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Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age

EFSA Scientific Committee

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Abstract

Following a request from the European Commission to the European Food Safety Authority (EFSA), EFSA's Scientific Committee (SC) prepared a guidance for the risk assessment of substances present in food intended for infants below 16 weeks of age. In its approach to develop this guidance, the EFSA SC took into account, among others, (i) an exposure assessment based on infant formula as the only source of nutrition; (ii) knowledge of organ development in human infants, including the development of the gut, metabolic and excretory capacities, the brain and brain barriers, the immune system, the endocrine and reproductive systems; (iii) the overall toxicological profile of the substance identified through the standard toxicological tests, including critical effects; (iv) the relevance for the human infant of the neonatal experimental animal models used. The EFSA SC notes that during the period from birth up to 16 weeks, infants are expected to be exclusively fed on breast milk and/or infant formula. The EFSA SC views this period as the time where health-based guidance values for the general population do not apply without further considerations. High infant formula consumption per body weight is derived from 95th percentile consumption. The first weeks of life is the time of the highest relative consumption on a body weight basis. Therefore, when performing an exposure assessment, the EFSA SC proposes to use the high consumption value of 260 mL/kg bw/day. A decision tree approach is proposed that enables a risk assessment of substances present in food intended for infants below 16 weeks of age. The additional information needed when testing substances present in food for infants below 16 weeks of age and the approach to be taken for the risk assessment are on a case-by-case basis, depending on whether the substance is added intentionally to food and is systemically available.

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Keywords: infants, neonates, ADI, health-based guidance values, development

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Amendment: An editorial correction was carried out that does not materially affect the contents or outcome of this scientific output. To avoid confusion, the older version has been removed from the EFSA Journal, but is available on request, as is a version showing all the changes made.

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Summary

Upon a request by the European Commission to the European Food Safety Authority (EFSA), a guidance was prepared by EFSA's Scientific Committee (SC) for the risk assessment of substances in food intended for infants below 16 weeks of age, taking into particular consideration the procedures to derive health-based guidance values (HBGV).

The EFSA SC considered that for the purpose of this guidance, the term 'infants below the age of 16 weeks' was used to describe the particular infant subpopulations where HBGV had traditionally not been considered applicable. This population includes preterm neonates receiving enteral feeding.

In preparing this guidance, EFSA's SC considered the physiological, developmental and nutritional aspects that are specific for infants below 16 weeks of age, with particular attention to the latest scientific developments in the field.

The EFSA SC concluded that high infant formula consumption per body weight is derived from 95th percentile consumption with the first weeks of life being the time of the highest relative consumption on a body weight basis. Therefore, when performing an exposure assessment for substances in infant formula, the EFSA SC proposes to use the highest consumption figure reported for the period of 14 to 27 days of life, which corresponds to a value of 260 mL/kg bw per day. This value would also cover the potential high consumption rates of preterm infants on enteral (formula) feeding and can be used when carrying out an exposure assessment of substances intentionally or not intentionally added to food, such as contaminants. For the exposure assessment, infant formula is expected to be the only source of nutrition for the first 16 weeks of life in non-breastfed infants.

Gastric, pancreatic and biliary functions are not fully mature at birth. However, preterm and term neonates display a mature-type intestinal epithelium. The local effects of a substance on the gastrointestinal tract, its absorption and its systemic fate will depend on local pH, transit time, size of the absorptive surface or its metabolism by the gastrointestinal wall and by the bacterial microbiome, all parameters changing during postnatal maturation.

Absorption of substances in general might be slower in infants below 16 weeks of age than in older ones, but the amount absorbed is not dependent on the age. Once absorbed and systemically available, the distribution of the substance may be different from that in adults owing to the age-dependent changing of body composition (neonates: larger extracellular and total-body water spaces and lower fat content). Other factors to be considered are the differences in regional blood flow, organ perfusion and cardiac output between infants and adults. In addition, total plasma protein binding capacity (in particular albumin) is lower in infants.

Infants show a unique pattern of development for individual enzyme isoforms involved in phases I and II metabolism, with a reduced capacity at birth. However, owing to overlapping substrate specificities, the overall impact on the clearance and half-life of substances (decrease in clearance, increase in half-life) is limited, being generally up to 2- (full-term) to 3-fold (preterm) lower when comparing infants with healthy adults. Likewise, renal function is also reduced at birth and increases in the first year of life. Therefore, if the precise metabolic pathway is not known and if it is not known to which extent the substance is excreted by metabolic elimination or by renal excretion an additional uncertainty factor of 3 should be considered as a conservative option to account for the reduced elimination/excretion. When the precise metabolic pathway for a given substance is known, reported age-specific ratios child/adult can be used. In rare instances where the data are available for the metabolic clearance for this age group, then chemical-specific uncertainty factors can be used. Preterm infants under medical supervision would not be sufficiently protected by the additional uncertainty factor of 3 due to the considerable immaturity of the ADME system, and would necessitate a case-by-case approach. For compounds, for which effects are depending on long-term body burden (e.g. cadmium, dioxins), the impact of immaturity of metabolism and excretion is likely to be minimal.

The developing blood brain barrier (BBB) is similar to the adult BBB, in that it possesses functional tight junctions and influx and efflux transporters. Hence, contrary to previous assumptions, the healthy BBB in the first 16 weeks, although immature, is considered functional and not leaky. However, the expression/activity of transporters and metabolising enzymes appears to vary from adult patterns to meet the needs of the developing brain. The movement of molecules across the BBB depends on their physicochemical properties, the local physiological milieu (e.g. pH) and the need for

transporters and their ontogeny, activity, and specific location. The differences may result in greater vulnerability to chemicals than in the adult. Uncertainty in predicting specific exposure of the Central Nervous System (CNS) to chemicals derives from knowledge gaps concerning the degree to which alternative pathway barriers such as the blood choroid plexus barrier are functional and the precise differentiation state of the BBB.

The developing immune system in fetal and early postnatal life is particularly sensitive and exposure to immunotoxicants may result in persistent effects on the immune system that last or appear only long after exposure and in general, may also occur at lower doses than during adult exposure. In addition, effects on immune function seen upon perinatal or adult exposure may be different. A number of compounds or types of exposure may produce different ranges or severities of outcomes depending on the time of exposure during the immune system development. Different and unpredictable arrays of alterations (quantitative and qualitative) may be expected when the exposure occurs *in utero* or in the early neonate versus adult exposure. They may be associated with chronic immunological conditions such as autoimmunity, immune deficiency, inflammation and allergic reactions. Altogether, neonates have specific features in the development and maturation of their immune system, which make their response to an immunogenic/allergenic compound different from that of an adult and therefore, deserve specific studies.

The development of the female reproductive system in infants below 16 weeks of age is relatively quiescent. However, for the development of the male reproductive organ, the testis, this is a sensitive period. The early postnatal testis exhibits a high proportion of immature Sertoli cells, which proliferate from birth until 6 months of age when the adult number of Sertoli cells is achieved. Effects on the development of the reproductive organs generally may become apparent later in life.

Infants below 16 weeks of age have specific endocrine profiles that are different from those in adults. This suggests that such differences may require particular attention for risk assessment of chemicals. Effects at any early developmental stage may be reflected in deficits at later time points, indicating that the window of exposure, the window of maximum sensitivity and the window of effect require a lifespan approach to generate a fully protective risk assessment.

The EFSA SC notes that during the period from birth up to 16 weeks, infants are expected to be exclusively fed on breast milk and/or infant formula. The EFSA SC views this period as the time where health-based guidance values for the general population do not apply without further considerations. The EFSA SC made the following considerations when developing a testing strategy for substances in food for infants below 16 weeks of age. Testing strategies for substances present in food for infants need to take into consideration the differences in development stages of the relevant organ systems and the related critical windows of maximum sensitivity between humans and laboratory animals used in safety testing. The safety evaluation of substances that may be present (intentionally or not) in the foods consumed by infants below the age of 16 weeks of age should follow the general principles set for the safety evaluation processes used for older infants and adults and can make use of information already obtained in such studies. The testing programmes for the safety evaluation of substances in food used for the general population will depend on the applicable guidance and legislations. Typically, these studies will provide information on ADME, sub-chronic and chronic toxicity, genotoxicity, carcinogenicity and reproductive toxicity. However, in view of the potential greater sensitivity during the first few weeks of life, the EFSA SC considers that additional studies may be necessary for the risk assessment of substances present in food consumed by this population.

The EFSA SC has endorsed the following approach to evaluate the safety of substances in food for infants below 16 weeks of age and has developed the following decision tree approach to support a risk assessment.

For substances added intentionally to food for infants below 16 weeks of age, an Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) would be required. The EOGRTS should include cohorts to assess the potential impact of a test substance on the reproductive and developmental system, on the developing nervous system and on the developing immune system. As exposure through mother's milk in experimental studies is usually substantially lower than the exposure through feed, the resulting doses in the neonatal animals may be insufficient for hazard characterisation. Therefore, for the purpose of this guidance, it is advised that direct dosing of the neonatal animals should be considered as soon as possible after birth. When standard toxicological studies do not show adverse effects in adult animals and the ADME studies show that a substance is

not absorbed, only a repeated dose study with direct oral administration to neonatal animals [preferably the (mini)pig] is needed. This latter study should include analysis of possible local effects on the gastrointestinal tract and on a possible reduction in the bioavailability of nutrients (minerals and vitamins) that are normally contained in food for infants. Human data (epidemiological, clinical studies and case reports, where available) should be included in the evaluation. The general principles underpinning the risk assessment performed according to the guidance or recommendations established by the different EFSA panels and units and prevailing legislations should be applied.

For substances not intentionally added to food for infants of this age group, the EFSA SC concludes that their presence in food for infants should be avoided as much as possible. The EFSA SC furthermore considered that current knowledge of the ontogeny of the metabolic and excretory pathways permits now to predict the toxicokinetic behaviour of substances if their metabolism in the adult is known. Even if their metabolic fate is not known in detail, the overall potential impact of the toxicokinetic variability in infants can be addressed by using an additional kinetic uncertainty factor (UF) to account for the differences between the adult population and the infants, in particular the very young ones. However, the EFSA SC is of the opinion that general quantitative and qualitative predictions of adverse effects in infants of this age group cannot be made and that the uncertainty surrounding the toxicodynamic variability in infants of this age group does not allow identification of appropriate additional UFs. Therefore, additional considerations on a case-by-case basis are needed to decide whether there is a health concern. In case studies on neonatal animals are available, these are expected to address the variability discussed above.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Background

The composition of foods intended for infants and young children is regulated at EU level and such rules include requirements concerning the use and/or presence of substances such as food additives, pesticide residues, contaminants and substances migrating from food contact materials in those foods. Adopting legal provisions on those matters that are in line with the current scientific knowledge requires a comprehensive evaluation to be carried out by EFSA on a number of aspects, including the appropriateness of health-based guidance values for infants below 16 weeks of age.[1]

Relevant legal framework

FOOD ADDITIVES IN FOOD FOR INFANTS AND YOUNG CHILDREN

The use of food additives is regulated by Regulation (EC) No 1333/2008[2] on food additives. Only food additives that are included in the Union list, in particular in Annex II to that regulation, may be placed on the market and used in foods under the conditions of use specified therein. Some of these are specific uses of food additives in food destined for infants and young children (food category 13.1) including uses in food/formulas for infants from birth onwards.

PESTICIDES IN FOOD FOR INFANTS AND YOUNG CHILDREN

Regulation (EU) No 609/2013[3] on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control foresees that the specific requirements on pesticides for infant formula, follow-on formula, processed cereal-based food, baby food and food for special medical purposes developed to satisfy the nutritional requirements of infants and young children should be updated regularly and include, inter alia, provisions to restrict the use of pesticides as much as possible [Article ll (l)(b)].

CONTAMINANTS IN FOOD FOR INFANTS AND YOUNG CHILDREN

EU rules ensure that food placed on the market is safe to eat and does not contain contaminants at levels which could threaten human health. In order to protect public health, and specifically the vulnerable group of infants and young children, strict maximum levels are established for certain contaminants in foods for infants and young children in the frame of Regulation (EEC) No 315/93.[4]

FOOD CONTACT MATERIALS FOR INFANTS AND YOUNG CHILDREN

Food comes into contact with many materials and articles during its production, processing, storage, preparation and serving, before its eventual consumption. Such materials and articles are called Food Contact Materials (FCMs). FCMs should be sufficiently inert so that their constituents neither adversely affect consumer health nor influence the quality of the food. To ensure the safety of FCMs for consumers including infants and young children, specific migration limits are established for certain substances for example in the frame of Regulation (EU) No 10/2011.[5]

Terms of reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002,[6] the European Commission requests the European Food Safety Authority to provide a scientific opinion on scientific and technical

[1] Opinion of the Scientific Committee on Food on the applicability of the ADI (Acceptable Daily Intake) for food additives to infants (expressed on 17/09/1998); Opinion of the Scientific Committee for Food on: A maximum residue limit (MRL) of 0.01 mg/kg for pesticides in foods intended for infants and young children (expressed on 19/09/1997) and Further advice on the opinion of the Scientific Committee for Food expressed on the 19 September 1997 on a Maximum Residue Limit (MRL) of 0.01 mg/Kg for pesticides in foods intended for infants and young children (expressed on 4/06/1998).
[2] OJ L 354, 31.12.2008, p. 16
[3] OJ L 181, 29.6.2013, p. 35
[4] OJ L 037, 13.2.1993, p.1
[5] OJ L 12, 15.1.2011, p. 1
[6] OJ L 31, 1.2.2002, p. 1

guidance for the risk assessment of substances – food additives, pesticide residues, contaminants and substances migrating from food contact materials - present in food intended for infants below 16 weeks of age. In particular, EFSA is requested to provide advice to the European Commission on the generic criteria and/or requirements that need to be considered by EFSA when performing the risk assessment of substances present in food intended for infants below 16 weeks of age taking into account the relevant provisions of the entire above mentioned legal framework.

