called extremely preterm; 28 to <32 weeks very preterm; 32 to <37 weeks late preterm, and >37 weeks term neonates[205]
[c]Level 1 with neonatal special care; level 2 with high dependency care, short-term intensive care, and low birth weight care, and level 3 with comprehensive intensive care for extremely low birth weight infants available[201,202]

The likelihood ratio statistic for testing the null hypothesis of zero between-group variance confirmed the need for the addition of department to the models as a random effect to adjust for between-department variance.

4.5.2 Product substitution

The substitution was defined as a replacement possibility of an EOI-containing product with an EOI-free counterpart; a list of products created in the SES and complemented with products reported only in the PPS was used. Only APIs used in ≥10% of units in the SES with at least one product containing EOI were included (paper III, Figure 1). Products considered as an alternative for substitution had to be free of any EOIs.

Substitution analysis did not include topical medicines due to the limited number of products. Also, multivitamins were excluded as defining adequate substitution possibilities proved unfeasible due to high variability in the composition of the multiple products used.

Opportunities for product substitution were evaluated in three stages (Figure 2).

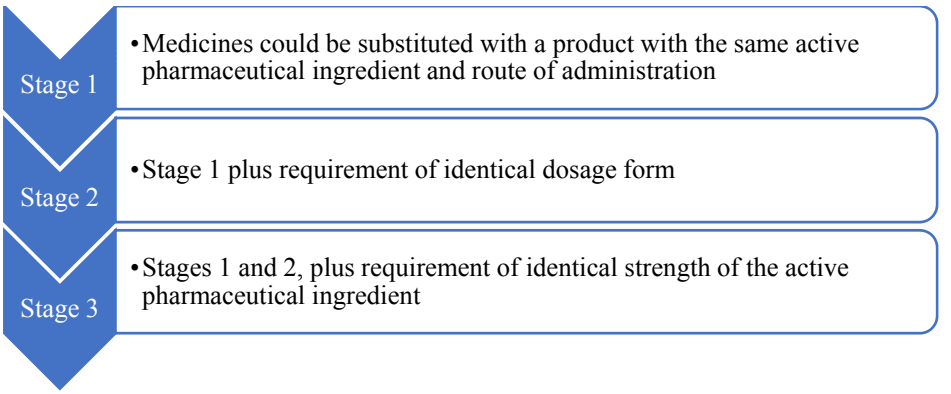


Figure 2. Criteria for substitution according to the stages of analysis

The first stage was based on similarity of API and route of administration. In the second stage, dosage form was included to provide differentiation between liquid and solid forms of enteral medicines: "solution for injection", "powder for reconstitution", and "solution for injection/ oral" were all considered equivalent forms for parenteral administration; "oral solution", "oral drops", "oral suspension", "granules for reconstitution", "powder for reconstitution", "syrup", "solution for injection/ oral", and "emulsion" were considered equivalent forms for enteral administration. The third stage also included the strength of API to identify the extent of an "ideal" substitution available. Here we assumed that the strength of API in substituted product was optimal for the NICU where it had been used.

Subsequently, PPS data were used to explore potential reduction of individual exposure to EOI when all product substitution possibilities based on the second stage criteria were applied.

4.5.3 Effect of study methodology in medicine use assessment

Primary endpoints of the comparative methodology analysis were to find the average probabilities of the units to use any given API in the SES versus PPS and thereby to identify the effect size of the study method.

Secondary endpoints were to describe the implementation of each method and to explore the correlation between medicines' exposure at unit and individual levels.

Exploratory data analysis with ten most often used APIs was done to find potential confounders for further analysis. Population averaged Poisson regression model with robust standard errors[206] was used (Table 14). The analysis included all units in both studies and was adjusted for department level, the status of teaching or non-teaching hospital, and European region; the p-values were corrected for multiple testing using Holm correction[207].

Table 14. Statistical analyses in comparative study

Model	Included APIs	Tested variables
Population averaged Poisson regression model with robust standard errors[206]	10 most often used APIs	Hospital teaching status Geographical region[a] Level of care[b]
Multilevel mixed effects logistic regression models with crossed random effects[208]	APIs used in more than one unit in the SES	Geographical region[a] Level of care[b]
		Unit size[c] Frequency of use[d] Duration of the prescription[e]
Polynomial regression model with square root transformation		None

API, active pharmaceutical ingredient; SES, service evaluation survey
[a]Northern, Southern, Western, and Eastern Europe according to the United Nations Statistics department[204]
[b]Level 1 with neonatal special care; level 2 with high dependency care, short-term intensive care and low birth weight care, and level 3 with comprehensive intensive care for extremely low birth weight infants available[201,202]
[c]Described by number of annual admissions
[d]Described by proportion of units using given API
[e]Described by the average time from the start of prescription to the PPS study day

Considering the data structure with repeated observations on both department and API level, to eliminate possible deficiencies in statistical power multilevel mixed effects logistic regression models with crossed random effects[208] were used (Table 14) to identify the effect size of the study method. Since the sample of departments in the two studies was partly overlapping, department and API were added to the model as a random effect when comparing the average odds of using an API between the two study methods. The outcome variable was a binary indicator showing whether an API was used in a specific unit in a specific study. All models were adjusted for potential confounders identified in an initial analysis.

As the assessment is likely to be affected by a number of additional parameters related to the use of a given drug, unit size, frequency of the use, and duration of the prescription were added into the analysis as covariates.

As nonlinear relationship between the number of units using each API and the number of prescriptions for that API was suggested, a polynomial regression model with square root transformation was used to study given relationship. The analysis was based on the PPS data.

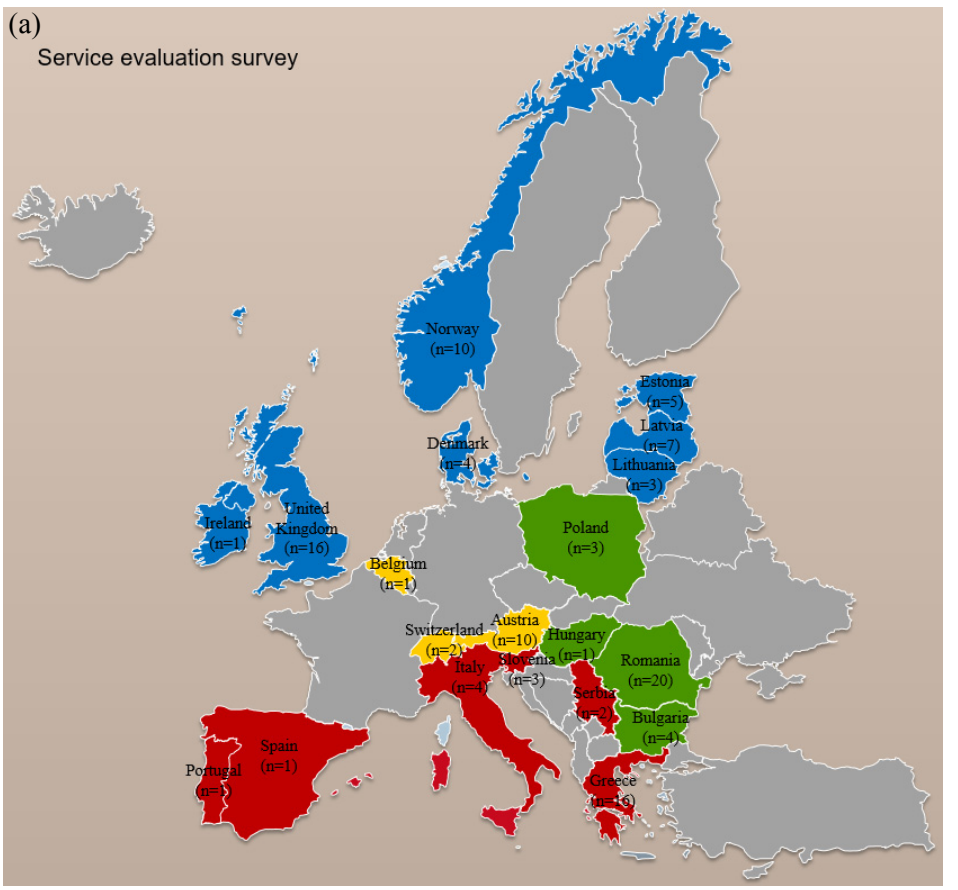
The use of caffeine and morphine was reported differently in the two studies. UK units using "special" manufactured[209] preparations, rather than extemporaneous formulations used in the rest of European countries, were over-represented in the PPS. Accordingly, these two APIs were excluded from the analysis.

5. RESULTS AND DISCUSSION

"Supposing is good, but finding out is better." (Mark Twain; 1835–1910)

5.1 Participating units in the SES and PPS

Altogether 20 and 21 countries (Figure 3) with 115 and 89 neonatal units joined the SES and PPS, respectively. The number of units per country varied from one to 20 (Figure 3). Data on drug use from Sweden were available only with respect to the whole country from a 4-day national study of drug consumption in 38 NICUs conducted previously in 2008; data by units were not available and thus could not be used in analysis of drug use frequency and variations, but drug list from this study was used in product substitution analysis.



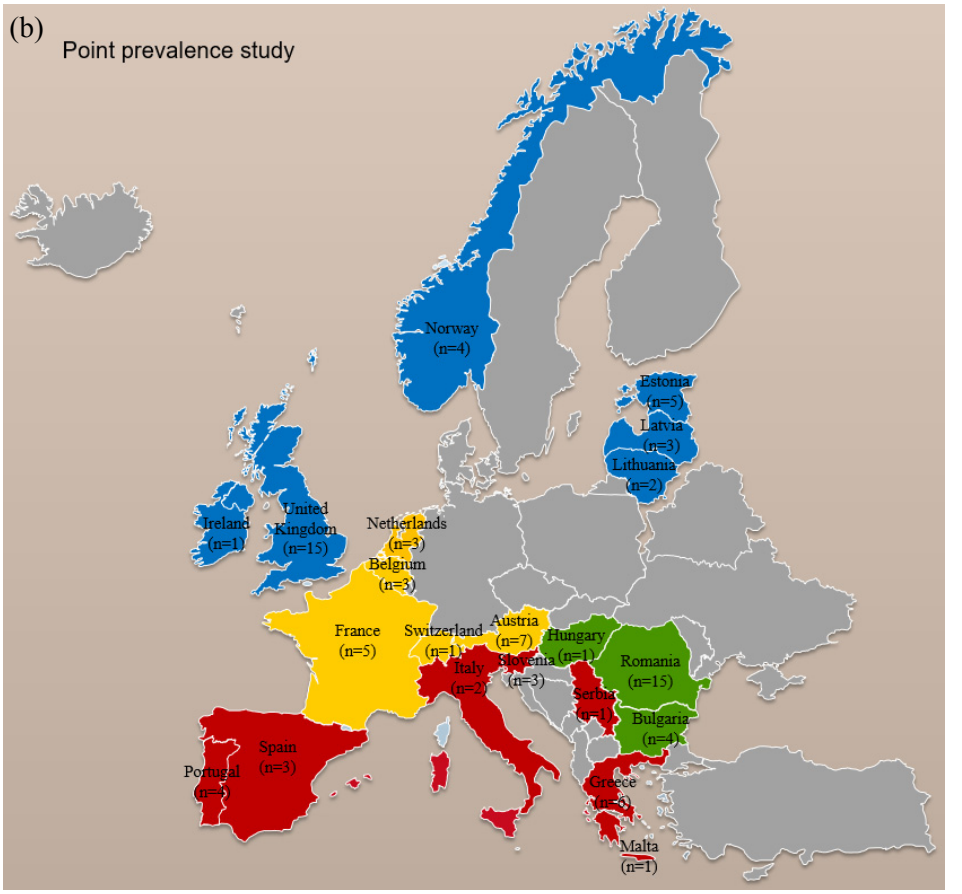


Figure 3. Number of units participating in the SES (a) and PPS (b) by country. Blue color indicates Northern, yellow Western, green Eastern, and red Southern European region according to the United Nations Statistics Department.[204] Number of neonatal units participating from each country is shown in parentheses.

In the SES a higher proportion of annual live births was covered compared to the PPS – 11% vs. 6% of 5,572,859 live births in invited countries in 2010, respectively; OR 1.89; 95% CI 1.87–1.89. Similarly, the proportion of annual admissions in participating units covered by the study was higher in the SES than the PPS (3% and 2% of 90,235 and 61,392 admissions, respectively; OR 1.58; 95% CI 1.48–1.68). Regional distribution was similar in both studies (paper I, Table 1). No difference in the prevalence of different levels of care between the two studies was observed. The lower prevalence of teaching hospitals in the SES as compared to the PPS (OR 0.47; 95% CI 0.24–0.92) (paper I, Table 1) makes the speculative assumption that physicians from bigger hospitals are more keen to be involved in more detailed and individualised approach. Prepopulation of a major part of the PPS database with no doubt makes it more attractive for researchers as well.

We have recruited so far one of the largest international cohorts of neonatal units from 21 European countries. Of note, the study did not involve some large European countries such as Germany. Altogether, 71% of invited countries participated. The number of units (by country coverage of live births) varied from 0.8% to 100%.

Due to the aim of attracting as many units as possible, hospitals were not randomly selected, that probably caused overrepresentation of tertiary or teaching hospitals (61% in the SES, 77% in the PPS compared to less than 10% according to available national hospital statistics[210]). Nevertheless, data on the distribution of the levels of neonatal care allowed appropriate adjustments and both the SES and PPS have achieved representative sample size with a margin of error being 1.73% and 2.63%, respectively (CI 95%; response distribution 50%). However, it would be valid under the condition of randomisation that remained unfeasible in our studies due to the lack of comprehensive list of neonatal units as well as relatively modest response rate. Still, current retrospective calculation of study power gives a notion of good coverage of the studied population in the SES and PPS.

5.2 Medicines consumption in European NICUs

In the SES 313 APIs representing 1065 products from 332 manufacturers were registered. Parenteral formulations predominated with 616 (58%) products, followed by enteral and topical formulations with 325 (31%) and 124 (12%) products, respectively (Table 15).

In the PPS 1382 neonates received 2608 prescriptions for 624 products containing 280 APIs (Table 15). Excluding neonates > 28 days of age, without any drug prescription during the study day, and those receiving only prescriptions with unavailable excipient content data, the further analysis included 726 neonates, 2199 prescriptions, 562 products, and 246 APIs. Demographic characteristics of patients and prescription data are shown in paper II, Table 2. Slightly lower prevalence of enteral and higher prevalence of topical formulations was noted in the SES compared to the PPS with no difference in parenteral medicines use (paper I, Table 2).

Table 15. Drug use variability

	SES	**PPS**
No of prescriptions during the study period	NA	2608
Median (IQR) No of prescriptions per neonate	NA	2 (1;4)
No of APIs prescribed*	313	280
No of trade names (by name, manufacturer, pharmaceutical dosage form, and strength)	1065	624
No of manufacturers	332	235

*all components in multicomponent drugs are counted separately
API, active pharmaceutical ingredient; IQR, interquartile range; NA, not applicable; PPS, point prevalence study; SES, service evaluation survey

Thus, the longer study period (period prevalence in the SES vs. point prevalence in the PPS) and larger number of participating units in the SES expectedly resulted in a more comprehensive list of APIs as well as drug products.