1.2. Interpretation of the Terms of Reference

The Terms of Reference require a framework applicable to all relevant working areas of EFSA. Therefore, EFSA's Scientific Committee (SC) will prepare a guidance for the risk assessment of substances in food intended for infants below 16 weeks of age, taking into particular consideration the procedures to derive health-based guidance values (HBGV).

1. The term 'infants below the age of 16 weeks' is used to describe the particular infant subpopulations where HBGV such as ADI, TDI or ARfD have traditionally not been considered applicable. This follows the earlier conclusions by the former EU Scientific Committee on Food (SCF) and the World Health Organization (WHO) that HBGVs do not apply to populations of infants below the age of 16 and 12 weeks, respectively.

2. The rationale for considering and defining a cut-off value for the age from when a HBGV may apply is complex. It includes different considerations:

- Physiology: maturation of the different physiological processes and organs in the body is completed at different ages, and the time frame may be extremely variable between individuals. Moreover, this population presents a great variability because differences between gestational age and chronological age may be present, e.g. whether the child is born preterm or at term; the chronological age may not be representative of the actual development.
- Nutrition: diet changes with age after birth, from restricted to milk in the first months of age to a more complex one with the introduction of complementary feeding over time.

The EFSA SC considered that the respective SCF and WHO conclusions seem to be pragmatic rather than scientifically based. Therefore, for the purpose of this guidance, the term 'infants below the age of 16 weeks' is used to describe the particular infant subpopulations where HBGV have traditionally not been considered applicable. This population includes preterm neonates receiving enteral feeding.

3. The guidance should clarify the issues and information requirements that need to be considered when performing a risk assessment of substances in food intended for the population of interest. As specified in the request from the Commission, the Risk Assessment guidance offered is expected to apply also to assessing potential risks from food additives, pesticides, contaminants and food contact materials.

4. The guidance should consider the physiological and developmental aspects that are specific for infants below 16 weeks of age, with particular attention to the latest scientific developments in the field. The physiological processes and organ systems to be included are the gastrointestinal tract, the metabolic and renal capacities relevant for the elimination of chemicals, the immune-, endocrine- and reproductive systems, the nervous system including the brain and brain barriers.

5. The guidance focusses on the oral route of exposure because the Terms of Reference relate to substances present in food.

6. The guidance should address specific experimental systems that would be appropriate to characterise the hazard of substances present in food intended for infants below 16 weeks of age.

As regards how to perform an adequate risk assessment for a population of this age, the following points will be discussed in this guidance:

- What makes this population different from the older ones?
- What kind of relevant information can be retrieved from the currently available data from biological experimental studies?
- What kind of data is needed?

- What methodology can be used for risk assessment for this age group? Can a tiered approach defining successive steps of knowledge be applied? Would additional uncertainty/safety factor(s) be required?

1.3. Additional information

This guidance is primarily aimed at EFSA Units and Panels performing risk assessments of substances present in food intended for infants below 16 weeks of age. The EFSA SC considers that the application of this guidance document is unconditional for EFSA and for all parties submitting assessments of substances present in food intended for infants below 16 weeks of age to EFSA for peer-review (EFSA Scientific Committee, 2015).

2. Data and methodologies

Literature searches were performed in Web of Science (http://wok.mimas.ac.uk/) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) to identify publications in the public domain in the following areas relevant to the developing infant.

- Toxicology
- Nutritional needs
- Physiological and biochemical considerations
    - Physiology of the gut
    - Metabolic and excretory capacities relevant for the elimination of chemicals
    - The nervous system, including brain and brain barriers
    - The immune system
    - The male and female reproductive systems
    - The endocrine system

Comprehensive reviews covering the above areas were identified in order to recognise recent developments since the latest comprehensive assessments of infant toxicology and development by national and international authorities and agencies. The periods covered ranged from January 2012 to February 2016 and the searches were performed in Web of Science. The search limits were document type = review and search terms in title. The search terms combined were 'intestin*' OR 'gastro*' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 98; selected 5); 'metabolism' OR 'cytochrome' OR 'CYP*' OR 'glucuronidation' OR 'glucuronosyl*' OR 'sulfation' OR 'sulphation' OR 'sulfonyl*' OR sulphonyl*' OR 'acetylation' OR 'conjugation' OR 'glutathione OR 'transporter' or 'clearance' OR 'ADME' AND 'development*' OR 'ontogeny' OR 'infant' OR 'neonat*' (retrieved 121; selected 10); 'brain' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 256; selected 21); 'immun*' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 289; selected 14); 'reproduct*' OR 'gonad*' OR 'sexua*' OR 'endocrin*' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 105; selected 25). The selection criteria included coverage of the perinatal or postnatal period and relevance to the assessment. Additional primary references of particular relevance were identified by the working group members (up to May 2016).

In addition, publicly available guidance documents and reports produced by committees and international authorities such as the JECFA, SCF, EFSA and the European Medicines Agency (EMA) that were relevant to risk assessment of substances in food intended for infants were considered.

3. Approach taken to develop this guidance

3.1. Risk assessment of substances present in food intended for infants: Past and current approaches

An FAO/WHO meeting on additives in baby foods was convened in 1971, and its draft report was subsequently modified and adopted by the JECFA (FAO/WHO, 1972). The report made an arbitrary distinction between children aged less than 12 weeks and older children, and considered it prudent

that food intended for infants under 12 weeks of age should not contain any additives at all. However, if it were deemed necessary to use additives in food intended for young infants, the JECFA concluded that 'particularly for infants under 12 weeks, toxicological investigations should be more extensive and include evidence of safety to young animals.' The JECFA also concluded that 'the establishment of acceptable residue levels of pesticides or other contaminants likely to be present in milk and cereals for infant foods should be based on toxicological evaluation in very young animals' (FAO/WHO, 1972).

These concepts were further elaborated by WHO in (International Programme on Chemical Safety (IPCS), 1987) where the need for toxicological studies covering the corresponding period of life and the limitation of current testing approaches were noted for cases when young infants are a target population for an additive. This WHO report stated that 'scientific evidence indicates that newborns and very young children are particularly sensitive to the harmful effects of foreign chemicals' due to, inter alia, 'immaturity of enzymatic detoxifying mechanisms, incomplete function of excretory organs, low levels of plasma proteins capable of binding toxic chemicals, and incomplete development of physiological barriers such as the blood-brain barrier. Moreover, there appears to be a general vulnerability of rapidly growing tissues, which is particularly important with regard to the developing nervous system' (International Programme on Chemical Safety (IPCS), 1987).

In a workshop organised by the ILSI Europe Acceptable Daily Intake Task Force on the applicability of the acceptable daily intake (ADI) to infants and children held in 1997, the differences between infants or children and adults from susceptibility and food intake points of view and the adequacy of testing methods and safety factors were discussed (Larsen and Pascal, 1998). Among the recommendations, it was proposed that no special safety factors, and consequently no special ADIs, should be used for infants and children and that any evidence of enhanced sensitivity of this age group to a particular food additive must drive the derivation of the ADI. However, because the usual toxicological test battery does not mimic the human situation with ingestion of infant formula, the ADI should not be considered directly applicable to infants below 12 weeks of age.

In 1998, the SCF expressed an opinion on the applicability of the ADI for food additives to infants (Scientific Committee on Food (SCF), 1998). The SCF noted that the scientific support for the 12 week cut-off was not presented in any details in the 1972 JECFA report (FAO/WHO, 1972). While acknowledging the importance of the physiological immaturity of many of the organ systems in the first weeks and hence an enhanced vulnerability, the SCF advocated a 16-week cut-off because of the nutrition habits up to that age (i.e. mother's milk or infant formulae intended for use as the sole source nutrition for infants). Furthermore, the standard animal studies recommended by the JECFA and SCF to assess food additives do not address the exposure situation i.e. exclusive feeding via infant formula, relevant to this specific age group.

A systematic analysis of the scientific principles to be considered in assessing health risks in children from exposures to environmental agents during distinct stages of development, is reported in (International Programme on Chemical Safety (IPCS), 2006). In 2009, the WHO re-iterated its recommendations that 'very young infants are a particularly sensitive subgroup because their metabolic capacities are not yet fully developed' and that 'health-based guidance values are not considered applicable to infants under the age of 12 weeks who might be at risk at lower levels of exposure. Accordingly, risk characterization of exposure of such infants to chemicals (e.g. in infant formula or occurring as contaminants) has to be considered on a case-by-case basis' (International Programme on Chemical Safety (IPCS), 2009).

At its 79th meeting (FAO/WHO, 2014), the JECFA evaluated three food additives intended for use in infant formulae and reported general considerations for use of the margin of exposure (MOE) for the evaluation of such food additives. The JECFA noted that the ADI concept does not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups and that risk characterisation for very young infants has to be considered on a case-by-case basis. The report noted that while reproductive and developmental studies address possible impacts of *in utero* and lactational exposure on neonates, studies involving direct oral administration to neonatal animals are required for the evaluation of additives to be used in infant formulae. If the food additive is intended for use in infant formula at relatively high levels (e.g. 0.1% or greater), then conducting toxicological studies in neonatal animals at doses two or more orders of magnitude greater than the anticipated human exposure, which is the approach commonly taken for food additives, may not be feasible. Therefore, the JECFA considered the MOEs between the respective NOAEL and the

estimated daily exposures to the food additives, and described toxicological and exposure considerations that need to be taken into account in interpreting the MOE (see Table 1).

Table 1. Toxicological and exposure considerations to be taken into account in interpreting the MOE in the evaluation of food additives used in infant formula, as proposed by the JECFA.

Considerations related to the toxicological point of departure[1]
Absorption, distribution, metabolism and excretion in the neonatal organism
Overall toxicological profile of the substance (including identification of critical effects)
Potential effects of exposure during life stages in experimental animals of relevance to human infants
Relevance for the human infant of the neonatal animal models used in toxicological testing
Adverse effects: identified in studies in neonatal animals or is the NOAEL the highest dose tested?
Design and outcome of any clinical studies conducted with infants
Reports of adverse reactions in post-marketing surveillance
Considerations related to the dietary exposure assessments
Formula is the only source of nutrition for the first 12 weeks of life in non-breastfed infants
The additive is used at the maximum proposed level
An energy density of 67 kcal/100 mL (280 kJ/100 mL) is used to convert energy to the volume of formula ingested daily
High infant formula consumption is derived from 95th percentile energy intakes
Variability of exposure among infants is small
Duration of exposure is for a limited time, and exposure decreases on a body weight basis during the exposure period

[1] The point of departure (PoD) is equivalent to the Reference Point (RP) used by EFSA.

3.2. Working definitions for stages in early human development for this guidance

Several classifications of the paediatric population into age categories exist and are to some extent arbitrary due to considerable overlap in developmental (e.g. physical, physiological, cognitive and psychosocial) issues across the age categories. From an EU legislative consideration,[7] the term 'infant' means a child under the age of 12 months and 'young child' means a child aged between 1 and 3 years. For the purpose of this guidance, the population of interest corresponds to infant subpopulations where health-based guidance values such as ADI have traditionally not been considered applicable, herein referred to as 'infants below 16 weeks of age'. Concerning the stage of physiological development, it is to be noted that this population includes term and preterm neonates (Figure 1).

[7] REGULATION (EU) No 609/2013 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009

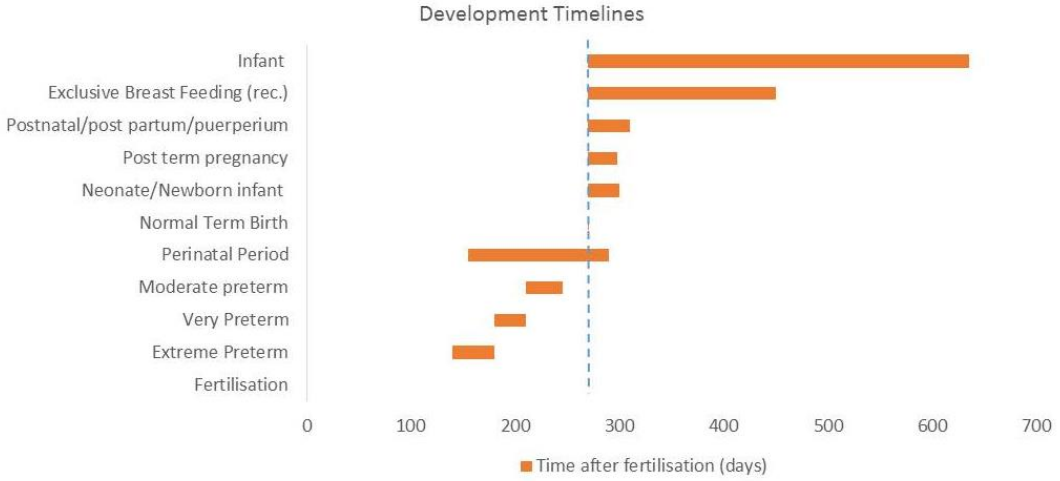

Figure 1. Key life stages

The continuous vertical line represents fertilisation and the dotted vertical line represents normal term birth. Abbreviation: rec., recommended.