In the PPS the number of prescriptions per neonate was inversely related to GA (Spearman's rank correlation coefficient –0.3949; $p<0.0005$), the average (SD) count per neonate being 4.53 (2.33); 3.95 (2.59); 2.68 (1.91), and 2.31 (1.93) in the extremely preterm, very preterm, late preterm, and term neonates, respectively. Higher drug utilization rates in preterm neonates as compared to term neonates have been described in previous studies. [24,131] That in combination with organ immaturity is putting preterm neonates at a higher risk of ADRs, including those related to excipients. For example, potentially toxic cumulative doses of benzyl alcohol and propylene glycol received by neonates during routine treatment were reported by Shehab et al.,[134] where neonates received up to four medicines containing benzyl alcohol (midazolam, phenobarbital, pancuronium, dexamethasone) and up to three medicines with propylene glycol (lorazepam, phenobarbital, digoxin).

The list of ten most often used medicines included nine APIs presented in both studies and also furosemide in the SES and caffeine in the PPS (Table 16). Not surprisingly, this list contains four antibacterials for systemic use. Antibacterials were found to be the most commonly prescribed medicines in NICUs almost in all studies.[20,24,131,136]

Table 16. Frequency of use and variety of drug products for ten most frequently prescribed APIs

ATC class (2nd level)	Active substance	% of units using		No of products	
		SES	PPS	SES	PPS
Antibacterials for systemic use (J01)	gentamicin	74.1	51.7	26	21
	ampicillin	60.2	40.5	20	22
	vancomycin	40.7	28.1	21	20
	benzylpenicillin	32.4	26.9	7	11
Antihemorrhagics (B02)	phytomenadione	73.2	31.5	11	11
Antianemic preparations (B03)	iron	57.4	37.1	27	18
Blood substitutes and perfusion solutions (B05)	amino acids	56.5	30.3	11	5
	lipids	44.4	25.8	7	6
Diuretics (C03)	furosemide	39.8	14.6	20	9
Vitamins (A11)	colecalciferol	37.9	41.6	16	22
Psychoanaleptics (N06)	caffeine	24.1	34.8	17	19

API, active pharmaceutical ingredient; ATC, anatomical therapeutical class; PPS, point prevalence study; SES, service evaluation survey

Among these commonly prescribed APIs EOI were previously reported in gentamicin, caffeine, iron, phytomenadione, and furosemide.[14–16,135,211]

Great variation in products was observed for frequently used APIs in both studies (Table 16, Figure 4).

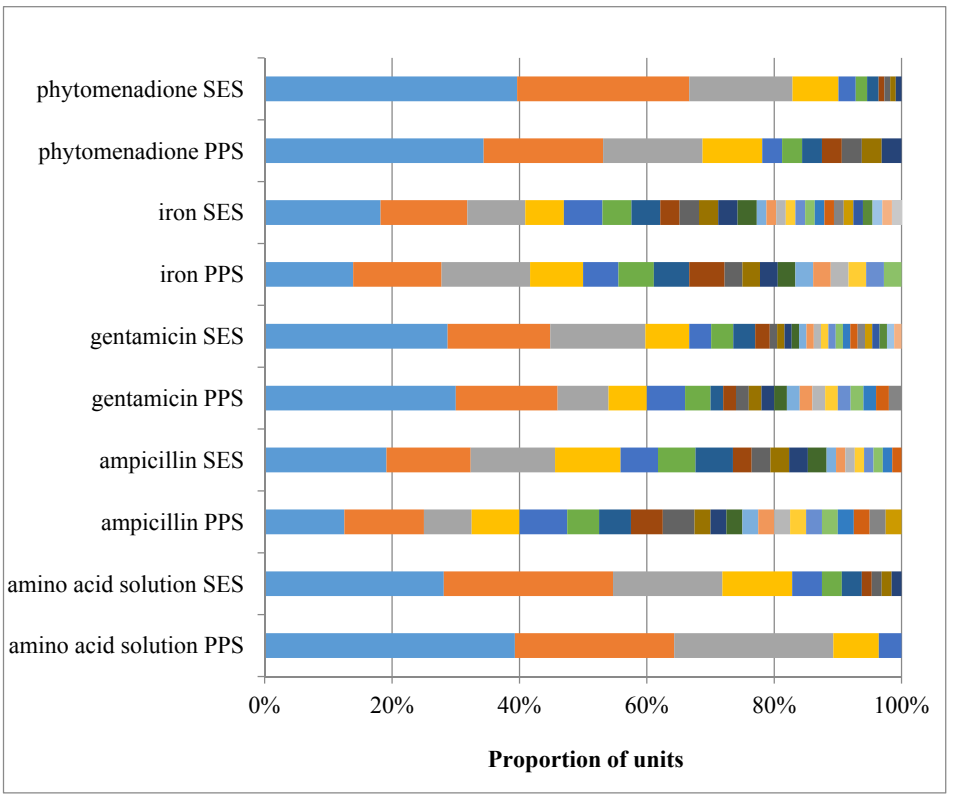


Figure 4. Variation of drug products for five most commonly used active ingredients (API) with proportion of units using each specified product*

PPS, point prevalence study; SES, service evaluation survey
*Each color indicates a single product. Similar color does not indicate identical products neither in the SES and PPS nor for different APIs

Three most frequently used formulations usually covered over 50% of units in both studies (mean%; SD: 64; 18 and 60; 20 in the SES and PPS, respectively). This means that in the case of the presence of EOI in these formulations substitution of a relatively small number of products may noticeably decrease neonatal exposure to these excipients.

5.3 Excipients in prescribed medicines – the scope of exposure

5.3.1 Describing the content of excipients in medicines

SmPCs were attainable for 927 (87%) of the 1065 reported medicines; for 77 (7%) products excipient data were obtained from queries. Thus, information on excipient composition was available for 1004 (94% of 1065) products. Usually, only qualitative data were presented, with quantitative excipient data available from public sources for only 6% of medicines. After unification of synonyms according to the European Pharmacopeia and integration of excipients with identical chemical base into one, overall 396 different excipients were identified. Among products without any excipients in the powder form prior administration (n=117) almost all were for parenteral use (n=111) with only six enteral formulations (Figure 5).

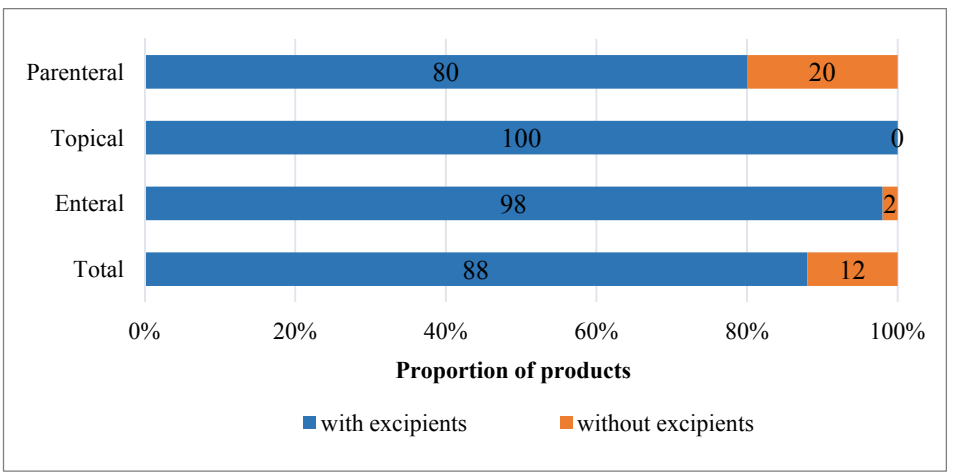


Figure 5. Proportion of products containing at least one excipient by route of administration in the SES

Of 2199 prescriptions and 562 products administered to 726 neonates in the PPS, excipient composition was available for 530 (94%) products with 2095 (95%) prescriptions.

5.3.2 The presence of EOI in medicines prescribed to neonates

Altogether 305 (30%) products contained at least one and 132 (13%) two and more EOIs (Figure 6). The median number of EOI per formulation was one (IQR 1; 2) with a maximum number of six (excipients from one group were counted once, e.g. methyl- and propylparabens). A higher proportion of products with EOI was observed in Northern NICUs compared with Western units (OR 1.83; 95% CI 1.24–2.68) with no differences between other European regions.

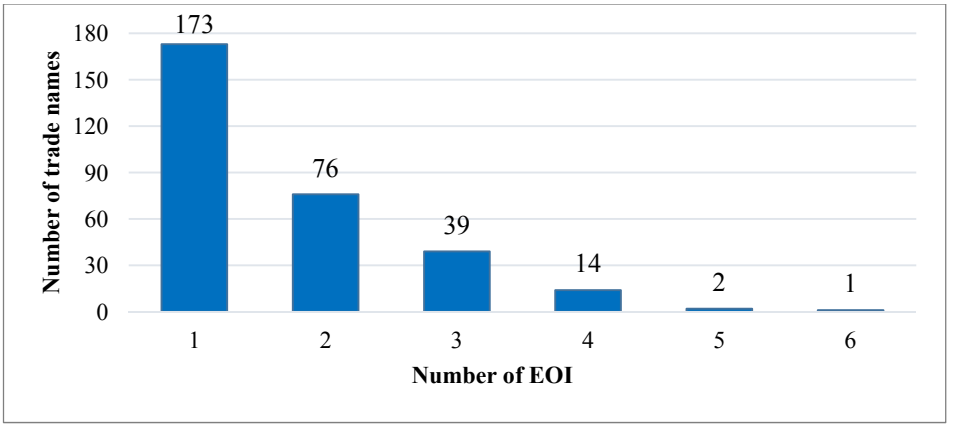


Figure 6. Number of products with one or more EOI in the SES

EOI, excipient of interest; SES, service evaluation survey

At least one EOI was found more frequently in enteral and topical compared to parenteral medicines (OR 8.4; 95% CI 6.1–11.7 and 6.2; 4.02–9.5, respectively) but no difference was found between enteral and topical formulations (OR 1.4; 95% CI 0.9–2.1) (Figure 7).

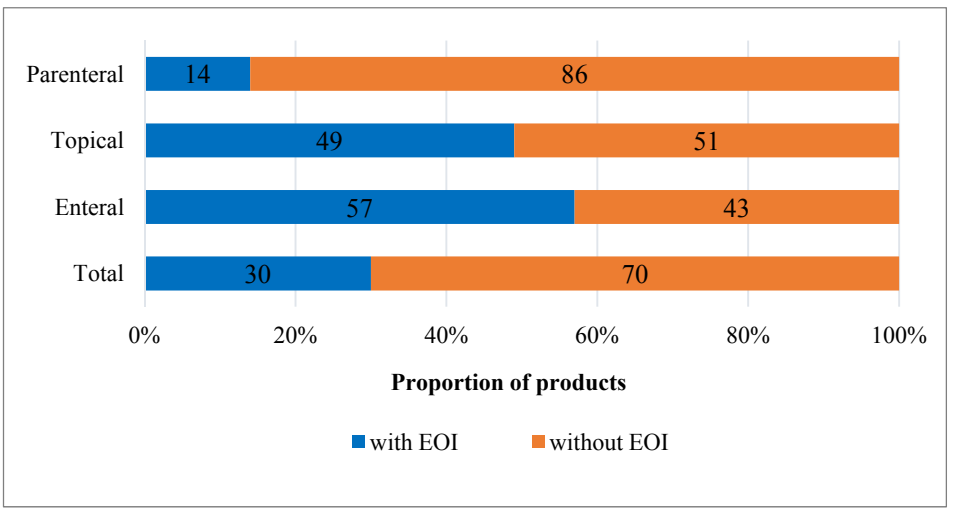


Figure 7. Proportion of products containing at least one EOI by route of administration in the SES

EOI, excipient of interest; SES, service evaluation survey

Similar tendency was found for every single EOI with the exception for benzalkonium chloride present exclusively in topical formulations (Figure 8).

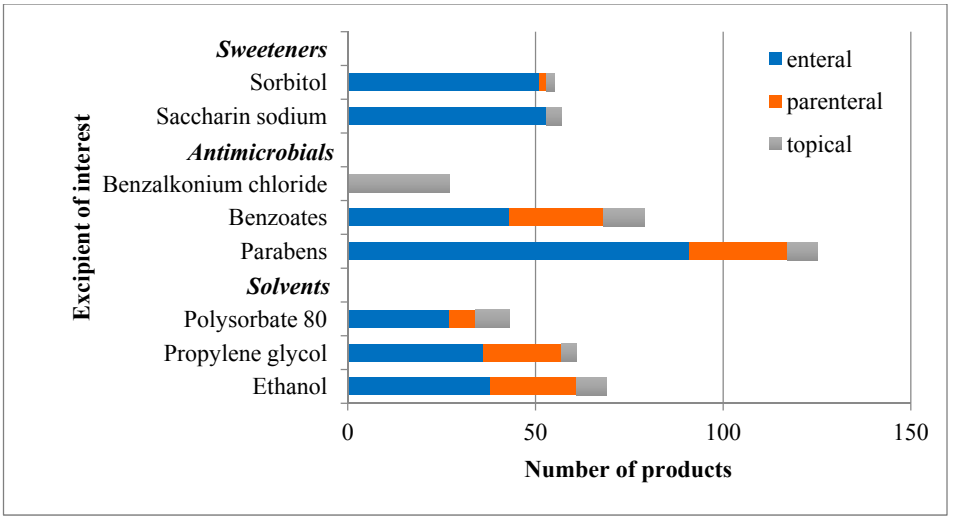


Figure 8. EOI in prescribed medicines by route of administration in the SES

Benzoates include benzyl alcohol, sodium benzoate, and benzoic acid; parabens include propyl- and methylparabens; EOI, excipient of interest; SES, service evaluation survey

The presence of sweeteners in almost all enteral formulations is logical; taste-masking is needed to increase compliance with therapy among children.[149] As the most common type of paediatric products are oral liquid solubilized multiple-use formulations,[149] the dose and volume may be limited by the solubility of API requiring the addition of cosolvent and surfactant excipients; additionally, physical, chemical, and microbiological stability must be assured with buffering agents, antioxidants, and antimicrobial preservatives.[212] The use of potentially harmful solvents can be avoided e.g. if an API is water-soluble and chemically stable in an aqueous formulation.[149] Additionally, solvent e.g. propylene glycol may be included in formulation to dissolve preservatives.[213]

Regarding the presence of EOI according to the route of administration our data are partly similar to the data from the Netherlands, where 52% of oral liquid formulations and 7% of all parenteral medicines in the country authorized for one or more paediatric age groups contained EOI.[186] The lower proportion of products with EOI compared to our study is likely explained by the methodological differences; polysorbate 80, saccharin sodium, and sorbitol were not included in the EOI list. In contrast, in Estonian[15] and Spanish[150] studies, looking at medicines prescribed to neonates within a single country and a single hospital, 62% and 32% of parenteral, and 100% and 62% of enteral products contained EOI, respectively. However, in our and the Dutch studies only "known to be harmful" excipients were taken into the analysis, while in Estonian and Spanish studies a very conservative approach was taken and the excipients were classified into the "potentially harmful" category even if only some data on human toxicity had been published. Interestingly, Garcia-Palop et

al. did not include polysorbate 80 known for the "E-Ferol syndrome" in the list of harmful excipients.[150]

5.3.3 Individual exposure to EOI

The presence of EOI in products by route of administration in the PPS was similar to that observed in the SES – enteral and topical formulations contained EOI more frequently than parenteral (OR 6.3; 95% CI 4.1–9.7 and OR 10; 95% CI 4.4–22.9, respectively). Out of 638 prescriptions with EOI, 328 (51%), 276 (43%) and 34 (5%) were administered enterally, parenterally, and topically, respectively. In 452 (22% of all and 71% of those with EOI) prescriptions and 89 (17% and 63%) products, more than one EOI was involved. Distribution of EOI by product, prescription, and neonate is shown in Table 17.