4. Nutritional needs of infants

4.1. Feeding pattern of infants during the first months of life

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) advises that exclusive breastfeeding for around six months is a desirable goal for nutrition of infants (Agostoni *et al.*, 2010). Similarly, the WHO recommends that 'Exclusive breastfeeding for 6 months has many benefits for the infant and mother' and 'Around the age of 6 months, an infant's need for energy and nutrients starts to exceed what is provided by breast milk, and complementary foods are necessary to meet those needs' [(WHO, 2001; 2016); see also (Kramer and Kakuma, 2001)]. If breastfeeding is not possible, infants should receive an infant formula. Regulation (EU) No 609/2013[8] defines infant formula as 'food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding' [Article 2(2)(c)].

In its Scientific Opinion on the appropriate age for introduction of complementary feeding of infants, EFSA concluded that the introduction of complementary food into the diet of healthy term infants in the EU between the age of four and six months is safe and does not pose a risk for adverse health effects (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009). Therefore, in non-breastfed infants, infant formula should represent the sole source of nutrition until at least the age of four months. The essential composition of infant formula is laid down in EU law. Currently, Commission Directive 2006/141/EC[9] applies but will be replaced by Commission Delegated Regulation (EU) 2016/127[10], applicable from 22 February 2020, except for infant formula and follow-on formula manufactured from protein hydrolysates, to which it shall apply from 22 February 2021.

[8] Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, p. 35

[9] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC.

[10] Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information

The first months of life are characterised by high requirements of energy intake per kilogram body weight (kg bw) due to the rapid growth rate and increase in body weight (Table 2). While energy intake per kg body weight peaks in the first two months and decreases afterwards, total energy intake (kcal/day) increases during infancy. Similar changes occur in consumption of breast milk or infant formula whilst they are the sole source of food for infants in the first four to six months of life.

Table 2. Reference values for body weight and energy requirements of infants in the first months of life (taken from (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2013).

Age	Body weight[1]		Average energy requirement		Average energy requirement	
Months	Kg		kcal/kg bw per day		kcal/day	
	Boys	Girls	Boys	Girls	Boys	Girls
0 to <1	---		109	103	359	329
1 to <2	4.5	4.2	112	107	505	449
2 to < 3	5.6	5.1	95	92	531	472
3 to < 4	6.4	5.8	78	79	499	459
4 to < 5	7.0	6.4	78	79	546	503

[1] 50th percentile of WHO growth standards.
Abbreviation: bw, body weight.

4.2. Consumption data in use by EFSA for the risk assessment of substances present in food for infants

For infants under six months of age, EFSA based its exposure estimates to contaminants present in breast milk and infant formula on standard values for three-month-old infants using a weight of 6.1 kg, an estimated average daily consumption of 800 mL and a high consumption of 1,200 mL [e.g. (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010)]. These figures were derived from the following sources and considerations: 'According to the Institute of Medicine of the U.S. National Academies of Sciences (IOM), average breast milk consumption is about 750 to 800 g per day (range: 450 to 1,200 g per day) for the first four to five months of life (Institute of Medicine (IOM), 1991). Infant birth weight and nursing frequency have been shown to influence consumption (Institute of Medicine (IOM), 1991). The WHO related breast milk consumption to body weight rather than age with an estimated 125 mL/kg bw or 763 mL per day for a three-month-old child weighing 6.1 kg (Onyango *et al.*, 2002). According to the German DONALD study, mean consumption of infant formula for a three-month-old child weighing on average 6.1 kg, was 780 mL/day with a 95th percentile consumption of 1,060 mL/day (Kersting *et al.*, 1998). The DARLING study, performed in the USA in the early 1990s, points out significant differences in milk intake between formula-fed infants and breastfed infants: mean milk intake was lower at 3 months (812 ± 133 mL vs 905 ± 162 mL) and 6 months (769 ± 171 mL vs 941 ± 238 mL) in breastfed compared with formula-fed infants, respectively (both $p<0.05$) (Heinig *et al.*, 1993).

In 2011, EFSA started to use data from the EFSA Comprehensive European Food Consumption Database for the assessment of dietary exposure to hazardous chemicals. This database includes food consumption data for infants up to and including 11 months. In 2011, information on infants were available from only two Member States (Italy and Bulgaria), whereas in 2015 food consumption data from additional four Member States (Germany, Finland, Denmark and United Kingdom) were added. Consumption statistics of infant formula (liquid) in infants in the first year of life from the EFSA Comprehensive European Food Consumption Database are reported in Table 3. Food consumption data on infants below four months are only available from one survey carried out in Bulgaria, with a 95th percentile of consumption equal to 1120 mL/day (274.2 g/day per kg bw) for consumers of infant formula. Dietary surveys on infants are currently ongoing in different EU countries within the EU Menu project and their data will be included in the Comprehensive Database as soon as they are made available to EFSA.

requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding.

544 At present, data for infants in use by EFSA do not allow time course quantitation of formula
545 consumption during the first sixteen weeks of life.

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Table 3. Consumption of infant formula (liquid)[1] in infants from the EFSA Comprehensive European Food Consumption Database.

Country	Survey	Age class (months)	Subjects	Consumers (%)	All subjects				Consumers only			
					Mean		95th percentile		Mean		95th percentile	
					mL/day	mL/day per kg bw	mL/day	mL/day per kg bw	mL/day	mL/day per kg bw	mL/day	mL/day per kg bw
Bulgaria	NUTRICHILD	0 - 3	200	95 (48%)	343	73	1095	245	722	153	1120	274
		4 - 6	227	111 (49%)	345	53	1040	182	706	109	1173	196
		7 - 12	432	154 (36%)	109	13	560	65	306	36	700	87
Germany	VELS	6 - 12	157	33 (21%)	42	5			199	24		
Denmark	IAT 2006_07	3 - 6	38	25 (66%)	276	35			420	53		
		7 - 12	788	519 (66%)	202	23	615	73	306	34	686	78
Finland	DIPP_2001_2009	6 - 12	500	293 (59%)	252	32	747	90	430	54	813	99
United Kingdom	DNSIYC_2011	4 - 6	76	61 (80%)	511	66	1029	140	637	83	1029	140
		7 - 12	1293	698 (54%)	275	31	777	93	510	57	854	102
Italy	INRAN_SCAI_2005_06	3 - 6	3	1 (33%)	208	32			625	96		
		7 - 12	9	1 (11%)	28	3			254	25		

[1] Infant formula powder transformed into liquid by means of a ratio of 1:8
Abbreviation: bw, body weight.

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4.2.1. Formula consumption over time in healthy non-breastfed term infants during the first 16 weeks

Fomon *et al*. (1971) reported daily formula consumption in healthy infants throughout the interval of 8 to 111 days of life (Fomon *et al*., 1971). The study was carried out between 1966 and 1970, in a sample of 142 Caucasian full-term infants (65 males, 77 females), in a university community in Iowa, USA. Ready-to-feed cow's milk based formula was delivered to the participating families in 120 or 240 mL units to supply for 48 to 72 hours; bottles including unconsumed portions were collected and re-weighed. The energy density of the formula (67 kcal/100 mL) was similar to the energy density of breast milk as reviewed by the EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2014) with 65 kcal/100 g and fits well into the 60 – 70 kcal/100 g range required for infant formula by the present Commission Directive (Commission Directive 2006/141/EC) and the Commission Delegated Regulation (EU) 2016/127, and as recently proposed by the EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2014).

During days 8 to 111, daily formula consumption (mL/day) in high consumers (P90) increased up to a maximum of around 1000 mL/day, which was achieved in boys by the age of 28-41 days and in girls by the age of 84-111 days. Formula consumption related to body weight in high consumers (P90) peaked by the end of the first month at about 200 mL/kg bw (Table 4). Consumption at P95 was not reported for this study sample. However, for a larger sample of this study (380 boys, 340 girls) energy intake per kg bw during the first 8-111 days of life was reported including P95 values (Fomon, 1993). Using the energy density of the formula applied in the study (67 kcal/100 mL) it is possible to re-calculate formula consumption as mL/kg bw at P95 in the subsample of the 142 infants from which the original consumption data are available (Fomon *et al*., 1971) (Table 4). The re-calculated P95 consumption values reach a peak by the end of the first month of life of about 220 mL per kg bw in both, boys and girls. It can be assumed that this approach is robust, as the re-calculation of the P90 (and P50) formula consumption per kg bw from the reported P90 (P50) energy intake resulted in almost identical consumption values as reported originally for the subsample (Table 4).

Table 4. Consumption of formula in healthy term infants during the first 16 weeks of life

Age (days)	8-13	14-27	28-41	42-55	56-83	84-111
Boys (n=65)						
mL/day						
P50[1]	573	696	774	790	846	890
P90[1]	741	865	988	1028	1033	1030
mL/kg bw per day						
P50[1]	166	181	172	161	148	137
P90[1]	208	214	209	198	178	155
P95[2]	*216*	*222*	*213*	*202*	*179*	*164*
Girls (n=77)						
mL/day						
P50[1]	558	657	695	721	741	791
P90[1]	745	757	854	855	909	975
mL/kg bw per day						
P50[1]	160	169	159	153	137	133
P90[1]	208	197	195	182	164	-
P95[2]	*213*	*218*	*215*	*198*	*180*	*166*

[1] daily measurements of liquid formula consumption, 142 infants (65 boys, 77 girls), study period: 1966-1970 (Fomon *et al.*, 1971).
[2] re-calculated from reported energy intake (kcal/kg bw) and a mean energy density of 67 kcal/100 mL formula; energy intake data are from a larger group of infants (boys: n=380; girls: n=340) (Fomon, 1993), which included the sample[1] for which original consumption data are presented in this table.
Abbreviation: bw, body weight.

4.2.2. Simulating a consumption time course in healthy non-breastfed term infants during the first 16 weeks with data used by EFSA

While the Fomon data can provide evidence for choosing the one-month-old infant to reflect high consumption in the first 16 weeks of age, it is not clear whether the data from a study carried out 50 years ago represent today's consumption habits. In the long-term observational German DONALD study, 3-day weighed dietary records were collected between 1990 and 1996 in infants aged 3, 6, 9 and 12 months (Kersting *et al.*, 1998). In the diet records formula consumption was reported as (instant) powder. In the group of consumers of formula (n=59) at the age of 3 months, the P95 value was 24.4 g/kg bw (maximum 24.6 g/kg bw) and a mean value ($\pm$ SD) of 17.1±4.7 g/kg bw, P50 not being reported.

To overcome the problem of different methods used to assess formula consumption in the older study where ready-to-feed bottles were administered (Fomon *et al.*, 1971) and the newer study where powder amount per bottle to be mixed with water was recorded (Kersting *et al.*, 1998), information on today's practice of formula preparation from powder is needed.

Recently, EFSA carried out a search of the Global New Products Database (GNPD) to collect information on preparation instructions on the labels of infant formulae introduced on the European market in the last 24 months (111 products from 13 countries). For most products, one scoop of infant formula powder is to be used for 30 mL of water and the use of the 4.3 grams of powder per spoon is the most frequently mentioned scoop size. If this amount (4.3 g) is added to 30 mL of water, it corresponds to 12% (1:8) of the final liquid infant formula. In a recent market search of package instructions of infant formula in Germany (June 2016; 8 products, 5 companies) a similar ratio was found referring to feeding the youngest infants (around one month old).

A re-calculation of the potential liquid formula consumption (12%) from the P95 powder consumption 24.4 g/kg bw at the age of 3 months results in a consumption of 195 mL/kg bw (Table 5) which is 18 % higher than reported in the Fomon study 50 years ago at the age of around 3 months (Table 4). Starting with this re-calculated newer liquid consumption figure at the age of 3 months and using the same course of consumption as in the earlier study, the peak at age 1 month is about 260 mL/kg bw per day. As P50 values of powder consumption (per kg bw) at age 3 months were not reported in the newer study (Kersting *et al.*, 1998), a similar higher consumption by 18 % was also assumed for the P50 level and a time course was estimated accordingly (Table 5).

Taking into account all of the available information, the EFSA SC recommends values of 200 and 260 mL/kg bw per day as conservative mean and high level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age. These values are derived from data for infants aged 2-4 weeks, when formula consumption is highest, expressed on a body weight basis.

For assessing the exposure to substances with a long half-life and therefore accumulate in the body, it may be appropriate to select consumption values representing a longer period of formula feeding. For example, consumption values for infants of around 2 months of age (56-83 days, Table 5) could be used, i.e. around 170 (P50) or 210 (P95) mL/kg bw.

Table 5. Estimated consumption of formula using old consumption data during the first 16 weeks of life (Table 4) and newer data on formula consumption at 3 months of age (Kersting *et al.*, 1998).

Age (days)	8-13	14-27	28-41	42-55	56-83	84-111
			mL/kg bodyweight per day			
Boys						
P50[1]	196	214	203	190	175	162
P95[1]	254	261	251	238	211	195[2]
Girls						
P50[1]	189	199	188	180	162	157
P95[1]	251	257	253	233	212	195[2]

[1] estimated as 18 % higher than the old data throughout the time course of the first 16 weeks of life based on the 18 % higher consumption at P95 in the newer study at the age of 3 months.
[2] as boys and girls were not separated in the newer study the P95 value at age 3 months was applied for both sexes.

4.2.3. Feeding of preterm infants

Present-day nutrition guidance for preterm infants aims to achieve growth similar to fetal growth coupled with satisfactory functional development (Agostoni *et al.*, 2010).

ESPGHAN (2010) recommends a 'reasonable upper limit' of fluid intake of 200 mL/kg bw (range 135-200 mL/kg bw) for enteral intake of stable-growing preterm infants up to a weight of 1800 g (Agostoni *et al.*, 2010). This ESPGHAN recommendation (135-200 mL/kg bw) was confirmed for the 'micropreterm infant' (<30 weeks gestation, < 10th P birth weight) (Tudehope *et al.*, 2013). For the very low birth weight neonate (<1000 g), full enteral feeding with 150-180 mL/kg bw was recommended, and should be achieved by about 2 weeks in <1000 g birth weight, and by about 1 week in 1000-1500 g birth weight (Dutta *et al.*, 2015).

Since these consumption values are lower than those for term infants in Table 5, application of the high consumption value of 260 mL/kg bw in infants below 16 weeks of age would also cover the potential high consumption rates of preterm infants on enteral (formula) feeding as recommended at present.

5. The developing infant: Physiological and biochemical considerations

Most organ and organ systems lack structural or functional maturity at birth. The degree of immaturity may be aggravated due to prematurity, intrauterine growth retardation or any potential pathologic condition affecting the infant. Immaturity of physiological processes and organ function predispose neonates to altered toxicokinetics and toxicodynamics, leading to potentially enhanced sensitivity of infants towards substances present in food.

Organ maturation is rapid in the post-natal period, and as a result can yield a high degree of both inter-individual and intra-individual variability in the infant population, which might be greater than later in life.

Data are available (ICRP, 2002) from which the relative organ weights can be taken for a newborn compared to the adult. For example, the relative liver weight is 37 g/kg bw in newborns and 25 g/kg bw in adults, the relative weight of the kidneys is 7.1 g/kg bw in newborns and 4.3 g/kg bw in adults. The greatest difference exists for the relative brain weight which is 108 g/kg bw in the newborn and 20 g/kg bw in the adult.

The WHO (International Programme on Chemical Safety (IPCS), 2006) reviewed extensively the aspects of organ maturation in infants. These are summarised below, with a particular focus on recent developments in the areas that are pertinent to the risk assessment of substances present in food intended for infants.

5.1. The developing gut

There are reviews available on the intrauterine development of the gastrointestinal tract (Grand *et al.*, 1976; Wells and Spence, 2014). The development is a complex process to which all three germ layers contribute. Among them and most important is the endoderm-derived epithelium, which houses specialized intestinal stem cells, which are important for the renewal of the cellular epithelium during the turn-over of the mucosal layer (Sato and Clevers, 2013). Smooth muscle, vasculature and lymphatic are mesoderm-derived, and the nervous system of the gastrointestinal wall has its origin in ectodermal tissue. Besides its role in digestion and absorption of nutrients, the intestine is also a major lymphoid organ through the gut associated lymphoid tissue, which is responsible for the immune tolerance to dietary antigens.

Recently, the colonisation with microbiota of the intestinal tract has become a field of interest also for the postnatal development of the structure and function of the intestine (Di Mauro *et al.*, 2013). However, limited knowledge is available concerning the postnatal development of the human gastrointestinal tract, and whether it is affected by differences in the microbiota.

Local effects in the gastrointestinal tract and absorption and systemic fate of a substance may depend upon its release from the ingested organic matrix, local pH, transit time, size of the absorptive surface or its metabolism by the gastrointestinal wall and also by the bacterial microbiome.

Gastric emptying and intestinal motility

The functional gastric capacity of infants is quite limited. It ranges from 38 to 76 mL in term neonates (Zangen *et al.*, 2001). The coordination of antral contractions is developing after birth, resulting in a marked increase in gastric emptying during the first week of life (Gupta and Brans, 1978). Similarly, increases in the frequency, amplitude, and duration of propagating contractions have been observed to occur in preterm infants (Berseth, 1989; Ittmann *et al.*, 1992). In most studies, the emptying pattern is exponential and can be fitted using the Elashoff model (Elashoff *et al.*, 1982). In preterm infants (24-34 weeks), gastric half-emptying time (T1/2) is in the range of 70 min for formula and 35 min for human milk. In full term infants, data are scarce and T1/2 is in the range of 80 minutes for formula. Many factors, including caloric density, may affect emptying rate [reviewed by (Bourlieu *et al.*, 2014)].

The small bowel motility patterns are poorly developed before 28 weeks of gestation, showing a disorganised motility pattern between 27 and 30 weeks, progressing to more mature migrating myoelectric complexes by 33-34 weeks of gestation (Berseth, 1996). Consequently, total transit time is longer in preterm than in term infants. However, inter-individual variance is high and sample sizes are small (Bode *et al.*, 2004).

Gastric secretion and digestive enzymes

Gastric acid secretion is active at birth even in very preterm neonates (gestational age (GA) = 24 weeks), and the gastric pH decreases below 4 in the first day of life (Avery *et al.*, 1966). However, the high buffering capacity of human milk and formulae stabilises the gastric pH in the 5-6 range; this pH level lasts longer as the meal frequency increases. The pH of gastric content after ingestion of human milk or formulae is optimal for gastric lipase, but not for pepsin activity (Armand *et al.*, 1996). However, extensive proteolysis occurs in the term infant's stomach during the first two weeks of life (Dallas *et al.*, 2014).

The secretion of gastric and pancreatic enzymes is not developed to adult levels at birth. In infants, secretion outputs and enzyme activity levels mature at very different rates: gastric lipase activity already reaches adult levels by 3 months (Menard *et al.*, 1995), whereas pancreatic α-amylase only does so after 3 years of age (Zoppi *et al.*, 1972). Of note, gastric lipase reaches about 75% of adult maximum levels in preterm newborns (Roman *et al.*, 2007). By one month postpartum, term and preterm infants' trypsin activity is similar to that of 2-6 years old children (Lebenthal and Lee, 1980). Pancreatic secretion rate of fluids is generally higher in preterm than in term infants. This was also reported for enzyme production in infants of the same postnatal age (Zoppi *et al.*, 1973). Duodenal pH is in the 5.2-7.6 range. In newborn infants, bile salt metabolism is active, but immature owing to a not yet fully developed conjugation capacity and slower transport into the intestinal lumen (Poley *et al.*, 1964; Suchy *et al.*, 1981). Therefore, the bile salt pool is limited and the primary bile salt concentration in the duodenum is low (Norman *et al.*, 1972). In contrast, the small intestine of human

infants is mature at birth and can handle the various nutrients (Shmerling, 1976). The intestinal surface area is even proportionally greater in infants than in adults because of the rapid prenatal small intestine growth rate (Weaver *et al.*, 1991). Figure 2 provides an overview of the changes in gastrointestinal structure and function with age.

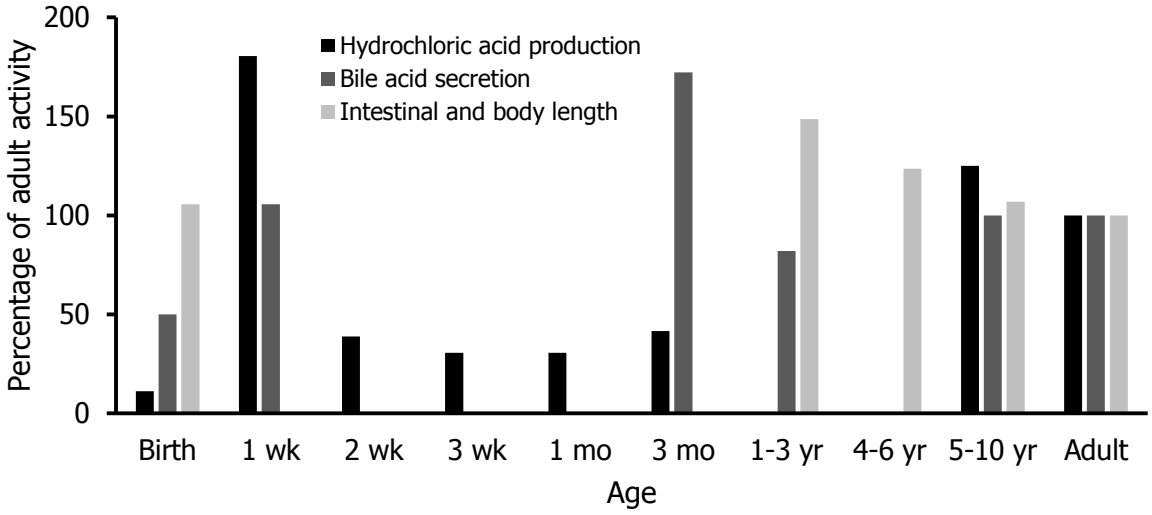


Figure 2. Overview of the age-dependent changes in gastrointestinal structure and function with age. Modified from (Kearns *et al.*, 2003).

The analysis of ileo-caecal samples in infants aged 10 days to 5 months and fed 150 mL breastmilk per kg bw per day showed that proteins are almost fully digested and absorbed (Hirata *et al.*, 1965). Balance studies in moderately premature infants (30-36 weeks of gestation) fed either human milk or cow's milk derived formulae have shown that premature infants are also able to digest and absorb proteins (Senterre, 1976). Similarly, the coefficient of absorption of fat in breastfed infants is about 90-95% at one week of age and 96% at 1.5 months of age (Williamson *et al.*, 1978). In contrast, fat digestion is less efficient with cow's milk derived formulae as indicated by a 70-85% coefficient of absorption at 3 months of age (Manson *et al.*, 1999). Fat absorption may be even lower in premature infants (Schmitz, 1977). Despite the slow maturation of pancreatic amylase, most cooked starches are digested and absorbed almost completely as early as one month after birth. However, high intakes (40 g/d) induced fermentative diarrhoea in 40% of the infants (DeVizia *et al.*, 1975).

Intestinal permeability

Some animal species (e.g. mouse, rat) retain fetal-type enterocytes at birth. These cells present many cytosolic vacuoles (related to the apical tubular system, ATS) actively involved in the uptake of luminal material. In mice and rats, fetal-type enterocytes are completely replaced by mature-type ones by the end of weaning, a phenomenon called 'closure'. On the contrary, human neonates have mature-type enterocytes at birth, characterised by an adult-type microvillus enzyme pattern (e.g. sucrase) and the absence of vacuoles (Moxey and Trier, 1979; Zabielski *et al.*, 2008). The change from fetal-type to mature-type intestinal epithelium is realised by 18-22 weeks and the ATS is undetectable by 24 weeks of gestation (Milla and Bisset, 1988). Infants born at 34 to 37 weeks of gestation achieve a 'mature' passive intestinal permeability to lactulose (MW 342; thought to cross the intestinal wall by a paracellular route) within four days after starting oral feeding. 'Mature' intestinal permeability is achieved earlier in exclusively breastfed than in formula fed infants (Catassi *et al.*, 1995; Taylor *et al.*, 2009). Infants born before 34 weeks of gestation have a higher lactulose permeability than term babies. However, preterm infants show a progressive decrease in lactulose absorption during the first week of oral feeding (Weaver *et al.*, 1984), irrespective of feeding mode (breast milk vs. formula) and prebiotic supplementation (Westerbeek *et al.*, 2011).

Gut microbiota

The microbiome in infants is highly dynamic and differs from the microbiome in adults, which is stable and resilient. Schematically, soon after birth, the bacterial microbiome switches rapidly from predominantly facultative anaerobic bacteria towards a diverse community of anaerobes. However, its composition can be notably affected by the mode of delivery (vaginally vs. caesarean section), the mode of feeding (breast milk microbiome might be its main physiological template), the environment (from household to geographic location), the host genetics and the use of antibiotics, which may significantly and permanently alter the developing microbiome (Lim *et al.*, 2016). It should be stressed that the reference compositions of the healthy infant and adult microbiota are still unknown.

In the preterm infant, gestational age, gender and mode of feeding (mother's milk or not), as well as the intensive care unit environment, seem to be the major factors influencing microbiota development (Cong *et al.*, 2016). Schematically, preterm infants display a delay in colonisation with typical commensal bacteria. They show reduced microbiota diversity, reduced levels of strict anaerobes and a high abundance of *Proteobacteria.*

5.1.1. Summary

Gastric, pancreatic and biliary functions are not fully mature at birth. However, this does not preclude term infants thriving on breastfeeding. It may be particularly relevant to the evaluation of exposure to various chemicals that term neonates display a mature-type intestinal epithelium.

The local effects of a substance on the gastrointestinal tract, its absorption and its systemic fate will depend upon its release from the ingested organic matrix, local pH, transit time, size of the absorptive surface or its metabolism by the gastrointestinal wall and by the bacterial microbiome, all parameters changing during postnatal maturation.

5.2. Ontogeny of the processes involved in the absorption, distribution, metabolism and excretion of chemicals

5.2.1. Absorption

This guidance focuses on oral exposure, and therefore, the term absorption relates to absorption from the gastrointestinal tract.

Only a few studies have been published on the oral absorption of drugs and other substances in infants below 16 weeks of age (Heimann, 1980); reviewed by (Bowles *et al.*, 2010; Batchelor *et al.*, 2014; Batchelor and Marriott, 2015).