Table 17. Count and proportion of products and prescriptions containing each EOI and number of neonates exposed

EOI	No. (%) of products N = (530)[a]	No. (%) of prescriptions (N = 2095)[a]	No. (%) of neonates (N = 726)
Solvents and solubilising agents			
Polysorbate 80	18 (3)	139 (7)	138 (19)
Propylene glycol	26 (5)	129 (6)	120 (17)
Ethanol	20 (4)	47 (2)	47 (7)
Antimicrobials			
Parabens[b]	71 (12)	397 (19)	313 (43)
Benzoates[c]	27 (5)	93 (4)	82 (11)
Benzalkonium chloride	10 (2)	27 (1)	26 (4)
Sweeteners			
Saccharin sodium	31 (6)	104 (5)	90 (12)
Sorbitol	24 (5)	64 (3)	57 (8)
At least one EOI	**142 (27)**	**638 (31)**	**456 (63)**

[a]number of products and prescriptions with available excipient content data
[b]parabens include propyl-, ethyl-, and methylparabens;
[c]benzoates include benzyl alcohol, benzoic acid, and sodium benzoate
EOI, excipient of interest

More than a half of neonates were exposed to at least one EOI. Furthermore, in some cases simultaneous administration of up to nine EOI from six different medicines was observed; 9% (n = 39) received five and more EOI during study day. Our results suggest poor compliance with existing administrative requirements/ recommendations. While European Commission guidelines pursuant to Article 65 of Directive 2001/83/EC contain warning statements relating to the

presence of certain excipients in medicinal products (e.g., parabens, topical propylene glycol, sorbitol, benzalkonium chloride, benzyl alcohol, and benzoates)[98] and the EMA Reflection paper has cautioned about the use of benzoates in neonates,[10] in this study parabens, propylene glycol, and benzoates (including benzyl alcohol) were administered to 43%, 17%, and 11% of neonates, respectively (Table 17). Parabens were the most and benzalkonium chloride least frequently administered EOI. The largest proportion of parabens use was associated with parenteral gentamicin for which also paraben-free formulations were available. Similar results were shown by Lass et al. – paraben-containing parenteral gentamicin was given to 57% of treated neonates being the main source of this excipient.[214]

It is noteworthy, that in the context of such extensive exposure of neonates to EOI, "only" 27% of products contained given excipients. This suggests that the major part of exposure to EOI are from most commonly used products, meaning that significant reduction in administration of EOI to neonates may be achieved through substitution or reformulation of a relatively small number of products.

5.4 Covariates associated with EOI administration

In univariate logistic regression analysis by EOI route of administration, GA category, geographical region, and department level were identified as significant covariates of administration (data not shown). Variations of exposure in relation to GA and geographical region on the example of parabens and ethanol are shown in Figure 9. Compared to East, parabens were administered more frequently in all other regions, and ethanol in North and West Europe ($p < 0.05$). Term neonates were exposed more frequently to parabens, and term and late preterm newborns to ethanol compared to extremely preterms ($p < 0.05$).

The effects of significant covariates from univariate analysis on EOI administration in multivariate logistic regression analysis are shown in Table 18 and Table 19.

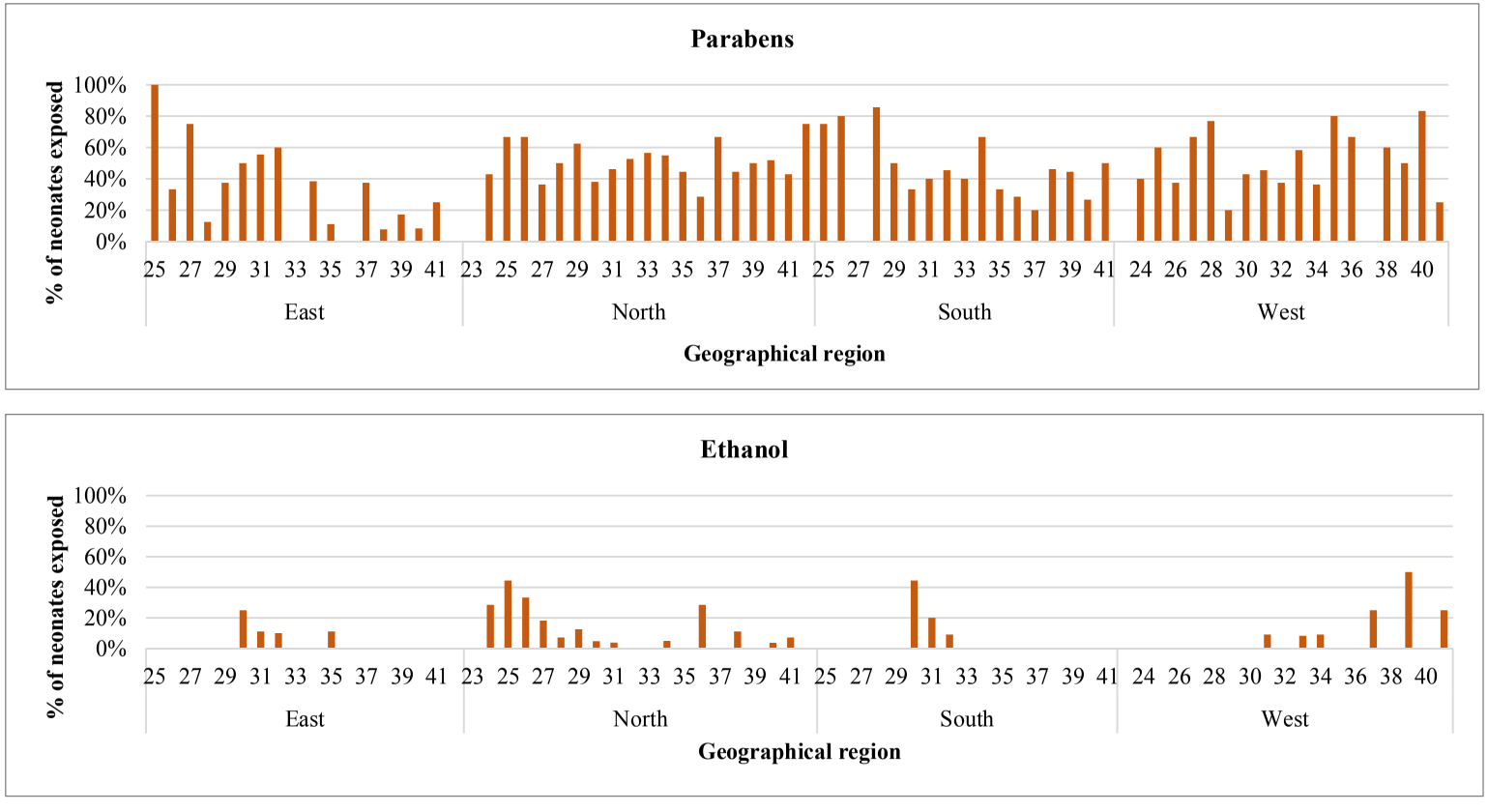


Figure 9. Administration of EOI by gestational age and region

Numbers on the x-axes indicates gestational age in weeks

Table 18. Influence of covariates on EOI administration by neonates in multivariate logistic regression analysis (model 1)[a]

Covariate	Solvents and solubilising agents			Antimicrobials		Sweeteners
	Polysorbate 80	Propylene glycol	Ethanol	Parabens[c]	Benzoates[d]	Saccharin sodium
Region						
East	1	1	1	1	1	1
North	0.9; 0.2–3.2	**0.04; 0.01–0.2**	0.7; 0.1–3.4	**3.2; 1.1–8.9**	0.7; 0.1–5.6	**31.2; 2.5–396.6**
West	0.4; 0.1–1.8	2.1; 0.6–7.0	0.6; 0.1–4.1	3.2; 0.98–10.7	2.0; 0.2–19.9	**153.8; 10.8–2201.4**
South	**0.1; 0.1–0.5**	0.4; 0.1–1.2	0.5; 0.1–3.1	2.3; 0.8–7.2	1.2; 0.1–11.4	**48.8; 3.6–657.2**
Gestational age[b]						
Extremely preterm	1	1	1	1	1	1
Very preterm	2.0; 0.8–5.0	1.0; 0.3–3.1	0.6; 0.2–1.7	1.5; 0.8–2.7	1.4; 0.5–4.4	**3.3; 1.3–8.4**
Late preterm	2.2; 0.9–5.5	0.9; 0.3–2.6	**0.3; 0.1–0.9**	0.95; 0.5–1.7	0.3; 0.1–1.01	1.3; 0.5–3.5
Term	1.3; 0.5–3.1	1.2; 0.4–3.4	**0.3; 0.1–0.7**	**0.5; 0.3–0.9**	**0.3; 0.1–0.8**	0.4; 0.1–1.2
Department level						
1	1	1	0	1	1	1
2	3.2; 0.2–50.2	0.8; 0.1–6.3	1	0.96; 0.1–8.0	0.7; 0.01—52.2	0.2; 0.01–4.6
3	2.1; 0.2–28.8	0.4; 0.1–2.5	5.3; 0.7–39.8	1.7; 0.2–12.4	1.5; 0.03–76.5	0.2; 0.01–3.1

[a]adjusted for geographical region, gestational age, and department level and presented as Odds Ratio (OR) and 95% Confidence Interval (CI)
[b]extremely preterm, very preterm, late preterm, and term denotes gestational age bands of <28, 28 to <32, 32 to <37, and >37 weeks, respectively
[c]parabens include propyl-, ethyl-, and methylparabens
[d]benzoates include benzyl alcohol, benzoic acid, and sodium benzoate
Header row represents excipients of interest (EOI); first column represents studied variables; one (1) indicates reference group; the administration of benzalkonium chloride and sorbitol was not associated with studied variables and not included in the table; significant ORs are in bold

Table 19. Influence of covariates on EOI administration by prescriptions in multivariate logistic regression analysis (model 3)[a]

Covariate	Solvents and solubilizing agent		Antimicrobials		Sweeteners	
	Polysorbate 80	Propylene glycol	Parabens[c]	Benzoates[d]	Saccharin sodium	Sorbitol
Region						
East	1	1	1	1	1	1
North	1.03; 0.1–8.6	**0.02; 0.004–0.1**	**2.97; 1.2–7.1**	1.3; 0.3–6.1	**32.7; 2.5–426.5**	2.3; 0.3–18.2
West	0.1; 0.01–1.2	0.6; 0.2–1.7	2.2; 0.8–5.9	1.3; 0.3–7.1	**60.3; 4.2–861.6**	0.2; 0.01-2.9
South	**0.03; 0.002–0.6**	0.6; 0.2–1.8	**4.1; 1.6–10.6**	1.7; 0.3–9.2	**101.3; 7.3–1403.6**	5.0; 0.6–44.8
Gestational age[b]						
Extremely preterm	1	1	1	1	1	1
Very preterm	1.1; 0.4–3.02	0.9; 0.3–2.4	1.1; 0.7–1.8	1.2; 0.5–3.1	2.04; 0.7–5.6	2.3; 0.6–9.0
Late preterm	**3.02; 1.1–8.5**	1.8; 0.7–4.7	1.04; 0.6–1.7	0.7; 0.3–1.98	1.9; 0.7–5.1	3.1; 0.8-12.3
Term	**11.3; 3.9–32.1**	**7.7; 2.98–19,8**	1.1; 0.7–1.97	1.3; 0.5–3.8	2.01; 0.6–6.5	**6.9; 1.6–29.8**
ATC group						
A	1	0,7; 0,4–1,2	1	1	1	1
B	1,8; 0,96–3,4	1	0,9; 0,6–1,3	1,6; 0,8–2,9	**0,1; 0,1–0,4**	**4,8; 2,1–11,02**
C	0	0,9; 0,2–4,7	**0,1; 0,01–0,5**	0	1,1; 0,1–12,6	0
H	0	0,8; 0,1–7,3	0	**21,4; 1,8–252,3**	0	131,7; 0,3–60500
J	0,1; 0,01–1,4	**0,1; 0,004–0,5**	0,3; 0,1–1,3	0,6; 0,1–4,97	0,2; 0,01-3,6	3,5; 0,3–40,4
J01F	0	0,5; 0,03–7,7	0,98; 0,1–15,7	**84,5; 8,8–812,7**	0	0
J01G	0	0	**26,0; 14,3–47,4**	**0,3; 0,1–1,7**	0	0
N	0	1,2; 0,5–2,8	**0,1; 0,02–0,2**	0,6; 0,2–1,8	**0,2; 0,04–0,97**	0,8; 0,1–4,9
V	34,2; 1,2–958,7	0	0	0	5,1; 0,3–96,4	0

Table 19. Continuation

Covariate	Solvents and solubilizing agent		Antimicrobials		Sweeteners	
	Polysorbate 80	Propylene glycol	Parabens[c]	Benzoates[d]	Saccharin sodium	Sorbitol
Department level						
1	1	1	1	1	1	0
2	0.3; 0.002–47.7	0.2; 0.02–2.7	0.2; 0.03–1.7	0.2; 0.01–5.6	0.3; 0.01–7.96	1
3	0.1; 0.001–18.9	**0.1; 0.01–0.7**	0.4; 0.05–2.6	0.4; 0.02–9.7	0.5; 0.02-11.2	7.7; 0.8–76.5
Route						
Enteral	1	1	1	1	1	1
Parenteral	**0.4; 0.2–0.95**	**0.4; 0.2–0.7**	**0.2; 0.1–0.3**	**0.3; 0.2–0.7**	0	**0.003; 0.00003–0.03**

[a]adjusted for geographical region, gestational age, department level, ATC group and route of administration and presented as Odds Ratio (OR) and 95% Confidence Interval (CI)
[b]extremely preterm, very preterm, late preterm, and term denotes gestational age bands of <28, 28 to <32, 32 to <37, and >37 weeks, respectively
[c]parabens include propyl-, ethyl-, and methylparabens
[d]benzoates include benzyl alcohol, benzoic acid, and sodium benzoate
ATC, anatomical therapeutic chemical classification; header row represents excipients of interest (EOI); first column represents studied variables; one (1) indicates reference group; zero (0) indicates variable not included in the analysis due to the insufficient number of prescriptions; ethanol and benzalkonium chloride were not included in this analysis due to small sample sizes and lack of variability; only those ATC groups with EOI are presented; significant ORs are in bold

5.4.1 EOI administration by geographical region

Region was a determinant of EOI administration regardless of excipient class. Significant variations were observed in the use of parabens, polysorbate 80, propylene glycol, and saccharin sodium (Table 18). Adjusting for ATC group and route of administration did not change the regional effect (Table 19). This suggests that medicines of the same ATC group and same route of administration but various EOI content are used in different regions. Hence, EOI-free formulations are feasible and available on the European market and therefore some EOI administration could be avoidable if different products containing the same API were used.