In a series of studies, Heimann investigated the absorption of several therapeutically used drugs (sulphonamides, phenobarbital, digoxin, and ß-methyldigoxin) in paediatric patients in relation to their age (some days until 1 year) (Heimann, 1980). The amount of absorption, as expressed by the area under the curve (AUC), was not age dependent whereas the rate of absorption was slower in neonates and increased within the first year to the values observed in adults. Heimann also investigated the absorption of L(+)-arabinose and D(+)-xylose. The amount absorbed of D(+)-xylose, a substance that is taken up through the gastrointestinal wall by active transport, was independent of the age whereas the rate of absorption was age dependent. The same findings were reported by Heimann for L(+)-arabinose, a substance that is taken up by passive diffusion. Thus, from the findings with drugs and some test substances it can be anticipated that the absorption of substances in general might be slower in young infants than in older ones but that the amount absorbed is not dependent on the age.

5.2.2. Distribution

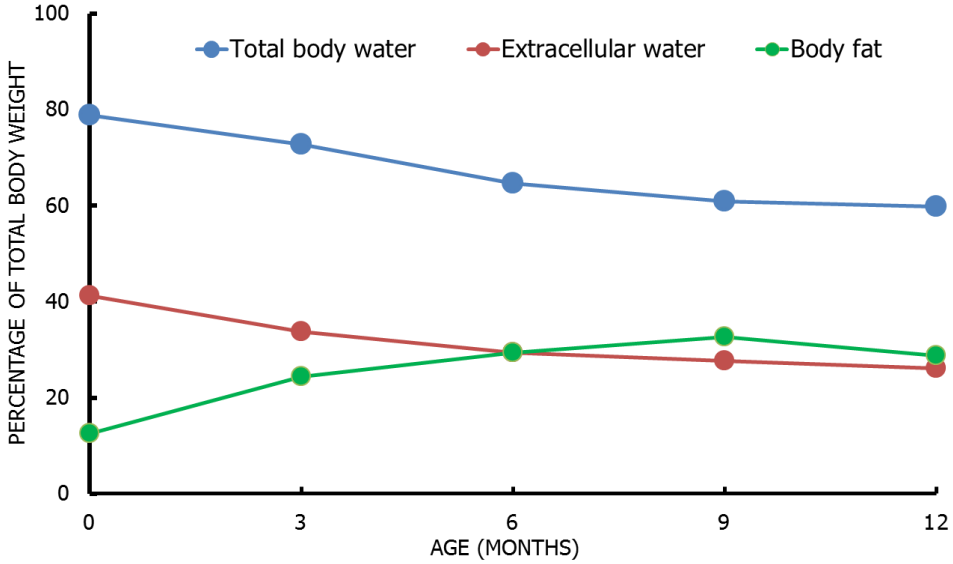
Body composition changes with age and hence the physiological spaces to which substances distribute can change. The volume of distribution of a substance, which is not congruent with a physiological space, can change. Factors associated with development such as regional blood flow, organ perfusion, and cardiac output, and also changes in relative organ weights can influence distribution. For example, extracellular and total-body water spaces are larger in neonates and young infants compared to adults (Friis-Hansen, 1983). Also the adipose stores might be different whereby the

253 percentage of fat is less than 20% in neonates and in young infants and increases in the next months
254 to reach 30% at the age of nine months. The percentage of extracellular water decreases from 40%
255 in neonates to 30% at nine months (see Fig. 3). Details on organ weights and blood flows through the
256 organs in neonates, infants, children, adolescents and adults are given in several sources (ICRP, 2002;
257 Abraham *et al.*, 2005; Mielke and Gundert-Remy, 2009).

258 As a consequence of the rising body fat, whereas total body water and extracellular water declines it
259 can be predicted that the concentration of water soluble, polar substances in plasma will become
260 higher and that of fat soluble, non-polar substances in plasma will become lower.

261 A special case are highly plasma protein-bound substances for which altered protein content and
262 binding may have influences on the kinetics of these substances. The quantity of total plasma proteins
263 (in particular albumin) is reduced during the neonate and young infant period. A higher concentration
264 of bilirubin in neonates might lead to displacement of protein-bound substances from the binding site
265 thus increasing the non-protein-bound fraction. There are some examples in which this caused an
56 increase in the non-protein-bound fraction of the substance (Ganshorn and Kurz, 1968; Ehrnebo *et*
57 *al.*, 1971; Fredholm *et al.*, 1975; Windorfer *et al.*, 1978).

268 The distribution of a xenobiotic can mechanistically be understood as resulting from passive diffusion
269 through membranes. The non-protein-bound, free concentration in plasma is in equilibrium with the
270 free concentration in the tissue (Pelkonen *et al.*, 2008; Smith *et al.*, 2010). Thus, the driving force is
271 the concentration gradients between the binding of the drug in blood and the resulting free
272 concentration and the free concentration in the tissue, which depends on the binding of the drug to
273 tissue components. Depending on the physico-chemical properties of the substance and the tissue
274 composition, different tissue to plasma/blood relationship will be established for a substance.
275 However, some tissues, also the intestinal wall, express transporters that may transport substances
276 into the cells or out of the cells of the tissue. It is assumed that the activity of the transporters is age-
277 dependent. However, the paucity of experimental data does not allow drawing firm conclusions on
278 this issue (Mooij *et al.*, 2016).



279

280 **Figure 3.** Age-dependent changes in body composition and its influence on the apparent volume of
281 distribution for chemicals. Total-body water and extracellular water, expressed as a percentage of
282 total body weight, is much larger in infants in the first six months of life as compared with older
283 infants. Modified from (Kearns *et al.*, 2003).

284 ### 5.2.3. Metabolism and excretion

285 The main excretory pathways are via metabolism in the liver and renal excretion by the kidney.
286 Volatile agents as well as CO_2, as an ultimate product of metabolism, may be excreted by exhalation.
287 Biliary excretion is for most of the substances a minor excretory pathway in Man. Despite the

relatively higher weight of the excretory organs in newborns, their function for the excretion of substances is not yet at the level of the adults with the exception of a few metabolic pathways.

The ontogeny of transporters, xenobiotic drug metabolising enzymes and renal excretion as well as their pharmacokinetic consequences in humans have been reviewed extensively, particularly with the data rich pharmaceutical literature (Renwick *et al.*, 2000; Alcorn and McNamara, 2002; Dorne *et al.*, 2005; Lee *et al.*, 2012; Valcke and Krishnan, 2013; Doring and Petzinger, 2014; O'Hara *et al.*, 2015). Mathematical and pharmacokinetic (PK) models such as physiologically-based PK (PB-PK) models have been developed to predict age specific differences, age specific uncertainty factors and age- and clearance pathway-specific Infant Scaling Factors (ISFs) (Renwick *et al.*, 2000; Alcorn and McNamara, 2002; Dorne *et al.*, 2005; Valcke and Krishnan, 2013; Salem *et al.*, 2014).

Metabolism

Metabolism involves two large groups of enzymes catalysing phase I and phase II reactions.

Phase I enzymes catalyse key reactions such as oxidation, reduction and dealkylation (e.g. by cytochrome P-450) and hydrolysis (e.g. by epoxide hydrolase and esterases).

Phase II enzymes catalyse conjugation such as glucuronidation by uridine diphosphate (UDP)-glucuronosyltransferase isoenzymes (UGT) (the largest group of phase II enzymes), sulphation by sulphotransferases, glutathione and methyl conjugation by glutathione-S-transferases and methyl-S-transferases, amino acid conjugation (e.g. glycine conjugation) and N-acetylation (NAT-1 and NAT-2). A reduced activity of these enzymes may be responsible for the toxicity syndromes that have been seen in preterm infants, such as grey baby syndrome with chloramphenicol (UGT2B7), neonatal jaundice with bilirubin (UGT1A1) and gasping syndrome with benzyl alcohol (glycine conjugation) (O'Hara *et al.*, 2015).

Discussing the developmental pattern of each enzyme isoform known in humans is beyond the scope of this guidance document.

Generally speaking, each enzyme isoform for phase I and phase II reactions has a unique pattern of development; however, the following three groups have been proposed based on expression profiles in human livers (Hines, 2008; van den Anker *et al.*, 2011; de Wildt *et al.*, 2014) (see Figure 4).

1. Expression is present during prenatal development with silencing or low level expression within 1—2 years after birth. Specific isoforms include CYP3A7, FMO1, SULT1A3/4, SULT1E1, ADH 1A.

2. Expression is at relatively constant levels throughout fetal development and increases postnatally. Specific isoforms include ADH1C, ADH1B, CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/3A5, FMO3 and SULT2A1. As an example, the major isoforms of human cytochromes P450 are CYP3A isoforms (CYP3A4/CYP3A5/CYP3A7) which contribute to the metabolism of more than 50% of all marketed drugs and the expression patterns of these isoforms are subjected to developmental influence. As CYP3A7 is highly expressed in fetuses, there is a shift in expression from CYP3A7 to CYP3A4, which occurs within the first month after birth. The shift in expression is associated with a rapid decrease in CYP3A7 after birth, a sharp increase in CYP3A4 in the neonatal period in order to reach adult levels at one year of age (He *et al.*, 2016).

3. Expression with onset occurring mostly in the third trimester with substantial increase in the first 1–2 years after birth. Isoforms include ADH1C, ADH1B, CYP1A1, 1A2, 2A 6, 2A7, 2B6, 2B7, 2C8, 2C9, 2F1, 3A4, FMO3, SULT2A1, UGTs, NAT-2 (see Fig. 4) (van den Anker *et al.*, 2011).

In humans, total cytochrome P450 content in the fetal liver is between 30% and 60% of that found in the adult and approaches adult values by 10 years of age (van den Anker *et al.*, 2011). CYP1A1 is expressed during early gestation, but expression of most of the other CYP enzymes occurs at or near birth (CYP2B, CYP3A), immediately after birth and reaching adult levels within 30 days (CYP2E1) or after the first week after birth (e.g. CYP1A2, CYP2C). The CYP3A isoform is the most abundant CYP in the human liver (>50%) of all CYPs and gut and is responsible for the metabolism of more than 50% of known pharmaceuticals and xenobiotics. In terms of isoform, CYP3A7 activity is high immediately after birth; during the first days after birth, there is a shift from mainly CYP3A7 activity to CYP3A4 activity (Lee *et al.*, 2012). For a further account of the ontogeny of phase I and phase II enzymes, the

reader is referred to recent reviews (van den Anker *et al.*, 2011; de Wildt *et al.*, 2014; O'Hara *et al.*, 2015).

PB-PK models have been successful at predicting differences between neonates and adults for CYP1A2 using caffeine and theophylline and CYP3A isoforms but poorer predictions were obtained for CYP2D6 and CYP2C because of lack of model complexity or inadequate hepatic microsomal activity data to fully describe the maturational process of functional enzymes (Alcorn and McNamara, 2002; Ginsberg *et al.*, 2004). Recently, several authors reported clearance values in neonates predicted using in silico tools that incorporate *in vitro* enzyme PK parameters (maximum rate of metabolism [Vmax] and Michaelis-Menten constant [Km]), *in vivo* clearance data from the literature in neonates and ontogenic information of isoforms. In 70% of cases, the predicted median clearances in neonates were within 2-fold of the observed values (Johnson *et al.*, 2006; Salem *et al.*, 2014).

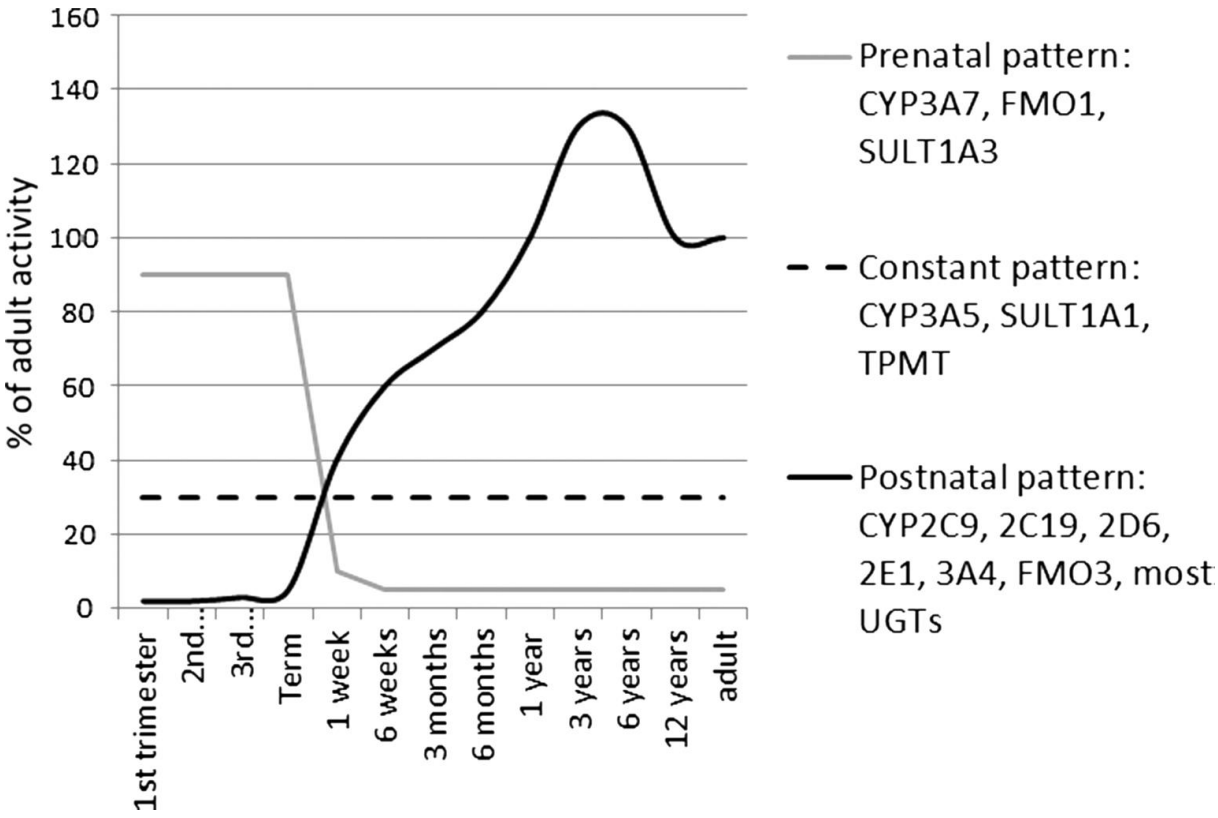


Figure 4. Schematic depiction of drug-metabolising enzyme ontogeny. The postnatal pattern is expressed as percentage of the adult level. Abbreviations used: CYP, cytochrome P450; TPMT, thiopurine S-methyltransferase; UGT, UDP-glucuronosyltransferases; SULT, sulfotransferases; FMO, flavin-containing mono-oxygenase. Reproduced with permission from (de Wildt *et al.*, 2014).

Finally, the extent of metabolism can be influenced by a number of factors including polymorphic expression of enzymes and transporters (CYP2D6, CYP2C9, CYP2C19, OATP, P-Gp) and the ontogeny of the rate of hepatic blood flow (particularly relevant for compounds with a high hepatic ratio). Immediately after birth, blood flow through the liver rapidly changes with the ductus venosus closing. In most cases, full closure will be within one week. With the first feed, portal blood flow increases leading to a rapid increase in hepatic functions and induction of a number of enzyme groups. With feeding bacterial colonisation of the gut begins, and varies with the type of feeding (i.e. breastfeeding or formula). Several authors have stated that because of such acute changes standard pharmacological models of hepatic extraction, metabolism and blood flow are not applicable to neonates and that they are relevant only once the ductus venosus is fully closed (van den Anker *et al.*, 2011).

Excretion

Excretion is a key step in ADME processes being the final removal of a xenobiotic and/or its metabolites from the body mostly via the renal (i.e. urine) or hepatic routes (i.e. faeces). Renal excretion is particularly important for drugs and xenobiotics that are excreted as parent compound without metabolism and water-soluble metabolites if they possess toxicological properties (Mooij *et al.*, 2016). When dealing with renal excretion of chemicals, renal clearance can be several-fold lower in neonates compared with older children and adults for lipophilic, hydrophilic, and organic ions (van den Anker *et al.*, 1995; Clewell *et al.*, 2002). Creatinine is a standard marker of renal function that becomes an accurate biomarker during the first weeks of post-natal life (O'Hara *et al.*, 2015).

PBPK models in neonates and young infants for drugs and environmental chemicals that are primarily cleared by the kidney have been developed using data for rates of glomerular filtration, tubular secretion, tubular reabsorption, and renal blood flow (Alcorn and McNamara, 2002; Johnson *et al.*, 2006; DeWoskin and Thompson, 2008; Salem *et al.*, 2014).

The maturation of the kidney, or nephrogenesis, is complete within 36 weeks of gestation followed by postnatal changes in renal and intrarenal blood flow. The glomerular filtration rate (GFR) at birth is approximately 2–4 mL/min/kg in term neonates (one third of the adult value expressed on the basis of body surface area) and as low as 0.6–0.8 mL/min/kg in preterm neonates. During the first 2 weeks of life, GFR increases rapidly to half of the adult value within 3 months and then follows a steep rise to adult values within 8–12 months. Such an increase in GFR in the first weeks of life is mainly driven by an increase in renal blood flow. Tubular secretion is around 25% of adult levels at birth, and increases more slowly and more variably than GFR, not approaching adult levels until 1-5 years of age. Limited data on renal plasma flow indicate neonatal rates of only 10-20% of adult values that rapidly increase to 50% by 6 months, and then approach adult levels by 1-2 years of age (Alcorn and McNamara, 2002; van den Anker *et al.*, 2011).

Kidney maturation in the neonate also involves changes in the expression of a number of transporter isoforms together with developmental changes in signal transduction affecting the capacity of renal tubules to reabsorb water and solutes. However, beyond expression levels, little is known about the ontogeny of human membrane transporters and its consequences on the PK of xenobiotics (Brouwer *et al.*, 2015; Gattineni and Baum, 2015).

The renal function reaches 50% of the activity of adults within the first two months of age. A number of analyses showed overall 1.5-3-fold differences between neonates, infants and healthy adults for renal clearances and total clearances and half-lives of substances that are cleared by the kidney (e.g. antibiotics) (Hattis *et al.*, 2003; Dorne *et al.*, 2005; Valcke and Krishnan, 2013) (Fig. 5). For the oral route, limited data showed that clearances were slightly higher in neonates and infants compared with healthy adults and associated with larger variability. For renal excretion, it is considered that an additional uncertainty factor of 2 to 3 would cover the differences between adults and infants below the age of 16 weeks. However, so far, the analyses from the literature have been based on compounds that are eliminated rapidly by the kidney and comparative elimination patterns between neonates, infants and healthy adults and for persistent compounds are as yet unavailable.

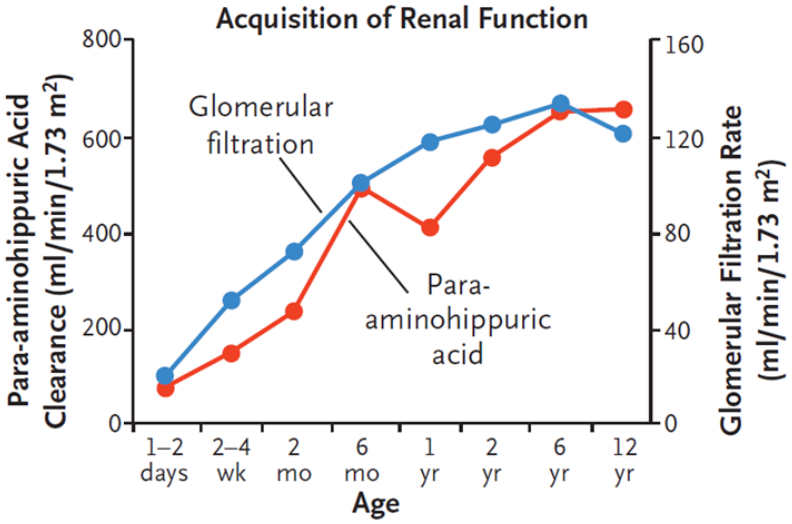


Figure 5. Maturation of renal function in the infants and young children. The figure shows para-aminohippuric acid clearance and glomerular filtration rate as a function of age. Reproduced with permission from (Kearns *et al.*, 2003).

To illustrate the influence of immature renal function and immature enzyme activity the example of the kinetics of propylene glycol should be mentioned. Propylene glycol is both eliminated by metabolism through alcohol dehydrogenase and by renal excretion. In adults, renal excretion accounts for 45% of the clearance (De Cock *et al.*, 2014). In a study by de Cock *et al.* (2013), infants with gestational ages between 24 and 41 weeks at birth, were investigated at an age between 1 and 82 days and a birth weight between 630 and 3980 g (De Cock *et al.*, 2013). The mean clearance in this infant population was 0.085 L/h whereas the clearance in adults is known from the literature to be 8.64–23.4 L/h/ 1.73 m^2 (Speth *et al.*, 1987). The clearance of propylene glycol was dependent on postnatal age and on body weight of the infants. This example also shows that this guidance cannot give general recommendations for preterm newborns and that HBGVs for substances used in this age group require special consideration.

Transporters

Membrane transporters can play an essential role in the transport of endogenous and exogenous compounds, and mediate the uptake, distribution, and excretion of many drugs. Historically, such transporters were not considered with phase I and phase II metabolism and over the past two decades, phase I and II metabolism have been extended to a concept of four phases that now includes phase 0, namely the cellular uptake of compounds, and phase III, the transport of compounds out of the cells. Cellular transporters belong to two main clusters of transporter families: the solute carrier (SLC) families and the adenosine triphosphate (ATP) binding cassette (ABC) carriers. SLC transporters include the human organic anion transporting polypeptides (OATPs) and human organic cation transporters (OCTs) and are often denominated as phase 0 uptake transporters. ABC transporters are often called efflux pumps or phase III and include examples of importance such as P-glycoproteins and multidrug resistance proteins (MRPs) (Hillgren *et al.*, 2013; Doring and Petzinger, 2014; EFSA, 2014). Transporters are mostly expressed in liver, kidney, the adrenal gland and the lung (Doring and Petzinger, 2014).

Examples of transporters of physiological importance in the kidney include the OATPs expressed in the kidney that are involved in the final excretion of phase II conjugates. OATPs have low activity at birth then increase rapidly to high levels, higher than those seen in adults, over the first few weeks of life and then begin to decline to adult levels. Such a change in secretion is independent of the changes in renal mass. Exposure to hormones and antibiotics, such as penicillin, also increases organic anion transporter activity (Mooij *et al.*, 2016).

Ontogeny of metabolic pathways and its impact on clearance

Ontogeny of metabolic pathways in neonates has been shown to be isoform-specific and the quantification of its impact on acute and chronic elimination of xenobiotics is an important aspect of

risk assessment (Hattis *et al.*, 2003; Dorne *et al.*, 2005; Valcke and Krishnan, 2013). If the enzymes involved in the metabolism of the substance are known the expression level of the enzymes can be taken into consideration. For example, newborns need only 25% of the dose of a one-year-old infant to get the same plasma concentration of morphine, which is metabolised by UGT2B7 and other UGT isoforms (Knibbe *et al.*, 2009; Sato *et al.*, 2012).

If the enzymes involved in the metabolism of a substance are not known, it should be assumed that the activity levels of the enzymes are lower compared to adults. Consequently, the clearance of substances may be lower compared with the adult population. Since the clearance is the product of the elimination constant (ln 2/half-life) and the volume of distribution, both age-dependent in infants, half-life and clearance are not linearly related. The pattern is illustrated in Figure 6 from Valcke and Krishan (2014) based on the data of (Ginsberg *et al.*, 2002) and (Hattis *et al.*, 2003).

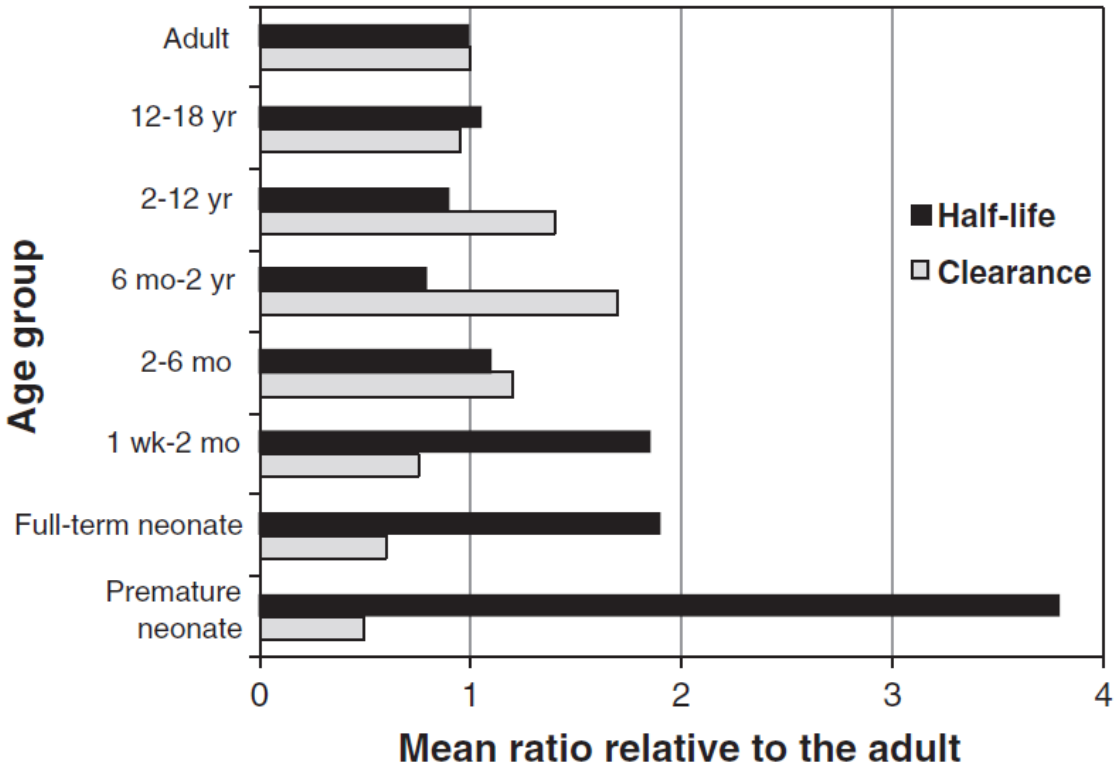


Figure 6. Half-life and clearance ratios (child/adult) as a factor of age. Reproduced with permission from Valcke and Krishnan (2013).