Regional differences in parabens administration were driven by choice of vitamin preparations (118/477 containing parabens), responsible for 118/397 paraben-containing prescriptions. The proportion of vitamin prescriptions containing parabens varied from 3–24% (3/98 in East and 33/137 in West) to a third (56/164 in North and 26/78 in South).

Domperidone, prescribed in North and South, and carnitine, prescribed in West, were responsible for 30% of prescriptions containing saccharin sodium but were not used in the East. No reasons could be identified for variations in polysorbate 80 and propylene glycol administration.

5.4.2 EOI administration by gestational age

In **univariate analysis** no association between GA and administration of at least one EOI was found; the median (IQR) number of EOI per neonate of 1 (0; 2) was identical for all GA bands. However, due to the decreasing number of prescriptions per neonate with increasing GA, the proportion of prescriptions containing at least one EOI was greater in term and late preterm compared to extremely preterm neonates (OR 1.9; 95% CI 1.4 to 2.6 and OR 1.5; 95% CI 1.1 to 2, respectively). The extent of EOI administration by GA band is presented in paper II, Figure 2.

In **multivariate analysis,** in model 1 term GA was associated with a lower likelihood of parabens, ethanol, and benzoates administration; late preterm with a lower likelihood of ethanol, and very preterm GA with a higher likelihood of saccharin sodium administration compared to extremely preterm GA (Table 18). When adjusting for ATC group (model 2), these associations were no longer significant except for saccharin sodium (OR 2.8; 95% CI 1.1–7.3). Thus, variations in the administration of parabens, benzoates, and ethanol were probably driven by variations in API distribution rather than by use of different drug formulations (routes of administration). After adding a route of administration to the model, the association was not significant for saccharin sodium anymore.

In contrast to model 1, in model 2 late preterm infants were more likely to receive polysorbate 80 (OR 4.5; 95% CI 1.6–12.2) and term newborns

polysorbate 80 (OR 13.8; 95% CI 4.9–39.2), propylene glycol (OR 8.4; 95% CI 3.2–21.8), saccharin sodium (OR 3.2; 95% CI 1.1–9.3) and sorbitol (OR 8.9; 95% CI 2.4–33.3) compared to extremely premature neonates. Except for propylene glycol, these excipients are found predominantly in enteral formulations (Figure 8). After adjusting for the route of administration, these associations remained significant for all EOI mentioned above except for saccharin sodium (Table 19). Together with major regional variation, this may reflect deliberate choices due to awareness of possible hazards considered more relevant in extremely preterm than term infants.

5.4.3 EOI administration by route of administration

Parenteral route was associated with a lower likelihood of administration for five studied EOI (parabens, polysorbate 80, benzoates, propylene glycol, and sorbitol) regardless of functional group (Table 19). Saccharin sodium, ethanol, and benzalkonium chloride were not included in this analysis due to small sample sizes and the predominance of a single route of administration – saccharin sodium was found exclusively and ethanol in 79% (37/47) of prescriptions in enteral formulations; benzalkonium chloride in 85% (23/27) of prescriptions in topical formulations.

The risks of ADR to the patient are proportional to the route of administration, approximately increasing in the following sequence: topical, oral and rectal, pulmonary/ inhalation, parenteral, ophthalmic, and preparations intended for use in open wounds.[166] The route of administration is also critical to defining the requirements for the excipient. It is accepted that oral route is the most preferred and appropriate route of administration in paediatric patients.[33] This may not always be a "gold standard" due to the extensive variability in children with special needs for drug formulations in different paediatric age groups and particularly neonates.[215] In case of oral medicines neonates should be given liquid formulations that could be easily swallowed. However, special attention has to be paid to the in-use quality of multidose preparations, regarding both microbial and chemical stability.[33] As showed by us and by others, EOI are often required in liquid products as solvents, antimicrobial preservatives, and/ or taste-masking/ sweeteners.[186,216]

5.4.4 EOI administration by ATC group

In the prescription-based multivariate analysis (Model 3), ATC group was a significant determinant of administration for all EOI except polysorbate 80 (Table 19). ATC groups with highest proportion and number of prescriptions containing each EOI are shown in Table 20.

Table 20. ATC groups with highest proportion and number of prescriptions containing EOI

EOI	ATC group with highest proportion of prescriptions	ATC group with highest number of prescriptions
Solvents and solubilising agents		
Propylene glycol	B (phytomenadione, iron)	B (phytomenadione, iron)
Ethanol	G (dinoprostone)	A (mainly enteral vitamins)
Antimicrobials		
Parabens[a]	J01G (gentamicin)	A (mainly enteral vitamins)
Benzoates[b]	J01F (lincomycin, clindamycin)	A (mainly enteral vitamins)
Benzalkonium chloride	S (tobramycin, chloramphenicol, ciprofloxacin – all topical)	S (tobramycin, chloramphenicol, ciprofloxacin – all topical)
Sweeteners		
Sorbitol	B (phytomenadione, iron)	A (mainly enteral vitamins)
Saccharin sodium	A (mainly enteral vitamins)	A (mainly enteral vitamins)

[a]parabens include propyl-, ethyl-, and methylparabens
[b]benzoates include benzyl alcohol, benzoic acid, and sodium benzoate
ATC, anatomical therapeutic chemical classification; EOI, excipient of interest

Thus, the main source of parabens, benzoates, ethanol, sorbitol, and saccharin sodium are enteral multivitamins. Practitioners should pay special attention to prescribing vitamins. Enteral multivitamin products were commonly prescribed in our study with 6% (n = 133) of all prescriptions; 81% (n = 108) contained at least one EOI. Similar results were reported from a Brazilian NICU with oral multivitamins making up 7% of all prescriptions.[135]

Medicines from ATC groups J01C (mainly ampicillin, benzylpenicillin), J01D (mainly cefotaxime, ceftazidime, meropenem), J01E (trimethoprim), J01M (ciprofloxacin), J01X (mainly metronidazole, teicoplanin, vancomycin), L (filgrastim), and M (pancuronium) did not contain any EOI.

5.5 Substitution of EOI containing medicines with EOI-free counterparts

Overall, 53 APIs were used in more than 10% of units. These APIs were administered through 564 products (53% of all products registered in the SES). Excluding topical medicines, multivitamins and APIs with no EOI, substitution analysis included 25 APIs in 318 products (paper III, Figure 1).

1. Substitution to reduce the number of products containing EOI

At least one EOI was found in 137 out of 318 (43%) products, 85 of these were enteral formulations.

When applying the first, second, and third stage criteria, the number of EOI containing products could be reduced by 88%, 66%, and 31%, respectively (Figure 10).

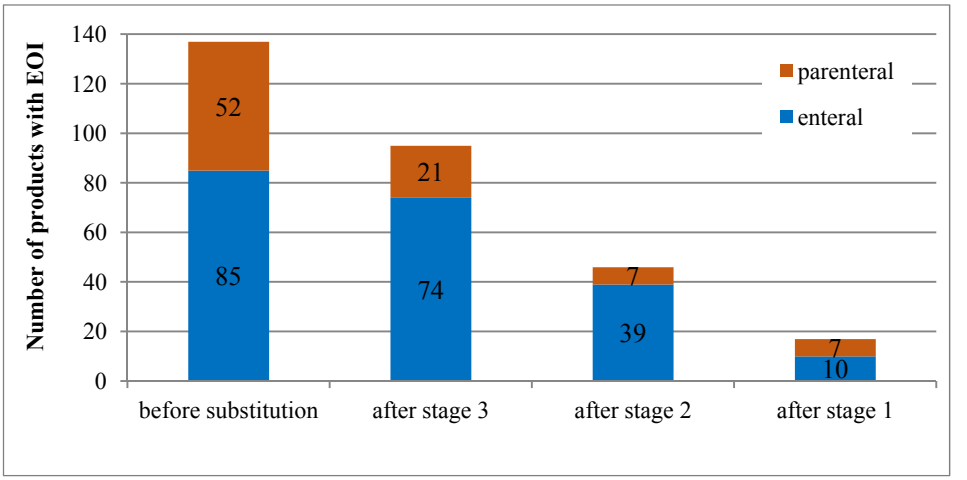


Figure 10. Product substitution possibilities[a] according to criteria of three stages

[a]presented as number of products; EOI, excipients of interest; Stage 1 – similar active pharmaceutical ingredient and route of administration; Stage 2 – stage 1 + similar dosage form; Stage 3 – stage 2 + similar strength of active pharmaceutical ingredient

Substitution opportunities with the list of countries in which EOI-free products were available are presented in paper III, Table I for parenteral and Table II for enteral products. Opportunity for substitution was available in several countries. For example, enteral colecalciferol products containing propylene glycol, ethanol, parabens, and benzyl alcohol could be substituted with six EOI-free products available in ten countries; for parenteral gentamicin with parabens there are EOI-free alternatives in use in four countries.

A major part of the reduction in number of products with EOI can be achieved by substitution of parenteral medicines – a greater proportion of parenteral products could be substituted compared to enteral medicines in both second stage (87% vs. 54%; OR 5.5; 95% CI 2.2–13.5) and third stage (64% vs. 14%; OR 10.4; 95% CI 4.5–24). However, even for enteral medicines, in the second stage substitution was possible for 54% (46/85) of products. Switching to parenteral administration would allow replacing an additional 21 of 39 enteral products (paper III, Table II).

The first stage of substitution analysis provides an estimate of how many excipients are required for the stability of the API – APIs not having any EOI-free

alternatives available are most likely to require one or more of them for manufacturing purposes and the use of product(s) with EOI is justified. For parenterally administered medicines from 12 APIs EOI-free products were available for all but alprostadil (paper III, Figure 3). For enteral medicines, from 17 APIs no substitution possibility was available for dexamethasone, domperidone, fluconazole, ibuprofen, metronidazole, nystatin, paracetamol, phenobarbital, and ranitidine. Parenteral EOI-free formulations are available for all above-mentioned APIs except for nystatin and domperidone (paper III, Table II).

The second stage provided discrimination between solid and liquid enteral formulations. For example, for nystatin an EOI-free tablet was available but should not be considered an adequate substitution possibility. Safe administration of tablets to neonates is at least questionable if not impossible.[32]

The third stage is important in neonatal practice because of possible limits on fluid administration but also dosing accuracy particularly in parenteral medicines.

In a recent study conducted by Garcia-Palop et al. in a university hospital in Spain, the authors showed, that in comparison with ADI estimated for adults, neonates receiving parenteral diazepam, sodium heparin, gamma globulin, and naloxone were exposed to higher amounts of propylene glycol, benzyl alcohol, sorbitol, and MPB, respectively.[150] Should be emphasized that for propylene glycol ADI for adults is 500 times greater than for neonates (500 vs 1 mg/kg/day).[66,107] According to our data, EOI-free products for all these APIs are available on the European market.

2. Substitution to reduce the number of neonates exposed to EOI

The analysis of potential product substitution for each neonate included 22 of the 25 APIs used in the analysis of products with 776 prescriptions (paper III, Figure 1). A half of these prescriptions (n = 372) contained EOI. Further results are presented in relation to all prescriptions (638/ 2095 with EOI) and neonates (456/ 726 exposed to EOI) from the PPS to indicate the overall benefit from the substitution of only the most commonly used medicines. After all available product substitutions of the 22 studied APIs the overall number of prescriptions with EOI in the PPS would be reduced by 50% (from 638 to 317; 95% CI 46–54%; paper III, Table 3) and the number of exposed neonates by 44% (from 456 to 257; 95% CI 39–48%; Figure 11).

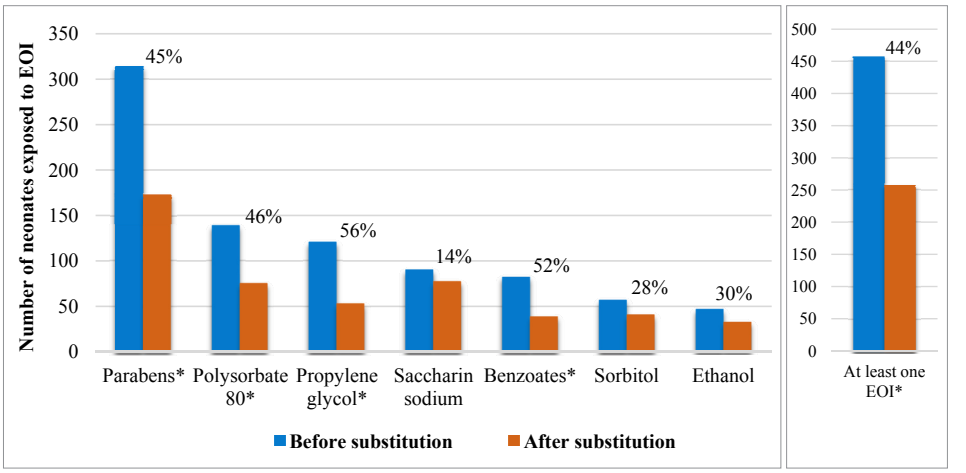


Figure 11. The number of neonates exposed to each EOI before and after product substitution and proportion of neonates favored from substitution[a]

[a]presented as overall reduction of neonatal exposure through the substitution of most frequently used medicines according to second-stage criteria – identical active pharmaceutical ingredient, route of administration, and dosage form; percentages indicate proportion of neonates favored from substitution; *lower odds of neonatal exposure after substitution ($p < 0.05$)
Parabens include propyl-, ethyl-, and methylparabens; benzoates include benzyl alcohol, benzoic acid, and sodium benzoate; EOI, excipient of interest

After potential product substitution, the odds of neonatal exposure were significantly lower for all EOI except for ethanol, saccharin sodium, and sorbitol (Figure 11; paper III, Figure 4). The availability of EOI-free products may be related to the role of the excipient in a formulation. For example, avoiding solvents like ethanol may be more difficult in the manufacturing process than antimicrobial preservatives like parabens in single-use parenteral products.[32] The situation is more complex for enteral products where multi-dose liquid formulations are frequently used and excipients are also needed to improve palatability and consistency (e.g. saccharin sodium and sorbitol).[32]

Additionally, possible cumulative risks associated with exposure to EOI from multiple sources[14,86,107,134] can be reduced by product substitution. Among 257 neonates still exposed to at least one EOI after product substitution, 70 neonates would benefit from being exposed to a lower number of EOI. Probably, overall cumulative gain from product substitution may be even greater. Iron, colecalciferol, and folic acid were prescribed in more than 10% of units. These products may contain all but one (benzalkonium chloride) EOI. Importantly, these liquid medications are commonly prescribed to preterm neonates for 3–6 months forming a "background" level of excipient exposure. We have shown that for all these drugs there are EOI-free products available on the European market.

5.6 Impact of study methodology on assessment of medicines use

The average comparative probability of the departments to use ten most common APIs within the study period as revealed by the two methods is shown in Table 20.

Table 20. The average probability of departments to use one of ten most common API in the SES compared to the PPS*

API	RR (95% CI)	p-value
Gentamicin	1.45 (1.18–1.78)	0.002
Ampicillin	1.38 (1.10–1.74)	0.02
Vancomycin	1.59 (1.17–2.17)	0.015
Benzylpenicillin	1.32 (0.93–1.86)	0.38
Phytomenadione	2.39 (1.75—3.27)	<0.0005
Iron	1.71 (1.29–2.26)	0.001
Amino acids	1.99 (1.45–2.72)	<0.0005
Lipids	1.89 (1.34–2.65)	0.001
Furosemide	2.81 (1.64–4.83)	0.001
Colecalciferol	1.05 (0.81–1.38)	0.705

*adjusted for department level, status of teaching or non-teaching hospital, and European region
API, active pharmaceutical ingredient; RR, risk ratio; PPS, point prevalence study; SES, service evaluation survey

For eight out of ten most frequently used APIs the likelihood of being captured in the SES was higher compared to the PPS. Further analysis included 99 APIs used in more than one unit in the SES. Almost all APIs were more frequently used in the SES compared to the PPS, but the 95% CIs were overlapping for the majority (paper I, Figure 1). High correlation in the frequency of medicine use between the SES and PPS for APIs used in more than one unit in the SES is shown in Figure 12.

The estimates in the frequency of medicine use in the PPS were systematically lower compared to the SES. However, knowing the relationship between the two an estimate for one can be extrapolated from the other.

In multilevel mixed effects logistic regression models with crossed random effects adjusted for geographical region and unit level the average probability of the departments to use each of the most common API (n = 99) was higher in the SES compared to the PPS (OR 2.36; 95% CI 2.05–2.73; $p < 0.0005$). The frequency of use and average duration of prescription further increased the likelihood of being registered in favor of the SES by 1.01 times (95% CI 1.01–1.02; $p < 0.0005$) and 1.02 times (95% CI 1.00–1.05; $p = 0.047$) per each additional percent in frequency and day in duration of prescription, respectively.

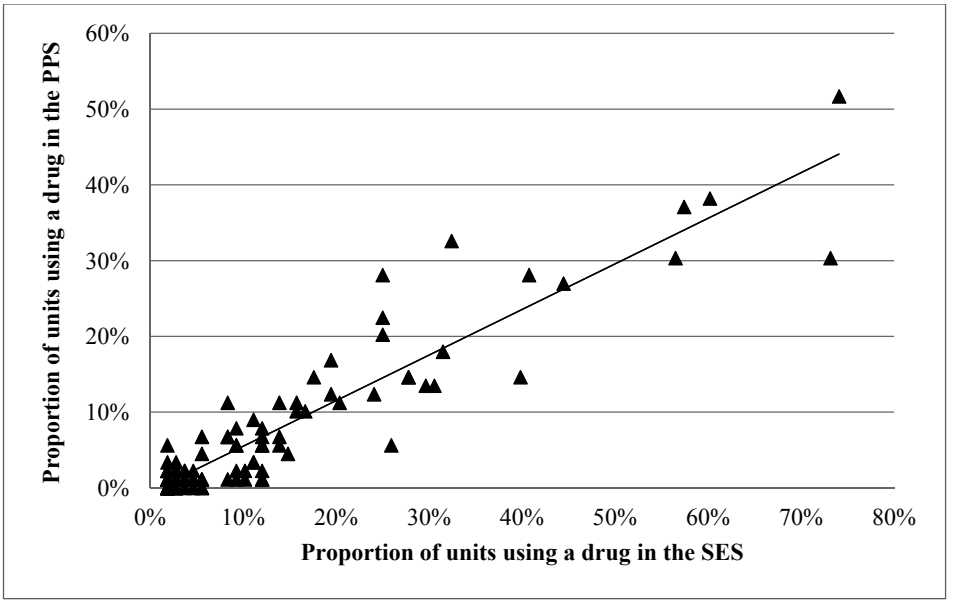


Figure 12. Correlation in the frequency of medicine use between the SES and PPS on unit level

Trendline for 99 active pharmaceutical ingredients: $y = 0.6019x - 0.0053$; $R^2 = 0.8605$
PPS, point prevalence study; SES, service evaluation survey

Size of the department did not influence the capture probability (OR 0.99; 95% CI 0.99–1.00; p = 0.652). Thus, simple data structure and longer study period used in the SES improved recruitment and the likelihood of capture of medicines consumption, giving a more comprehensive list of drug products.

We have also shown high correlation ($R^2 = 0.93$) between the number of units using each specified API and the number of prescriptions in the PPS (Figure 13).

Thus, the less demanding unit level approach like SES should allow an indirect individual exposure assessment under the condition of limited resources. Nevertheless, such calculations should be applied with caution and to estimate the exposure only for the studied population as a whole. It has been shown previously and also by us that medicines utilization pattern in extreme prematurity is different from that near or at term.[19,20,24,131] Therefore, in neonatal studies stratification by GA age allows a more meaningful risk assessment, especially when also considering the strong dependence of the maturity of metabolic pathways on the postmenstrual age of the newborn.[7,85]

Against the background of rising safety concerns of neonatal medicine/ excipient exposure, the extensive trade name list from the SES allows identification of substitution possibilities, while individual exposure data from the PPS provide a more precise quantification of the problem. Investigators need to balance the advantages of a PPS with the risk of bias, which depends on the frequency as well as the duration of medicine use. Simultaneous use of both

methods with merged data analysis will likely result in optimal coverage of both aspects of the problem.

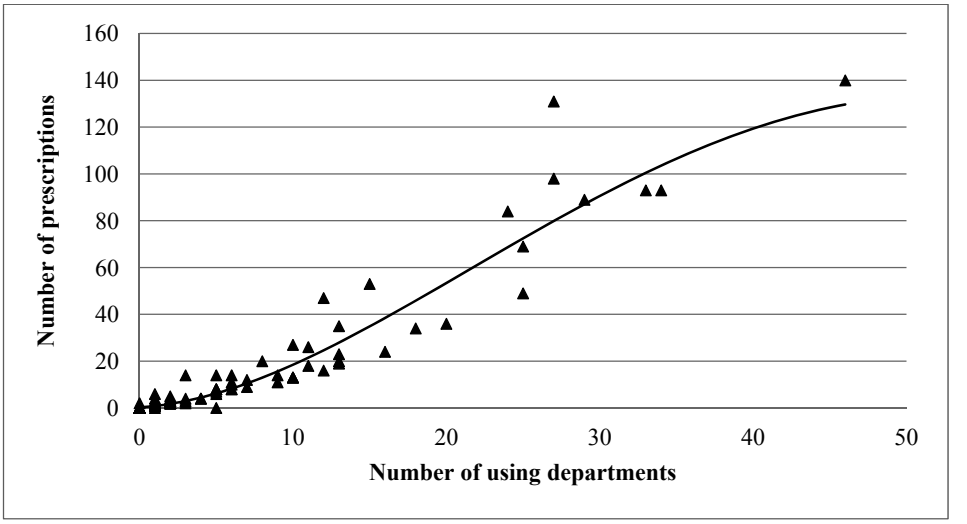


Figure 13. Correlation between medicines exposure at unit and individual levels

Number of units using each specified active ingredient (n = 99) in relation to the number of prescriptions in the PPS was observed. Polynomial regression trendline for active pharmaceutical ingredients used in more than one unit in the SES is shown. $\sqrt{\text{number of prescriptions}} = 0.421 * (\text{number of departments}) - 0.004 * (\text{number of departments})^2 + 0.485$; $R^2 = 0.93$

6. GENERAL DISCUSSION

Neonates are routinely given medicines with no specific paediatric information because of the lack of clinical trials in this population. The status of children as "therapeutic orphans"[217] has been increasingly recognised and has lead to a number of legislative initiatives in developed countries, including the US and EU over the last decades. The concept of the therapeutic orphan originates from 1962. The first objective evaluation of labelled drugs available for children was done in 1973 with further continuum of validations from multiple sources.[217] As a result, substantial unlicensed and off-label use of adult medicines in neonatal population has been actively addressed.[18,28] Pharmaceutical excipients were called "inactive ingredients" just a little while ago[46] with little attention paid to excipients safety until recently. Only from 2007 Paediatic Regulation requires excipient safety to be also considered by pharmaceutical industry when developing new medicines.[218,219] While safety issues related to the use of some excipients in neonates raised concerns, data regarding the scope of the problem on a large scale were not available to date. In this thesis, we aimed to address the excipient exposure via an integrated risk assessment approach to identify the extent of the problem (excipient use), determine related risk factors and propose possible solutions.

6.1 Unexplored field of pharmaceutical excipients in neonates

Although extensive work has been done by pharmaceutical companies to ensure that excipients are safe when used in adults, their safety in paediatric subpopulations is often unknown; some of them are known to be toxic and uncertainty about others, especially in neonates, exists.[57] In an analysis, carried out by EMA Paediatric Committee, of paediatric investigation plans (PIP) proposed in 2009, issues with excipients were identified in 102 (82%) out of 125 pharmaceutical forms – applications included insufficient justification of the chosen excipients related to age, daily dose of excipient(s), and inadequate discussion on the possibility to replace excipients with potential safety concern.[218] Currently, only 30% of PIPs include studies with neonates.[220] Comprehensive data about excipients included in medicines used in neonatal units is lacking and doctors are still prescribing medicines to neonates without knowing what excipients are included, whether they are safe, and whether there are opportunities/ needs to avoid excipients with safety issues.

This was the first study looking at neonatal administration of pharmaceutical excipients in a large multi-country setting and the biggest and most detailed prospective study of excipient use in neonates to date. Previous studies on excipient exposure among neonates were conducted within single unit/ country.[14–16,134,150,221] While frequent general administration of potentially

harmful excipients was emphasized, some results were contradictory probably due to small sample size and therefore cannot be extrapolated to the whole population. While Fister et al. showed no benzyl alcohol in medicines prescribed in Slovenian neonatal unit,[16] Garcia-Palop et al. reported this to be one of the most common EOI prescribed parenterally to neonates in Spain.[150] We found benzyl alcohol in both enteral and parenteral commonly prescribed drugs, e.g. enteral colecalciferol and iron and parenteral phytomenadione, phenobarbital, hydrocortisone, heparin, and amoxicillin.

In this study, data on the surprisingly broad range of medicines with highly variable excipient compositions, used in neonates in Europe, have been collected. Similarly to previous single country/ unit studies on excipient administration[15,16,135] we demonstrate that a substantial part (63%) of neonates treated in European NICUs is exposed to potentially harmful excipients called here EOIs.[214] Additionally, our data on both prescription and individual level showed that in particular the most commonly used medicines (e.g. gentamicin, iron, enteral vitamins) were responsible for a large part of the EOI "load" suggesting that substitution or reformulation of a relatively small number of medicines may spare a large number of neonates from unnecessary exposure.

Another element of exposure assessment not studied in this work is quantitative, i.e. understanding of excipient exposure patterns also requires descriptions of excipient kinetics comparable to PK for APIs. Recent observational studies suggest that neonates are exposed to significant amounts of excipients, some of which can exceed internationally recommended limits for exposure.[14,134] However, there have been very few attempts to measure excipients quantities in the bloodstream and assess PK parameters in neonates – the main impediment has been a limited amount of blood volume allowed for sampling. Most excipient exposure studies, including those presented in this thesis, measured exposure indirectly through prescription data. To date, only a couple of studies have been performed with the aim to estimate blood levels or PK parameters of excipients with known safety concerns – benzyl alcohol by LeBel et al. and propylene glycol by Roosmarijn et al. – in neonates using "wet" sample-based assays.[7,78] Although dry blood spot (DBS) method allows determination of drug levels in very small blood volumes,[222–226] this methodology has not been used before the ESNEE project to measure excipient (i.e. parabens) PK parameters.[227] Data collected during our study allowed to identify drug products containing methyl- and propyparabens with further inclusion of these medicines into PK study.

Today, even if some data on the safety/ toxicity of excipients are available, these are not easy to find from public sources. Moreover, Lass et al. reported no human safety/ toxicity data accessible in the literature for 16% out of 123 excipients identified in medicines prescribed to neonates in Estonia.[15] Global Research in Paediatrics (GRiP) popularizes the need for a comprehensive source of computerized information concerning the toxicity and safety of excipients for paediatric medicines.[228] To address this need, the European and US Paediatric Formulation Initiatives (Eu-US PFIs) are working together to

create and maintain a database of Safety and Toxicity of Excipients for Paediatrics (STEP) – evidence-based database addressing safety issues and toxicity aspects of pharmaceutical excipients in medicines for children.[229] The purpose of STEP is to serve as a free database for safety data and supporting toxicity studies for excipients, particularly in paediatric population.

A newly launched project "Safe Excipient Exposure in Neonates and Small Children" (SEEN) aims to explore the quantity of excipient exposure in neonatal and young paediatric patients in a Danish Hospital focusing on excipients known to be harmful.[230] Both registered and extemporaneous pharmaceuticals as possible sources of excipients will be studied. The success of this doubtless important study will depend on whether the manufacturer of each medicinal product included in the study will provide needed quantitative information on excipient content.

6.2 Methodological issues of the excipient exposure study

To date, generally accepted tools for excipient exposure assessment have not been identified. Most available tools for pharmacoepidemiologic studies, i.e. hospital or health insurance databases, lack details required for assessment of excipient exposure. Study designs allowing coverage of large study populations over relatively short period and limited dataset appear appropriate for initial estimation of the scope of the problem. In this thesis, to assess the exposure to excipients, we had to gather data about prescribed medicines first. The precision of estimation of the extent and nature of neonatal exposure to different medicines/ excipients was maximized by successive use of two different cross-sectional study designs, the SES and PPS – the first providing a comprehensive list of medicines/ excipients used in neonates and the latter adding detailed data on individual exposure. The strength of both methods used in this thesis is the simplicity and uniformity of data collection. In the SES the most time-consuming part of data analysis turned out to be the identification of excipient content from SmPC for each single drug product. Such approach is probably not sustainable for future researchers. Hence, time and resource consuming development of new tools, like the web-based prepopulated database in our study and/ or hospital electronic prescription databases including also excipient data, may prove necessary. Furthermore, given the possible organisational and economic implications, market size making these feasible (e.g. drug reformulation or substitution) should be aimed/ involved as in our study. Simple data structure used in the SES improved recruitment in general, while more detailed data capture, when facilitated by prepopulated tool as in the PPS appeared more attractive for physicians from teaching hospitals, usually taking care of more complex patients with broader spectrum of medicines used. However, as showed in our study, longitudinal component is needed to capture less commonly used medicines.