Other authors have given more detailed information, indicating the relative activity of specified enzymes in children of different ages versus the adult (Table 5).

Table 5. Estimates of age-dependent enzyme activity as ratio child/adult

Age	**Enzyme activity (ratio child/adult)**				
	CYP 3A4	CYP 1A2	CYP 2E1	UGT 2B7	UGT 1A6
Premature	0.1	0.02	0.1	0.015	0.015
Term	0.2	0.05	0.21	0.05	0.1
7 days	0.24	0.1	0.32	0.06	0.11
1 month	0.5	0.2	2.5	0.1	0.16

3 months	0.7	0.25	0.45	0.3	0.25
6 months	1.1	0.29	0.45	0.7	0.36
1 year	1.25 (1-3 years)	0.34	1.0	1.0	0.5
10 years	1	1 (8 years)	1	1	1

Modified from (Edginton *et al.*, 2006). Similar values as given by Edginton *et al.* (2006) have also been reported by other authors (e.g. (Cresteil *et al.*, 1985; de Wildt, 2011; Saghir *et al.*, 2012). Abbreviations used: CYP = cytochrome P 450; UGT = uridine diphosphate glucuronosyltransferase.

It should be noted that in contrast to other conjugation enzymes, sulfotransferases are expressed at the same level in infants as in young adults as indicated by semi-quantitative Western blot analyses of liver samples (Duanmu *et al.*, 2006).

As a generalisation, if the substance is mainly metabolised, the reduced metabolic activity of most of the enzymes will lead to higher concentration in plasma. If the toxicity is owing to the parent compound, the substance may have a higher toxicity owing to the higher internal exposure. A more complex situation is present if the toxicity is due to bioactivation of the parent compound. The resulting toxicity depends on the potential presence of parallel metabolic pathways with a more advanced development leading to non-toxic metabolites by which the parent compound will then mainly be eliminated and/or on the maturity of the kidneys, which may excrete the parent compound and the formed metabolites being toxic or non-toxic.

If the enzymes involved in the metabolism are not known, the general approach by Valcke and Krishnan (2013) can be applied. The authors provided half-life and clearances for different age groups as the ratio child to adult based on the data of Ginsberg *et al.* (2002) and Hattis *et al.* (2003) which analysed kinetic studies, mainly of drugs, in the different age groups. The data as given in Figure 6 (Valcke and Krishnan, 2013) are the means of the ratios from all the kinetic studies. If the metabolic pathway and the involved enzymes of a substance are known, available detailed information could be used to adjust for enzyme activity in the specific age group. The activity of several CYP-enzymes and of other enzyme can be taken from Table 5, which is based on a publication by Edginton *et al.* (2006).

5.2.4. Summary

Absorption of substances in general might be slower in infants below 16 weeks of age than in older ones, but the amount absorbed is not dependent on the age. Once absorbed and systemically available, the distribution of the substance may be different from that in adults owing to the age-dependent changing of body composition (neonates: larger extracellular and total-body water spaces and lower fat content). Other factors to be considered are the differences in regional blood flow, organ perfusion and cardiac output between infants and adults. In addition, the total plasma protein binding capacity (esp. albumin) is lower in infants.

Infants show a unique pattern of development for individual enzyme isoforms involved in phases I and II metabolism, with a reduced capacity at birth. However, owing to overlapping substrate specificities, the overall impact on the clearance and half-life of substances (decrease in clearance, increase in half-life) is limited, being generally up to 2- (full-term) to 3-fold (preterm) lower when comparing infants with healthy adults. Likewise, renal function is also reduced at birth and its function increases in the first year of life. Therefore, if the precise metabolic pathway is not known and it is not known to which extent the substance is excreted by metabolic elimination or by renal excretion an additional uncertainty factor of 3 could be considered as a conservative option to account for the reduced elimination/excretion. When the precise metabolic pathway for a given substance is known, the age-specific ratio child/adult (see Table 5) can be used. In rare instances where the data are available for the metabolic clearance for this age group, then chemical-specific uncertainty factors can be used. Special considerations may need to be taken into account if a substance is metabolised through a pathway that is polymorphic, such as for CYP2D6 substrates. The importance of the polymorphic expression of e.g. CYP2D6 depends on the relative contribution of this metabolic pathway to the

overall elimination. A case-by-case approach is advisable to account for a potential higher susceptibility of poor metabolisers in all age groups.

Specific situations may exist that will affect the impact of the immaturity of the infants ADME system on the magnitude of the difference in internal dose between infants below 16 weeks of age and adults and the potential toxicological consequences. For instance, extremely and very preterm neonates represent a special subpopulation under medical supervision that would not be sufficiently protected by the additional uncertainty factor of 3 due to the considerable immaturity of the ADME system, and would necessitate a case-by-case approach.

For compounds, for which effects are depending on long-term body burden (e.g. cadmium, dioxins), the impact of immaturity of metabolism and excretion is likely to be minimal.

5.3. The developing brain and brain barriers

When describing the development of the Central Nervous System (CNS), the differential role between the developing Brain Barriers (BBs) and the developing neuronal and glial cells needed considering. In the context of chemical risk assessment, a key impact of the BB is on the accessibility of compounds to their target neuronal and glial cells, and as such, the BB can be regarded as a major toxicokinetic modulator for the distribution of chemicals and other substances. Whereas the BB influences the distribution to the target cells and hence the concentration at the site of action, the ability of a chemical to induce adverse effects on neuronal or glial cells reflects the toxicodynamic properties of the compound.

5.3.1. The developing brain barriers

Once a potential neurotoxin has entered the systemic circulation, it will reach the CNS via cerebral blood vessels. The Blood Brain Barrier (BBB) is formed by endothelial cells, which in contrast to other endothelial cells have continuous intercellular tight junctions, lack fenestrations and undergo extremely low rates of transcytosis. Astrocytes, pericytes and extracellular matrix components contribute structurally and functionally to the BBB (Obermeier *et al.*, 2013). However, the brain and spinal cord are bathed in fluid that is chemically different from the blood (Saunders *et al.*, 2014). In a risk assessment, therefore, it is desirable to understand the Brain Barriers (BBs) and how they control access of substances, to the neuronal cells of the CNS at all stages of development.

Development of the BBB commences as the neural tube forms and mesenchymal cells surround and penetrate developing neuro-ectoderm. Because the brain grows and new blood vessels form throughout gestation and well into the post-natal period the barrier is continually forming fresh new areas and expanding. The barrier is probably functional from very early in the development of the CNS (Saunders *et al.*, 2014). The key component of the BBB is a continuous vascular endothelium controlling bidirectional transport of large and small molecules through regulation of transcellular and intercellular pathways between circulating blood and the neuronal compartment.

The observation of unconjugated bilirubin penetration to deep human brain structures in the condition of neonatal kernicterus was previously taken incorrectly as evidence for the BBB being leaky in the period shortly after birth (Goldmann, 1913; Gröntoft, 1954; Kumar *et al.*, 2010). However, it is now generally accepted based on considerable experimental evidence from vital dye and radioactive tracer experiments that this is not correct and that the barrier in the fetus and newborn should be described as 'immature' and not as 'leaky' (Saunders *et al.*, 2014).

Extent of the BBBs

There are several (albeit some relatively minor) routes that allow access to the CNS and brain that lie parallel to the BBB. One is the route to the hindbrain vomiting centre where substances derived from blood gain direct CNS access allowing them to create part of a neuro-chemical reflex that is protective against ingested poisons.

In dye injection studies in humans and animals, performed within hours of birth, the brain was generally unstained but dye did stain smaller specific parts including the choroid plexus and the circumventricular organs, which lie just outside the blood brain barrier. The substantially higher concentration of protein in CSF observed at the time of maximum growth of the brain is caused by specific transport from the blood to the CSF across the choroid plexus epithelium. Therefore, this

region has been called the Blood-CSF barrier (Adinolfi *et al.*, 1976; Adinolfi, 1985; Liddelow *et al.*, 2011a; Liddelow *et al.*, 2011b; Liddelow *et al.*, 2013). A Blood-Retinal barrier is also considered as a distinct interface, as are the roof of the third and fourth ventricles, capillaries in the pineal gland, the roof of the diencephalon and the pituitary gland where there is release of endocrine secretions directly into the blood stream. Uncertainty stems from the fact that study of transport at several of these sites has not been extensive.

Permeability and transport of substances across the BBB

Substances can gain access to the glial and neuronal cells by simple passive diffusion across the BBB (which physicochemically can be regarded as an interrupted phospholipid membrane) or by facilitated diffusion or by active transport, depending on their physicochemical properties. Lipophilic substances will cross the BBB by passive diffusion whereas as in the case of water-soluble compounds, specific membrane transport proteins play a major role for their transport across the BBB and in and out of the cells (Obermeier *et al.*, 2013). Small molecules such as amino acids and glucose are transmitted across endothelial cell plasma membranes by transporters, carriers and channels. Brain endothelia exhibit ion channels affecting Ca^{++} flux and vasoactive secretion of nitric oxide, histamine, substance P, endothelin-1, bradykinin also prostacyclin, haemostatic factors such as tPA and von Willebrand factor (Ennis *et al.*, 1996; Kimelberg, 2004). Macromolecules appear to use clathrin (cationic preference) and caveolin (anionic preference). These coated endocytic and transcytotic pathways may lead to lysosomal degradation of their contents or may carry their contents intact across the endothelium (Narushima *et al.*, 1999; Lossinsky and Shivers, 2004). Transport across the BBB can be paracellular through the intercellular space between the cells or transcellular.

Paracellular transport across the BBB is by passive or facilitated diffusion alone. It is based on electrochemical, hydrostatic and osmotic gradients, characterised by higher conductance and lower selectivity, has similar conductance and selectivity in either apical-to-basal or basal-to-apical directions and has well defined values of electrical conductance as well as charge and size selectivity.

Evidence that endothelial gene expression patterns differ in development, observed higher rates of synthesis of certain proteins at early time points and differential expression of membrane transporter proteins can account for the majority of the differences in solute composition of the CNS interstitial fluid and blood (Daneman *et al.*, 2010). This is consistent with evidence that even early simple endothelial cell sinusoid forming arrays form a 'tight enough' layer to restrict passage of polar molecules as small as sucrose (Saunders *et al.*, 2014). Nevertheless, there is uncertainty in predicting the handling of particular substances where their transport has not been specifically studied.

BBB enzymes such as ɣ-glutamyl transferase, alkaline phosphatase and aromatic decarboxylase are enriched in cerebral microvessels compared with similar sized vessels elsewhere in the body. They metabolise neuroactive compounds in blood and may be differentially expressed in apical and basal domains (Betz and Goldstein, 1978; Betz *et al.*, 1980).

Altered permeability of the barrier

The BBB usually constitutes an effective barrier to infection and within it is regarded as an immunologically privileged site (Muldoon *et al.*, 2013). Many pathological effects, infectious agents and physiological factors may disrupt BBB function leading to neurotoxicity, encephalitis, meningitis and brain oedema. Some of these have increased incidence in the newborn (Lee *et al.*, 1997; Kimelberg, 2004). Disruption of paracellular pathways (Chen *et al.*, 2003) may be more serious than e.g. pneumolysin effects on transcellular pathways (Nizet *et al.*, 1997). Toxins, viral infections, eukaryotic parasites and fungi penetrate the BBB rendering it permeable to other agents (Stamatovic *et al.*, 2008). Inflammatory mediators, matrix metalloproteinases, growth factors prostaglandins reactive oxygen and nitrogen affect the function of the BBB (Stamatovic *et al.*, 2008).

It is not totally understood how all the 'opening mechanisms' operate but opening by any means will affect local toxicokinetics and increase CNS exposure to neurotoxins. Some appear to affect only the paracellular route possibly through IL1-β and CXCL8 whereas compounds such as TNF-α increase only transcellular permeability (Butt *et al.*, 1990). Sometimes opening is transient as when histamine causes intracellular Ca^{2+} accumulation. Thrombin is one rather potent cause of cytoskeletal reorganisation that results in more prolonged opening of the BBB (van Hinsbergh and Amerongen, 2002). This pathway incidentally indicates a theoretical biologically plausible mechanism of damage to the BBB from birth trauma. Highly effective inducers of BBB hyperpermeability other than thrombin

(Lee *et al.*, 1997) are amyloid-β peptide (Mackic *et al.*, 1998) and intracellular Ca^{2+} (Brown *et al.*, 2004). Leukocytes in blood may cause BBB permeability and use diapedesis to enter the brain themselves (Alvarez and Teale, 2006). Exogenous pharmacological agents such as nicotine are known to affect the BBB and neonicotinoid pesticides may through their action on intracellular Ca^{2+} also disrupt BBB function. *In vitro* models of the BBB are in development but no model validated for risk assessment is currently available (Dehouck *et al.*, 1990; Garberg *et al.*, 2005; Culot *et al.*, 2008; Paolinelli *et al.*, 2013).