Data structure should be chosen in compliance with the research question. A study with the primary aim of describing the variability/ comprehensive list of medicines or excipients administered for short time periods may allow less sophisticated data structure/ volume to be reported, with additional benefit of a longitudinal component to maximise study power. When individual exposures of frequently used medicines are targeted, the increasing data volume (both structure and duration) needs to be weighed against evolving increase in resource consumption and possible decrease in compliance that may lead to underestimation of prevalence.[231] It has been shown that the longer the recall period, the greater the imprecision (especially underestimation) of prevalence estimates,[232,233] although this finding may be more relevant in non-medical informants.[234] Oppositely, short-term surveys like SES and PPS conducted on a single unit/ country level are likely to underestimate the extent and variety of medicines/ excipient exposure.[235] Instead, single country/ unit long term cohort studies may provide valuable complementary information as described by Lass et al.,[15,24] allowing more detailed individual risk assessment including quantitative/ cumulative exposure estimation, where wanted.[14]

Alternatively to our approach, nested study designs with longitudinal data collection would allow linkage between different datasets and could be the best option. In our study, nested design with detailed individual data collection performed as part of the SES was not feasible, as an interim between the two studies was needed to develop the prepopulated PPS medicines database from the data collected in the SES. This could potentially reduce the overlap between the studies. In that situation our data represent a "worst case scenario". The actual concordance in API reporting may be higher than described by us. To the best of our knowledge, nested design has hardly been used in paediatric pharmacoepidemiological studies.

Not only optimal recruitment of participants but also sample size maintenance over the course of a study becomes an issue, especially in multinational studies.[236] Recruitment of participating NICUs, regional distribution, and variability in levels of care and unit size should be considered.[57] As random recruitment is often unfeasible, cluster sampling approach was planned in the PPS.[237] Recruitment of randomly chosen regional clusters based for example on the EuroStat NUTS classification would have allowed adjusting for the variability of levels of care in NICU studies. Still in cluster design the cluster effect and intracluster coefficient of variance need to be considered.[57] Involvement of all units from chosen region is needed to achieve estimated sample size for each country and region and to ensure the randomisation and representativeness of the data. In our study, randomisation of participating hospitals remained unfeasible due to the lack of a full list of neonatal units in each country. Additionally, it is hardly possible to achieve 100% response rate in such a wide scale study.[196–199]

While pharmacoeconomic analysis remains beyond the scope of this study, economic considerations always play a role in the choice of a specific study design. There were no major cost differences in conducting the SES and PPS

studies except those associated with the development of the PPS database specifically for this study. Web-based data collection with automated checks allowed to save resources and to improve compliance and overall quality of data. We believe that whenever adequate to answer the research question, the simple data structure is favourable in terms of resource allocation. However, when a more complex format is required, reasonable additional expenditure on data collection tools may result in an improved cost-benefit ratio. We have shown that under the condition of limited resources, individual exposure estimates can be extrapolated from a less demanding approach like the SES. However, such extrapolation does not allow stratification by demographic characteristics, that is of great importance in the neonatal population.

6.2.1 Availability of excipient data in current sources

"Omnia venenum sunt, nec sine veneno quicquam existit; dosis sola facin ut venenum non sit." (In practice, every substance is a poison, at the right dose. Paracelsus; 1493–1541)

Even today, collecting information on the content of excipients in drug formulations remains a challenge. In this thesis, SmPC proved the most reliable data source because excipients present even in tiny amounts have to be included.[163] Nevertheless, in our study SmPC were unattainable for 13% (n=138) of products, leaving the practitioners with little if any reliable grounds to take decisions. Moreover, information about the composition of compound flavours is usually not included in SmPC; even in specialized pharmaceutical databases only qualitative data are presented: http://www.theriaque.org/apps/monographie/index.php?type=SP&id=6826. The information on excipients still not being readily available to the clinician derives from two main principles. Firstly, some medicines package inserts list no excipients, or the list is incomplete, ignoring regulatory requirements, for historical or inconvenience/ data availability issues. Secondly, manufacturers are not open to disclose this information, especially when quantitative content is concerned, claiming business confidentiality.[238] We identified reports stating that a full list of excipients was not available as part of the protection of intellectual property.

Guidances and recommendations from Regulatory Authorities regarding the inclusion of potentially harmful excipients in drug products intended (labelled) for use in children/ neonates are increasing in both the US and EU. Since 2010 disclosure of quantitative data on excipients with safety concerns has been required. Woefully this initiative will address only a small proportion of new/ newly authorised products for neonates while the majority of medicines are still prescribed off-label or unlicensed. In our study quantitative excipient information was available from SmPC in only 6% (n=58) of products. Notably, more than half of these products did not contain any EOI; among all products with EOI quantitative data were available for only 8% (25/305). Like us, Fister

et al. found insufficient data on the quantities of excipients in products administered to neonates.[16]

Making at least comprehensive qualitative but preferably quantitative excipients data available to healthcare professionals holds an opportunity to make "safer" choices. Knowledge of quantitative data would better guide the selection for only those products where replacement is an absolute necessity.

6.3 Rationality of adding EOI into medicines

Assessment of the rationality of excipient administration is a complex task involving multiple manufacturing as well as formulation target/ use related details, e.g. route of administration. Many excipients have the potential to fulfill multiple roles within a single or in different formulations. We believe that certain abstraction can be reached based on the (main) purpose of an excipient in a formulation, accepting that these assumptions can never be exhaustive. The pattern of covariates associated with the administration of EOI in our study suggests rational use in many medicines, while for some a margin for improvement is proposed and supported by availability of substitution possibilities.

Based on available data we hypothesised that solvents (polysorbate 80, propylene glycol, ethanol) are required for manufacturing process of a specific API not soluble in the water or other liquids.[48,149] In contrast, we showed that polysorbate 80 and propylene glycol use was associated with geographical region and enteral route of administration even after adjusting for potential covariates, including ATC group as a surrogate for API. Our results suggest the possibility to use/ produce medicines free of these excipients in one but not another country/ region. Although some regional variations may be driven by differences in API within the ATC group (e.g. enteral vitamins), many likely rise from hospital routines and policies. The latter is often based on traditions and expert opinions rather than evidence-based guidelines.[24,28,136] No regional variations were associated with administration of ethanol, found predominantly in enteral formulations, referring to its rational use. Nevertheless, we identified either enteral (i.e. colecalciferol, furosemide, iron, nystatin) or parenteral (i.e. metronidazole, phenobarbital, ranitidine) ethanol-free alternatives for all enteral ethanol-containing products. In some cases, judging the rationality of excipient use requires careful weighing of possible additional risks of an alternative formulation (e.g. different route of administration) against those related to excipient exposure.

Antimicrobials (parabens, benzoates, benzalkonium chloride) should not be required for sterile single-dose parenteral formulations. Accordingly, parenteral route was associated with lower administration of these excipients – 85% of parenteral prescriptions were paraben- and benzoate-free. The presence of parabens and/ or benzoates in 15% of parenteral prescriptions may suggest the use of multiple-dose formulations or, that some medicines require these excipients for other than antimicrobial, like manufacturing and stability pur-

poses. No conclusions can be made regarding the first explanation as data on single- or multiple-use were not available in SmPC. Therefore we propose this information to be included on the drug label and patient information leaflet. We can speculate that among frequently used APIs with antimicrobials dexamethasone (4 mg/ml), epinephrine (1 mg/ml), and gentamicin (80 mg/2ml) are single-use formulations. We found same strength products without any EOI for all above-mentioned APIs. In addition, we identified EOI-free alternatives for several commonly used enteral products containing antimicrobial EOI – amoxicillin, caffeine, colecalciferol, folic acid, iron, and tocopherol – indicating possibility to produce enteral liquid medicines, e.g. in the form of powder for reconstitution, without antimicrobials.

Sweeteners (sorbitol, saccharin sodium) were expected to vary only between routes of administration and GA. This was true for sorbitol; rational use was supported by the association with term GA, the extent of use being independent of geographical region and a lower rate in parenteral formulations. Among commonly used APIs sorbitol-free enteral products were available only for iron. The use of saccharin sodium, found almost exclusively in enteral medicines, varied between geographical regions, suggesting the availability of saccharin sodium-free formulations on the European market (e.g. iron and furosemide). One could also question the rationality of adding colouring and flavouring agents to formulations administered to premature neonates through a nasogastric tube. For example, strawberry flavour could contain 13 different substances including propylene glycol. As flavouring agents do not contribute to functions other than palatability,[37] their use can be avoided in products aimed at patients/ situations, where this is not relevant e.g. due to the method of administration.

6.4 Substitution as a way of avoiding EOI

Substitution of EOI-containing products with EOI-free formulations already available on the market is attractive[33] as the risks of importing and using licensed medicines are much lower than those associated with extemporaneous preparations.[182] To examine the feasibility of this approach, searches for alternative medicines with the same API, route of administration and dosage form have been done in this work. We have shown that EOI-free formulations are workable and available on the European market offering alternative to EOI-containing products in many cases. For example, we have put in use paraben-free parenteral gentamicin formulation in our neonatal unit in Tartu University Hospital.

Compared to parenteral formulations finding EOI-free alternatives for enteral products proved more complicated probably due to common use of multidose formulations and need to improve palatability. Accepting parenteral route of administration instead of enteral may be needed to avoid EOI administration. We recognise that switching to parenteral administration if the

oral route is available may pose unnecessary risks.[215,239] WHO has suggested the change of product, dosage form or even route of administration to be occasionally justified to avoid significant risk posed by excipients known to be toxic.[2,33]

Manufacturers should adopt existing best practices of safe and efficient use of excipients at the global level.[59] For example, if ethanol can be removed from certain oral liquid medicines in one country,[59,119] it should be carried out globally. We accept that there is a minimum set of excipients needed. However, our work indicated that 11 out of 12 parenteral and nine out of 17 enteral frequently used APIs do not need EOI to be included in the manufacturing process a priori. Changes in manufacturing processes to avoid EOI are possible without altering the pharmaceutical quality of the medicine.[57]

Klingmann et al. have shown the administration of uncoated mini-tablets to be a valuable alternative to syrup in term neonates.[240] This may be part of a strategy to avoid some toxic excipients, e.g. sweeteners, solvents, antimicrobial preservatives. Developing new products for the market is time-consuming and costly. The reformulation of ibuprofen or caffeine has resulted in products that are significantly more expensive than unlicensed alternatives. Such products may remain out of the reach of many NICUs, especially in less privileged countries.[241] Therefore, considering the concomitant costs related to the development of age-appropriate EOI-free medicines, neonatal formulations need to be limited to those that are absolutely needed. We have shown a significant potential to reduce neonatal exposure to EOI through substitution of only frequently used medicines with EOI-free alternatives available on the European market.

We recognise that implementation of systematic product substitution into every-day practice is not straightforward and can be only a part of an all-embracing strategy to provide safe and effective pharmacotherapy for neonates.[57] Even substitution with existing EOI-free products may incur additional costs due to the complexity of the requirements to be considered in a process involving multiple stakeholders (e.g., regulatory, financial, clinicians, etc.). If products are currently marketed in one European country, barriers to product substitution relate to licensing, distribution and marketing costs.[215,218] These costs may outweigh expected market return in small countries. Nevertheless, the knowledge of EOI-free products for a substantial number of APIs available in one but not other EU countries deserves further consideration by licensing authorities and should stimulate manufacturers, researchers, and healthcare professionals to make these products available in each country. Cooperation to ensure mutual consideration of data to allow licensing in all European countries is needed. Free movement of medicines inside the European market and committed collaboration between practitioners and stakeholders that extends across regions and countries becomes essential.[215]

6.5 Limitations of the study

Some limitations of the current thesis should be acknowledged. Out of the 31 invited countries, about two-thirds participated in each study, omitting some large European countries (e.g. Germany). Also, inability to randomly select hospitals has to be borne in mind. However, we believe that identified excipient administration patterns are appropriately adjusted and could be extrapolated to the European cohort.

Neonatal units were given the option of selecting the most appropriate day(s) for data collection. This could lead to underestimation of medicine use because less busy day(s) were probably more likely chosen. However, we believe that eventually this approach captured information on a larger number of neonates and medicines through improved compliance and a larger number of participating units.

Our study included eight excipients known to be harmful in neonates leaving a substantial number of excipients classified as potentially harmful, unexplored. We believe, that assuming the reformulation/ substitution processes not to be straightforward, those medicines containing the most problematic excipients should be studied first.

In risk factor analysis grouping of API to the 1st level of ATC classification only, with the exception of antibiotics, was feasible due to the relatively small number of prescriptions. Therefore some effects may be driven by intra-level variations in API use. These limitations still do not undermine the significance of regional variations, although may explain some findings on GA level.

As underlined before, the lack of quantitative information on excipient content in medicines did not allow detailed conclusions on quantitative exposure to EOI. Although this does not undermine our findings, large-scale quantitative exposure assessments are missing.

We recognise, that due to the general deficiency of age-appropriate formulations for neonates products used for substitution may not always be age-appropriate for this age group. We did not study the stability and validity of different formulations in detail and assume, that products prepared in different pharmacies may have variations in stability and shelf-life. Still, by identifying the substantial capacity for product substitution among marketed formulations in actual use in neonatal units, we believe this concept as a way to reduce EOI exposure in neonates well proven. Here, close collaboration of all stakeholders is required to resolve the technical and logistical issues surrounding practical achievement of substitution, such as import restrictions, legal and supply issues relating to off-label or unlicensed medicines, cost, age-appropriateness, dose volume and dose flexibility, interchange of route of administration, and prevention of medication errors. Economic and regulatory aspects of product substitution were out of the scope of the current analysis and need further evaluation.

6.6 The need for further research

An ideal neonatal prescription should represent an age-appropriate formulation of high pharmaceutical quality with appropriate knowledge on PK/ PD and safety profile of all compounds incorporated in a medicinal product. Future of age-appropriate formulations for neonates also involves excipients safety.[32] High rates of exposure to EOI in European neonates as was shown by us, underlines the relevance of the issue. We have proposed the way to reduce neonatal exposure to EOI through product substitution being fully aware this will not resolve the problem completely. If EOI-free formulation does not exist then careful balance between the evidence that an excipient may cause problems in the developing organism, especially in relation to the severity of patient condition, and expected benefit of the drug should be sought in each case; risk/ benefit ratio should be carefully weighted based on predicted dose, duration of exposure, and PK data. Limited data on the safety and/ or toxicity of excipients bind hand and foot in achieving this. Consequently, toxicokinetic research-based risk assessment in neonatal population becomes paramount, to identify true needs for reformulation/ substitution or where this is not possible, defining safe practice recommendations.

It is accepted that excipients may only show an effect above a certain dose[98] and the presence of an excipient in formulation does not necessarily mean (daily) intake exceeding the toxic threshold during routine treatment. To date, information on toxicity thresholds remains sketchy, especially in neonates. Need for excipient toxicokinetic studies has been increasingly recognised but only slow progress has been made so far.[78,227,229] Until recently, PK data in neonates were available only for propylene glycol and ethanol. For example, Allegaert et al. and Kulo et al. showed, that propylene glycol administration with a median of 34 mg/kg/day for a maximum of 48 hours seems to be well tolerated in neonates and does not affect short-term postnatal adaptation.[242,243] However, in extremely preterm neonates accumulation of propylene glycol may occur with co-administration of currently used phenobarbital and paracetamol formulations – birth weight was found to be the most significant covariate for propylene glycol clearance.[78] Similarly, we in the ESNEE clinical study have demonstrated low serum levels of parabens in neonates with some risk of accumulation only in the most preterm neonates.[227] As exact safe/ toxic concentrations are still unknown, further information on excipient PK in neonates is needed to estimate the level of tolerance considering the number of potential covariates as GA, postnatal age, birthweight, disease characteristics, treatment modalities (e.g. whole body cooling), route of administration, polypharmacotherapy/ drug-drug, drug-excipient interactions etc..[51] Toxicokinetic models should be developed to serve as a tool for individualised risk estimation in difficult to predict patient groups (e.g. organ failure, extreme prematurity). Studies in juvenile animals can provide relevant information, but cannot always be extrapolated to human neonates. To allow extrapolations, more complex

population pharmaco/ toxicokinetic studies, including physiology-based PK modelling, are needed.

Valid long-term neurodevelopmental outcomes of administration of potentially harmful excipients are entirely missing, even when favourable short-term outcome is demonstrated.[242,243] The applicability of outcome variables/ indicators applied to assess renal, hepatic and metabolic tolerance of administered excipients in adults needs to be validated in neonates.[79] As excipients are interacting with the body and vice versa, clinical pharmacologists with expertise ranging from PD, PK, rational prescribing, adverse drug effects, and toxicology should be key players in advancing drug formulation issues in children.[90]

Recent methodological advances including opportunistic sampling, population PK approach, micromethods for substance level estimation and DBS have made neonatal PK studies feasible. The ESNEE project has shown the feasibility of such methods for parabens.[227] This means that it is now possible to examine the net effects of age-specific features of excipient disposition.[79,244] The assessment of potential excipient exposure can include direct evidence about the concentrations that results from prescribed medicines administered during clinical care. The inclusion of this information in risk assessment will reduce uncertainty and inform the development of safety margins.[57] That, among other things, may save time and resources by identification of only those excipients/ excipient concentrations/ products where replacement or reformulation is necessary.

7. CONCLUSIONS

In the course of this research, a unique database of medicinal products used in neonates in European neonatal intensive care units (NICU) was created, which allowed us to assess the extent of excipients use, to determine related risk factors, and to offer possible solutions to reduce neonatal exposure to excipients of interest (EOI).

The following specific conclusions can be drawn from this research:

1. The two cross-sectional study designs used in this research have proven valuable complementary tools in assessing neonatal exposure to excipients, with good correlation between the two methodologies. Simple data structure and longer study period of the service evaluation survey (SES) allows better recruitment, resulting in more comprehensive overview of medicines used in NICUs. The frequency of use on unit level appears a good surrogate of individual exposure rates. However, detailed information on individual exposure, including demographic variables, relevant in excipient associated risk assessment, can be collected in the point prevalence study (PPS). Suitable tool/ database development may be required prior to implementation. Combining different methods with merged data analysis will likely result in optimal coverage of different aspects of the problem.

2. The broad range of medicines with over one thousand different products of highly variable excipient compositions administered to neonates in European NICUs highlights the relevance of excipient related research. Administration of excipients with known safety issues to neonates is common as a third of drug products, reported in our study, contained at least one EOI. Enteral medicines more likely contained all studied excipients except benzalkonium chloride. The largest number of prescriptions with parabens, benzoates, ethanol, sorbitol, and saccharin sodium was associated with enteral vitamins. This should be considered by clinicians when prescribing enteral formulations to neonates; enteral vitamins should be included in the priority list of drugs requiring data in preterm and term neonates.

3. Almost two-thirds of neonates were exposed to at least one EOI, over half of them received more than one. Gestational age (GA) related variations in excipient exposure were predominantly related to increasing prescription rate with decreasing GA and different active pharmaceutical ingredients (API) and formulations prescribed in different GA groups. Extreme prematurity was associated with higher exposure to EOI with antimicrobial properties (parabens and benzoates) compared to term infants, explained by the high use of parenteral antibiotics (i.e. gentamicin) and enteral multivitamin preparations. Term infants more likely received products containing

solvents (polysorbate 80 and propylene glycol) and sweeteners (sorbitol) compared to extremely preterm neonates. This may reflect some degree of deliberate avoidance of EOI in extreme prematurity.

Most commonly used medicines were responsible for most exposure to EOI, meaning that substitution or reformulation of a relatively small number of products would significantly reduce neonatal exposure.

4. Significant geographical variations were found in the use of EOI, independent of excipient functional class. Compared to the eastern region, the likelihood of paraben exposure was higher in the southern and northern, and of polysorbate 80 and propylene glycol lower in the southern and northern region, respectively. In contrast, exposure to saccharin sodium was the lowest in the eastern compared to all other regions. Geographical variations in the use of studied excipients suggest availability of EOI-free medicinal products and potential to reduce exposure through product substitution.

5. Two-thirds of the medicinal products containing EOI could be substituted with EOI-free medicine of the same API and dosage form. Substitution of a relatively small number of frequently used products would spare almost half of currently exposed neonates. The overall cumulative gain may be even greater as in many cases if not completely avoiding, substitution would reduce multiple administration of EOI. Our data support the rational use of studied excipients in many cases, but significant improvement is still possible without major reformulation costs. There is a need for common European market with free movement of medicines between different countries that will contribute to better product substitution opportunities and stimulate product reformulation if needed.

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9. SUMMARY IN ESTONIAN

Abiainete kasutamine vastsündinutele manustatavates ravimites Euroopas

Ravimite tootmisel on lisaks toimeainele vaja kasutada erinevaid farmatseutilisi abiaineid. Abiained on kõik ravimi koostisosad v.a. toimeaine: lahjendajad, täiteained, lahustid, emulgaatorid, sideained, disintegrandid, magusained, säilitusained, lõhna- ja värviained. Farmatseutilises tehnoloogias kasutatakse üle tuhande erineva abiaine, mis moodustavad keskmiselt 90% ravimvormi massist.[2] Abiainetel on ravimvormis/ ravimpreparaadis erinevaid funktsioone ja nad on vajalikud ravimite säilitamiseks, välimuse ja maitseomaduste parandamiseks, erinevate ravimvormide väljatöötamiseks, biosaadavuse parandamiseks jne.[1]

Ravimi ohutus on alati olnud prioriteediks, palju tähelepanu pööratakse eeskätt toimeaine kvaliteedile, efektiivsusele ja ohutusprofiilile. Abiainetel on oma kindel funkstioon ravimvormis ning seega on neil kindlad füsikokeemilised omadused. Viimasel ajal on üha selgem, et ka abiained võivad olla kõrvaltoimete põhjustajaks.[3–6] Tõsiste kõrvaltoimete kõrval võivad abiained põhjustada talumatust, allergiat ning ravimi toimeaine efekti vähenemist.

Arvestades ainevahetuse iseärasusi on teatud abiainete kasutamisega seotud riskid enam väljendunud vastsündinutel, kelle kesknärvisüsteem ja teised elutähtsad organid ei ole veel lõplikult arenenud[10]. Seetõttu võivad teatud ainete minimaalsedki annused põhjustada tõsiseid kõrvalekaldeid organite funktsioonis ja arengus.[13] Näiteks on varajases sünnijärgses perioodis kesknärvisüsteem erakordselt vastuvõtlik mõnedele neurotoksiinidele nagu propüleenglükool ja etanool.[75] Ainevahetuse ja organfunktsioonide iseärasusest tulenevalt on vastsündinutel võrreldes vanemate laste ja täiskasvanutega erinev ka manustatavate ainete s.h. abiainete farmakokineetika ja farmakodünaamika. Lisaks on vastsündinute, eriti enneaegsete puhul, sageli tegemist polüfarmakoteraapiaga; üheaegselt kasutatavate ravimite hulk on tugevas korrelatsioonis kõrvaltoimete tekkega.[130] Ühe metaboolse raja (nt. alkoholi dehüdrogenaas) mitme erineva substraadi (nt. propüleenglükool ja etanool) koos manustamine võib põhjustada toksilise aine (propüleenglükool) kuhjumist organismis.[9]

Vastavalt Euroopa Ravimiameti (EMA; *European Medicines Agency*) juhendile vastsündinutele ravimite väljatöötamiseks tuleb siin „erilist tähelepanu pöörata abiainete valimisele, kuna paljud nendest võivad olla toksilised".[18] Kuigi on andmeid, et mõned abiainetest võivad olla vastsündinutele toksilised ning põhjustada haigestumist või surma, puuduvad selle kohta põhjalikud ülevaated ning süstemaatilisi uuringuid on vähe. Üksikud uuringud on näidanud, et vastsündinutele manustatakse abiaineid märkimisväärsetes kogustes, mis sageli tunduvalt ületavad soovituslikke maksimumannuseid.[14,134] Lass et al. näitasid, et Eesti kahes neonatoloogia osakonnas manustati poole aasta jooksul 88%-le vastsündinutest vähemalt ühte teadaolevate kõrvaltoimetega abiainet.[15] Sarnased tulemused saadi ka Souza et al. poolt Brasiilias[135] ja Fister et al. poolt Sloveenias

läbi viidud uuringutes.[16] Üksikute riikide/ osakondade uuringud osutavad probleemi olemasolule, kuid ei võimalda hinnata selle tegelikku ulatust. Põhjalikud kogu Euroopat hõlmavad andmed abiainete kasutamise kohta vastsündinutel seni puuduvad. Samas võimaldaks need edaspidi planeerida meetmeid ohtlike abiainete kasutuse vähendamiseks vastsündinutel kasutusel olevate ravimite sobivama abiainete koostisega preparaatidega asendamise ning vajadusel ja võimalusel ka uute vastsündinutele mõeldud ravimvormide välja töötamise teel.

Vastsündinutel kasutatavate ravimite rahvusvaheliste uuringute läbiviimiseks võib kasutada erinevaid vaatlusuuringute liike, kuid mitte kõik farmakoepidemioloogias kasutatud meetodid ei sobi abiainete ekspositsiooni uurimiseks.[57,139] Abiainete koostis erineb ühe toimeaine erinevate ravimpreparaatide vahel ja seega tuleb spetsiifilise ravimpreparaadi identifitseerimiseks registreerida andmed nii toimeaine kui ka raviminimetuse, ravimtootja, ravimvormi ja manustamisviisi kohta. Abiainete manustamise kvantitatiivseks hindamiseks on lisaks vaja koguda detailsed andmed ravimkorralduse (annus, manustamissagedus ja -kestus) ning patsiendi demograafiliste parameetrite kohta. Läbilõikeuuringuid võib teostada kahel viisil. Esiteks, osakonna tasemel küsimustiku vormis, mis hõlmaks võimalikult palju osakondi ja annaks ülevaate kasutatavatest ravimitest ja abiainetest. Teiseks, hetklevimusuuringu vormis, mis kirjeldaks individuaalset ekspositsiooni ja selle variatsioone. Siiski on vähe teada erinevate uuringu metoodikate mõjust uuringu tulemustele vastsündinutele abiainete manustamise hindamisel.

Uurimistöö eesmärgid

Projekti peamisteks eesmärkideks oli iseloomustada ravimites esinevate teadaolevate kõrvaltoimetega abiainete (edaspidi EOI, *excipients of interest*) kasutamise ulatust Euroopa vastsündinute osakondades, teha kindlaks nende manustamisega seotud riskifaktorid ning hinnata ravimite asendamise võimalusi vähendamaks vastsündinute ekspositsiooni nendele abiainetele.

Konkreetsed eesmärgid

1. Selgitada välja kahe erineva uuringu metoodika nõrgad ja tugevad küljed Euroopa vastsündinutele manustatavate ravimite/ abiainete uurimisel
2. Koostada andmebaas Euroopa vastsündinutel kasutatavatest ravimitest ja abiainetest
3. Kirjeldada EOI manustamist ja seda mõjutavaid tegureid Euroopa vastsündinutel, selgitamaks välja kõige suurema riskiga olukordi
4. Kirjeldada geograafilisi ja osakondade vahelisi variatsioone EOI manustamises
5. Välja selgitada sageli kasutatavate toimeainete EOI sisaldavate ravimpreparaatide asendamise võimalused Euroopa turul olemasolevate neid abiaineid mitte sisaldavate preparaatidega. Järgnevalt hinnata nende abiainete vastsündinutele manustamise vähenemist juhul, kui kõik ravimpreparaatide asendamisvõimalused oleksid rakendatud

Metoodika

Uuringu eesmärkide saavutamiseks viisime läbi järgmised uuringud:

1. Kolmepäevane uuring Euroopa vastsündinute osakondades (*service evaluation survey*, SES), mille käigus registreeriti 3 päeva jooksul kõik vastsündinutele manustatavad ravimid. Iga ravimi puhul koguti andmed ravimi tootja, kaubandusliku nime (*trade name*), toimeaine, toimeaine kontsentratsiooni, ravimvormi ja manustamisviisi kohta. Uuring viidi läbi 30.05.2011-30.09.2011.
2. Ühepäevane hetkelevimusuuring (*point prevalence study,* PPS) Euroopa vastsündinute osakondades, mille käigus veebipõhises keskkonnas registreeriti kõik vastsündinutele manustatavad ravimid koos patsiendi demograafiliste andmete (sünniaeg, sugu, APGARi hinne, gestatsioonivanus, sünnikaal, kaal uuringu päeval, organpuudulikkused) ning ravimi annustamise detailse informatsiooniga (ravimi tootja, kaubanduslik nimi, toimeaine, toimeaine kontsentratsioon, ravimvorm, manustamisviis, annus ja intervall, ravi algus). Uuring viidi läbi 01.01.2012-30.06.2012.

Uuringutes registreeriti kõik ravimid v.a. verekomponendid, glükoosi ja elektrolüütide lahused, vaktsiinid, piimasegud, rinnapiima rikastajad, taimsed preparaadid ja käsimüügis olevad toopilised preparaadid. Lisaks ravimiinfole kogusime uuritavatest vastsündinute osakondades info osakonna ja teeninduspiirkonna suuruse, osakonna struktuuri ja haigete arvu kohta.

Osalema kutsuti kõik Euroopa Liidu riigid, lisaks Šveits, Island, Norra ja Serbia. Osakondade uuringutesse kaasamise ja andmete registreerimisega tegelesid igas riigis kohalikud koordinaatorid. Uuringutesse kutsuti kõik vastsündinutega tegelevad osakonnad, kus vastsündinud moodustasid >50% osakonna patsientidest, andmed koguti vastsündinute kohta vanuses kuni 28 päeva (k.a.).

SES ja PPS uuringute andmete statistilisel töötlusel kasutasime nii kirjeldavat statistikat kui ühe- ja mitmemõõtmelist regressioonanalüüsi.

Ravimite ekspositsiooni võrdlesime SES uuringus osakonna ja PPS uuringus nii osakonna kui ka individuaalsel tasemel. Kahe uuringu võrdlusanalüüsis kasutasime mitmetasandilise regressioonanalüüsi (*multilevel mixed effects logistic regression models with crossed random effects*). Tulemused olid kohandatud geograafilisele regioonile (Põhja-, Lõuna-, Lääne- ja Ida regioonid)[204] ja osakonna raviprofiili tasemele (esimene, teine ja kolmas etapp)[201,202] kui potentsiaalselt segavatele teguritele, mis tehti kindlaks ettevalmistavas Poissoni populatsiooni regressioonanalüüsis, mis hõlmas 10 sagedamini kasutatavat toimeainet. Võimalust hinnata individuaalset ekspositsiooni läbi osakonna ekspositsiooni vaatasime kasutades polünomiaalset regressioonanalüüsi.

SES ja PPS uuringus kogutud ravimite abiainete koostise leidmiseks töötasime läbi vastavate riikide ravimiinfo materjalid ja ravimite veebipõhised andmebaasid. Kui nendes puudus infomatsioon vastava ravimi kohta, siis küsisime andmeid uuringus osalevatest haiglatest. Üksikutel juhtudel saime infomatsiooni abiainete kvantitatiivse koostise kohta nende tootjatelt.

Edasine detailne analüüs hõlmas kaheksa EOI, mis valiti põhinedes olemasolevatele toksikoloogilistele andmetele. Siia kuulusid polüsorbaat 80, propüleenglükool ja etanool, mida valdavalt kasutatakse lahustuvuse parandamiseks; parabeenid, bensoaadid ja bensalkooniumkloriid, mida kasutatakse tänu nende antibakteriaalsetele omadustele, ning maitseparandajad naatriumsahhariin ja sorbitool.

Vastsündinutele manustatud EOI mahu ja individuaalse ekspositsiooni iseloomustamiseks kasutasime kirjeldavat statistikat ja ühemõõtmelist regressioonanalüüsi, et välja selgitada potentsiaalsed EOI manustamist mõjutavad tegurid. Erinevate faktorite mõju abiainete kasutusele uurisime kasutades nii lapsepõhiseid kui ka ravimkorraldustel põhinevaid binomiaalseid logistilisi segamudeleid, mis olid kohandatud järgmistele ühemõõtmelises regressioonanalüüsis kindlaks tehtud potentsiaalselt segavatele teguritele: geograafiline regioon, gestatsiooni vanus (<28 rasedusnädalat ehk erakordselt enneaegsed, 28 kuni <32 rasedusnädalat ehk väga enneaegsed, 32 kuni <37 rasedusnädalat ehk hilised enneagsed, >37 rasedusnädala ehk ajalised vastsündinud)[205] ja haigla tüüp. Ravimkorraldustel põhinev mudel kohandati lisaks ATC ravimklassile ja manustamisviisile.

Ravimitele, mis sisaldasid EOI ja olid kasutusel rohkem kui 10% osakondadest, hindasime asendamisvõimaluse olemasolu analoogsete ravimpreparaatidega, mis ei sisalda ühtegi EOI. Asendamisvõimaluste olemasolu uurisime kolmes etapis. Esimeses etapis pidi asendamiseks kasutatav ravimpreparaat olema sama toimeaine ja manustamisviisiga, teises etapis lisaks sama ravimvormi ja kolmandas etapis ka sama toimeaine kontsentratsiooniga. Teises etapis loeti kõik parenteraalsed ravimvormid (nt. lahus süstimiseks, pulber süstelahuse valmistamiseks) vastastikku asendatavaks; enteraalsete ravimite puhul olid asenduseks sobivad kõik vedelad ravimvormid (nt. suukaudne lahus, suukaudne suspensioon).

Hindasime ravimpreparaatide asendamise potentsiaalset mõju (teise etapi kriteeriumite järgi) vastsündinute EOI ekspositsiooni mahule, kasutades lihtsat logistilist regressioonanalüüsi.

Peamised tulemused

Kokku osales SES uuringus 20 ja PPS uuringus 21 Euroopa riiki ning vastavalt 115 ja 89 vastsündinute osakonda. SES uuringus registreeriti 313 toimeainet, 1065 ravimpreparaati 332 tootjalt. PPS uuringus osales 1382 vastsündinut, kellest 726-le (52,5%) manustati 2199 ravimikorralduse alusel 562 ravimit. Ühtekokku hõlmas uuring 246 toimeainet. Oodatavalt kasutati enamust ravimitest parenteraalselt (58%). Suukaudsed ja toopilised manustatavad ravimid moodustasid vastavalt 30% ja 12%.

Võrreldes SES ja PPS uuringu metoodikat selgus kõrge omavaheline toimeainete kasutussageduste korrelatsioon kahe uuringu metoodika vahel osakonna tasemel ($R^2 = 0.8605$; $y = 0.6019x - 0.0053$). Toimeaine kasutuse tõenäosus oli

SESis võrreldes PPSiga suurem (OR 2.36; 95% CI 2.05-2.73); seda mõjutas ravimi kasutamise sagedus ja kestvus, OR iga lisanduva kasutusprotsendi ja kasutamise lisapäeva kohta vastavalt 1.01 (95% CI 1.01–1.02) ja 1.02 (95% CI 1.00–1.05).

Polünomiaalses regressioonanalüüsis selgus igat toimeainet saavate laste ja seda kasutatavate osakondade osakaalude omavaheline kõrge korrelatsioon ($R^2 = 0.93$; $\sqrt{y} = 0.421x - 0.004x^2 + 0.485$).

Edasiselt uurisime detailsemalt abiainete kasutamist. Ühtekokku registreerisime kasutatavates ravimites 396 abiaine sisaldust. Vaid 12% manustatud ravimpreparaatidest ei sisaldanud abiaineid, nendest valdav enamus (95%) olid mõeldud parenteraalseks manustamiseks. Kõigist ravimitest 306 (31%) sisaldasid vähemalt ühte EOI ning enam kui üks EOI esines 173 (17%) ravimis. Ootuspäraselt sisaldasid parenteraalsed ravimid EOI harvem kui suukaudsed (OR 0.12; 95% CI 0.09–0.17) ja lokaalselt kasutatavad ravimid (OR 0.16; 95% CI 0.11–0.26). Kõige sagedamini kasutatavad EOI olid parabeenid (43% vastsündinutest eksponeeritud), mille järgnesid polüsorbaat 80 (19%) ja propüleenglükool (17%). Ravitud vastsündinutest 65% (456/726) said vähemalt ühte teadaolevate kõrvaltoimetega abiainet.

EOI kasutus oli seotud gestatsioonivanusega: parabeene, bensoaate ja etanooli kasutati ajalistel vastsündinutel (OR ja 95% CI vastavalt 0.5; 0.3–0.9; 0.3; 0.1–0.8 ja 0.3; 0.1–0.7) ja etanooli hilistel enneaegsetel (OR 0.3; 95% CI 0.1-0-9) erakordselt enneaegsetega võrreldes oluliselt vähem. Eelkõige oli see tingitud erinevate toimeainete kasutamisest erinevates gestatsioonigruppides, sest ravimkorralduste analüüsis, mis lisaks muudele faktoritele oli kohandatud ka ravimiklassile, ei olnud see mõju enam statistiliselt oluline. Seevastu, polüsorbaat 80 kasutati ajalistel ja hilistel enneaegsetel (OR ja 95% CI vastavalt 11.3; 3.9–32.1 ja 3.02; 1.1–8.5) ja propüleenglükooli ja sorbitooli ajalistel vastsündinutel (OR ja 95% CI vastavalt 7.7; 2.98–19.8 ja 6.9; 1.6–29.8) erakordselt enneaegsetega võrreldes oluliselt rohkem.

Mõnede abiainete kasutamises esinesid regionaalsed erinevused, mis jäid oluliseks ka võimalikele neid erinevusi põhjustavatele teguritele (gestatsioonivanus, toimeaine, manustamisviis) kohandatud mudelis. Võrreldes ida regiooniga kasutati polüsorbaat 80 vähem lõuna (OR 0.03; 95% CI 0.002–0.6) ning propüleenglükooli põhja regioonis (OR 0.02; 95% CI 0.004–0.1), parabeene kasutati rohkem põhja ja lõuna (OR ja 95% CI vastavalt 2.97; 1.2–7.1 ja 4.1; 1.6–10.6) ning naatriumsahhariini põhja, lõuna ja lääne piirkonnas (OR ja 95% CI vastavalt 32.7; 2.5–426.5; 101.3; 7.3–1403.6 ja 60.3; 4.2–861.6).

SESi andmebaasi alusel leidsime asendusvõimaluse analüüsi esimese, teise ja kolmanda etapi kriteeriumide järgi vastavalt 120/137 (88%; CI 81–92), 91/137 (66%; 95% CI 58–74) ja 42/137 (31%; 95% CI 24–39) ravimile. Teise etapi asenduse kriteeriumeid arvestades on võimalik vähendada parenteraalselt ja enteraalselt manustatavate EOI sisaldavate toimeainete arvu vastavalt 12lt ühele (92% vähenemine; 95% CI 65–99) ja 17lt üheksale (47% vähenemine; 95% CI 26–69).

Ainuüksi sageli kasutatavate ravimite asendamine vähendaks nende läbi EOI eksponeeritud vastsündinute arvu 85% (315-lt 46-le) ja kõigi EOI eksponeeritute arvu 44% võrra (456-lt 257-le). Kõikide EOI, v.a. etanooli, sorbitooli ja naatriumsahhariini manustamise tõenäosus vähenes asendusvõimaluste arvesse võtmisel statistiliselt oluliselt. Alprostadiil oli ainus parenteraalne toimeaine, mille ravimpreparaatide hulgas puudus etanoolivaba ravimvorm.

Järeldused

Antud uurimistöö käigus on koostatud unikaalne andmebaas Euroopa vastsündinutel kasutatavate ravimite kohta, mis võimaldab hinnata abiainete manustamise mahtu vastsündinutel, identifitseerida seotuid riske ja näidata võimalikke lahendusi vähendamaks vastsündinute ekspositsiooni teadaolevate kõrvaltoimetega abiainetele.

Uurimistöö konkreetsed järeldused:

1. SES metoodika oma lihtsama andmestruktuuriga võimaldab kaasata rohkem vastsündinutega tegelevaid osakondi ja annab ammendavama ülevaate vastsündinutel kasutatavate ravimite ja abiainete nimistust. PPS annab võimaluse kirjeldada vastsündinute individuaalset ekspositsiooni ja kindlaks teha selle võimalikud riskifaktorid Euroopa riikides. Kahe erineva metoodikaga kogutud tulemusi saab kasutada teineteist täiendavana. Metoodika valik konkreetse uuringu jaoks sõltub ennekõike püstitatud uuringuküsimusest. Üldise ravimikasutuse kirjeldamisel võiks eelistada SES metoodikat, mis võimaldab väiksema tööjõu ja ressursside kuluga saada ammendavama ülevaate; kogutud andmeid saab kasutada kaudseks individuaalse ekspositsiooni hindamiseks üldpopulatsioonis. Kui eesmärgiks on detailne demograafilisi parameetreid arvesse võttev ravimikasutuse analüüs, siis tuleks eelistada PPS metoodikat, mis võimaldab paremini iseloomustada ravimikasutusega seotud individuaalseid riskifaktoreid.

2. Euroopa vastsündinute osakondades on kasutusel üle tuhande erineva ravimpreparaadi, mille abiainete sisaldus varieerub ulatuslikult. Kolmandik ravimpreparaatidest sisaldas vähemalt ühte EOI. Suukaudsed ravimvormid sisaldasid kõiki uuritud EOI, v.a. bensalkooniumkloriidi, sagedamini kui parenteraalselt manustatavad. Eeltoodut tuleks arvesse võtta suukaudsete ravimvormide/ ravimpreparaatide määramisel vastsündinutele.

3. Peaaegu kahele kolmandikule vastsündinutest manustati vähemalt ühte teadaolevate kõrvaltoimetega abiainet. Gestatsioonivanusega seotud riski erinevused tulenesid peamiselt erinevate toimeainete ja ravimpreparaatide kasutamisest erinevates gestatsioonigruppides. Äärmiselt enneaegsetele manustati võrreldes ajaliste vastsündinutega enam antimikroobseid abiaineid

(parabeenid, bensoaat). Peamiseks põhjuseks on parenteraalsete antibiootikumide ja suukaudsete multivitamiinide laialdane kasutus selles grupis. Võttes arvesse erinevusi toimeainete kasutuses ja manustamisviisides, manustati ajalistele vastsündinutele sagedamini lahustite gruppi kuuluvaid abiaineid (polüsorbaat80, propüleenglükool) ja magusaineid (sorbitool). Nimetatud erinevus võib peegeldada mõningast teadlikku EOI kasutuse vältimist äärmiselt enneaegsetel vastsündinutel. Enamikul juhtudel manustati just sageli kasutatavate ravimite koostises EOI, seega võiks suhteliselt väikese arvu ravimite asendamine EOI-vabade ravimvormidega oluliselt vähendada vastsündinute ekspositsiooni nendele ainetele.

4. Kõikide uuritud abiainete klasside kasutuses esines regionaalseid erinevusi. Võrreldes ida regiooniga kasutati põhja ja lõuna piirkonnas parabeene suurema ning propüleenglükooli ja polüsorbaat 80 väiksema tõenäosusega. Magusainete kasutus oli ida regioonis kõikidest teistest piirkondadest madalam. Geograafiliste erinevuste olemasolu näitab, et mitte kõigis tänastes ravimites ei ole EOI olemasolu ilmselt hädavajalik. Lisaks viitab see ravimpreparaatide asendamisvõimalusele Euroopa turul juba olemasolevate ravimprepaatidega vältimaks EOI manustamist vastsündinutele.

5. Kaks kolmandikku sagedamini kasutatavatest EOI sisaldavatest ravimpreparaatidest oleks teoreetiliselt võimalik asendada EOI-vabade ravimvormidega. Selline asendamine Euroopa turul olemasolevate EOI-vabade ravimvormidega säästaks peaaegu pooled vastsündinud ebavajalikust ekspositsioonist. Ühtne Euroopa turg ravimite vaba liikumisega aitaks kaasa ravimite asendusvõimaluste kättesaadavuse parandamisele ja stimuleeriks uute ravimvormide välja töötamist, kus see osutub vajalikuks. Ravimasenduse võimaluste rakendamisega seotud tehniliste ja logistiliste küsimuste lahendamiseks on vaja kõikide osapoolte tihedat koostööd.

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PUBLICATIONS

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