5.3.2. The developing brain

The development of the human brain starts on day 19 in the human embryo and comprises a number of key stages that progress through the neonatal and infant period well into adolescence before the brain is fully mature (European Medicines Agency, 2009). These steps consist of neuronal proliferation, migration, organisation and myelination. In a first phase, neuronal proliferation and the generation of radial glia occur between gestation weeks 8 and 16, followed by a second phase between 5 months and 1 year of life, consisting of glial multiplication (while neurogenesis and neuroproliferation are continuing). Postnatal brain growth in humans is prolonged and proceeds at fetal growth rates for nearly a year or even longer (Neubauer and Hublin, 2012).

Neurotransmitters such as glutamate, γ-aminobutyric acid, dopamine, serotonin, acetylcholine, opiates and adenosine play a crucial role in the control and modulation of pre- and postnatal brain maturation. For instance, neurons with γ-aminobutyric acid are involved in migrational events following radial glial guides whereas N-methyl-D-aspartate receptors transduce excitatory signals in the developing brain, influence synaptic construction, and promote proliferation and migration of neuronal progenitors. The latter events play a crucial role in the development of brain plasticity, which results from a subtle balance between neuronal proliferation and physiological apoptosis.

The vulnerability of the immature brain derives from incomplete myelination and receptor-maturation as well as a lack of endogenous protectors especially in the preterm neonate. This vulnerability can be exacerbated by pre- and postnatal events, including maternal diseases and infections that affect brain maturation.

Increased intracerebral bilirubin concentrations may lead to bilirubin encephalopathy and severe brain damage (kernicterus). The pathogenesis of bilirubin encephalopathy is multifactorial and involves an interaction between unconjugated and free bilirubin levels, albumin binding capacity, acid-base status and neuronal susceptibility to injury (Watchko, 2016).

5.3.3. Summary

The developing BBB is similar to the adult BBB, in that it possesses functional tight junctions and influx and efflux transporters. Hence, contrary to previous assumptions, the healthy BBB in the first 16 weeks, although immature, is considered functional and not leaky. However, the expression/activity of transporters and metabolising enzymes appears to vary from adult patterns to meet the needs of the developing brain and apparently give rise to the measured differences in uptake (Ek *et al.*, 2012) and metabolism rates (e.g. CYP2D6) across the neonatal and infant BBB (Mann *et al.*, 2016). The movement of molecules across the BBBs depends on their physicochemical properties, the local physiological milieu (e.g. pH) and the need for transporters (i.e. influx and efflux transporters) and their ontogeny, activity, and specific location. The differences may result in greater vulnerability to chemicals than in the adult (Johansson *et al.*, 2008; Ek *et al.*, 2012; Neal-Kluever *et al.*, 2014).

Uncertainty in predicting specific exposure of the CNS to chemicals derives from knowledge gaps concerning the degree to which alternative pathway barriers such as the blood choroid plexus barrier are functional, precise differentiation state of the BBB.

5.4. The developing immune system

The immune system is a defence system designed to react to exposure to any foreign and 'non self' component. To this aim, a large number of cells and humoral mediators interplay and contribute to an adequate immune response, both by non-specific (innate)- and antigen-specific acquired (or adaptive) immune responses (Delves *et al.*, 2017). In the course of these responses, efficient mediators are released and potent mechanisms are triggered that need to be adequately regulated in order to avoid

any undesirable injury to the responding organism itself, including auto immunity and/or allergy (Delves *et al.*, 2017). Finally, it must be considered that the immune system includes both systemic and local (e.g. lungs, skin, gastrointestinal tract) mechanisms, which may react independently but remain interacting. In this regard, in the neonate the development of the gastrointestinal immune system may be especially vulnerable and deserves a specific attention (Brugman *et al.*, 2015).

At birth, the immune system is far from being completely functional and requires some time before being fully efficient. Newborns mostly rely on their innate (non-specific) immune system as their adaptive (specific) immunity and their mainly Th2 skewed adaptive (specific) immunity fully develop only later in life, as result of a delayed onset of the Th1 immunity and neonatal antibody responses. The imbalance between Th1 and Th2 responsiveness in the newborn is believed to contribute to the susceptibility of infants to infection and allergic reactions (Dietert and Piepenbrink, 2006; Siegrist and Aspinall, 2009; Zaghouani *et al.*, 2009; Krishnamoorthy *et al.*, 2012). The development and maturation of the immune system, are initiated *in utero* during the fetal period, and expand in time through the first years of childhood (Pettengill *et al.*, 2014). Accordingly, adequate building up of this system is especially prone to any factor disrupting the numerous mechanisms involved in these processes during the fetal life, in the neonate and the young infant (Gasparoni *et al.*, 2003; Kotiranta-Ainamo *et al.*, 2004; Hartel *et al.*, 2005; Dietert and Piepenbrink, 2006; Velilla *et al.*, 2006; Adkins, 2007; Morein *et al.*, 2007; Greer *et al.*, 2008; Saito *et al.*, 2008; Belderbos *et al.*, 2009; Dietert, 2009b; Walker *et al.*, 2011; Renz *et al.*, 2012; Sharma *et al.*, 2012; Ygberg and Nilsson, 2012; Neal-Kluever *et al.*, 2014; Valiathan *et al.*, 2016).

In addition, it should be considered that the immune system shares mediators, and therefore interferes with, for example, the endocrine and the nervous systems (Madhusudan *et al.*, 2013; Kabouridis and Pachnis, 2015); consequently, any alteration of one of these mediators may influence the development of any of the systems that share these mediators.

Environmental influences

From a risk assessment standpoint the issue at hand is whether exposure to immunotoxicants during development and maturation of the immune system produces more severe or persistent health aspects than similar exposure after the immune system is fully mature. It has been reported that adult exposure assessment does not always predict the risk of perinatal immunotoxicity (Dietert, 2009a; 2011; DeWitt *et al.*, 2012; Kollmann *et al.*, 2012; Krishnamoorthy *et al.*, 2012).

Although not established absolutely, it is generally recognised that the immature immune system is more or at least differently (qualitatively) susceptible to immunotoxicants than the fully mature system (Hrubee *et al.*, 2005; DeWitt *et al.*, 2012; Kollmann *et al.*, 2012; Krishnamoorthy *et al.*, 2012). Several studies using animal models have shown that the developing immune system is altered by (immuno) toxicants, such as 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD), tributyltin or Pb, at doses that were significantly lower than those required to produce effects in the adult. In addition, different effects can be observed depending on the age at which the organism is exposed. For instance, tributyltin affects a broader spectrum of immune function upon perinatal than adult exposure, and perinatal TCDD exposure mainly affects cell-mediated immunity (decreased, with males more sensitive than females), whereas adult exposure especially affects humoral immunity (enhanced) (Luebke *et al.*, 2006). Furthermore, sequels of exposure to immune toxicants during development may be particularly persistent in contrast to effects observed following adult exposure, which generally occur at higher doses (Holladay and Smialowicz, 2000).

Maternal influences

Being fed with breast milk in the first weeks of its life is for the newborn a significant opportunity for an efficient and harmonious development and function of its immune system (Marques *et al.*, 2013; Yong *et al.*, 2013). For instance, breastfeeding provides the newborn with immunoglobulins and other components or modulators (e.g. fatty acids, oligosaccharides) of the immune system. However, composition of the breast milk can be altered by contamination with substances that may be present in the foods eaten by the mother and that are able to cross from the blood to the milk, and this may result in disturbance of the beneficial properties of breast feeding.

In addition to direct beneficial effects on the immune system itself, breast milk favours an adequate colonisation of the gastrointestinal tract with a microflora that helps in the maturation of the immune system (Parigi *et al.*, 2015). The human gut microbiota, the mixed-species community of microbes

717 that reside in the gastrointestinal tract, plays a critical role in physiological and immunological
718 maturation and homeostasis (Donovan *et al.*, 2012). Epidemiological studies suggest that the common
719 feature of many allergy risk-related exposures is their influence on the founding and early
720 development of a child's gut. Perturbations to gut bacterial community composition during the
721 neonatal period have been associated with a variety of paediatric disorders, underscoring the
722 relationship between early life gut microbiota development and childhood health status. Microbial
723 exposures could alter the risk of subsequent allergic disease and asthma. The human microbiome
724 plays a role in immune regulation, which may impact against the development of allergic diseases
725 (Bengmark, 2013; Alegre *et al.*, 2014; Kabat *et al.*, 2014; Salzman, 2014). In this context, it should be
726 noted that the uterus may also play an indirect role in the immune development through fetal
727 microbial colonisation (Brugman *et al.*, 2015; Romano-Keeler and Weitkamp, 2015).

728 **Allergic reactions**

729 Data on the consequences of exposure to an allergen in infancy are available mainly from studies
730 investigating food allergy prevention involving older infants from the age of 4 months onwards.

731 The concepts of an 'optimal window' for oral introduction of allergens during which the chance for the
732 development of immune tolerance is greatest, have recently been discussed (Gray, 2015). They were
733 based on studies focussing on the timing of introduction of allergenic food components, which led to a
734 'paradigm shift' of strategies, away from the earlier concept of 'strict, total allergen avoidance',
735 towards a concept of 'tolerance induction' by early oral introduction of allergenic foods (de Silva *et al.*,
736 2014; Tey *et al.*, 2014; Gray, 2015; Perkin *et al.*, 2016). These studies, even if they were not without
737 methodological flaws, suggested that early introduction of allergens (e.g. from egg, wheat or milk),
738 between 4 and 6 months or below 7 months, respectively, may protect against allergies (Poole *et al.*,
739 2006; Katz *et al.*, 2010; Koplin *et al.*, 2010; Nwaru *et al.*, 2010). Similarly, a randomised control trial
740 on optimal timing of peanut introduction in the infant at risk of peanut allergy (Du Toit *et al.*, 2015)
741 showed that early introduction of peanut significantly decreased the development of peanut allergy
742 amongst children tested negative on a peanut Skin Prick Test (SPT) and those at high risk for this
743 allergy and modulated the immune response to peanuts. Other studies showed that feeding of
744 hydrolysed or partially hydrolysed cow's milk-formulae led to reduced allergic manifestations and food
745 sensitisations compared to standard cow's milk-based formulae (Muraro *et al.*, 2014; von Berg *et al.*,
746 2016).

747 As is reflected by current recommendations supported by the European Academy of Allergy and
748 Clinical Immunology (Muraro *et al.*, 2014), existing information reports that infants at high risk may
749 benefit from reduced exposure to substances being potential allergens in the first four months of their
750 lives (Halken, 2004; Poole *et al.*, 2006; Katz *et al.*, 2010; Koplin *et al.*, 2010; Nwaru *et al.*, 2010; Du
751 Toit *et al.*, 2015).

752 ### 5.4.1. Summary

753 The developing immune system in fetal and early postnatal life is particularly sensitive and exposure
754 to immunotoxicants may result in persistent effects on the immune system that last or appear only
755 long after exposure and in general, may also occur at lower doses than during adult exposure. In
756 addition, immune effects seen upon perinatal or adult exposure may be different. A number of
757 compounds or types of exposure may produce different ranges or severities of outcomes depending
758 on the time of exposure during the immune system development. Different and unpredictable arrays
759 of alterations (quantitative and qualitative) may be expected when the exposure occurs *in utero* or in
760 the early neonate versus adult exposure. They may be associated with chronic immunological
761 conditions such as autoimmunity, immune deficiency, inflammation and allergic reactions. Altogether,
762 neonates have specific features in the development and maturation of their immune system, which
763 make their response to an immunogenic/allergenic compound different from that of an adult and
764 therefore, deserve specific studies.

765 ## 5.5. Development of the reproductive system

766 Adequate measurements of reproductive function and development include endpoints early in life such
767 as development of sex organs and attainment of pubertal milestones. The reproductive system is

heavily influenced by the endocrine system, which facilitates the release of hormones that influence growth and development of many organ systems (Neal-Kluever *et al.*, 2014).

Reproductive organs develop *in utero* and are immature at birth (Lemasters *et al.*, 2000). Some of the critical steps in the formation of the reproductive system take place during embryogenesis. Sexual maturation and related events do not occur until puberty.

5.5.1. Female reproductive organ development

At birth, female infants possess their entire complement of oocytes and further oogenesis does not occur (Neal-Kluever *et al.*, 2014). There are many milestones to accomplish for female reproduction such as maturation of reproductive tissues, steroid hormone production, development of external genitalia, development of sexual behaviour and cyclic hormonal signalling events (Beckman and Feuston, 2003). The period of birth through 6 months is relatively quiescent in terms of female reproductive development, but chemical exposures during this period might have latent effects not detectable until reproductive maturation. For example, girls perinatally (*in utero* and via breastfeeding) exposed to high levels of polybrominated biphenyl due to an accidental contamination with a fire retardant in Michigan in 1973, had an earlier age at menarche compared to breastfed girls exposed to lower levels *in utero* or girls who were not breastfed (Blanck *et al.*, 2000).

5.5.2. Male reproductive organ development

The first six months after birth are considered a sensitive developmental window for the testes (Lemasters *et al.*, 2000). The early postnatal testis exhibits a high proportion of immature Sertoli cells (Mendis-Handagama and Ariyaratne, 2001) which proliferate from birth until 6 months of age when the adult number of Sertoli cells is achieved (Lemasters *et al.*, 2000). The number of Sertoli cells that develop during this period directly influence the number of spermatogonial cells that can be supported (Cortes *et al.*, 1987), hence heavily affects sperm count and fertility later in life (Lemasters *et al.*, 2000). Furthermore, the infant testes have a higher susceptibility for toxic injury compared to the adult, since the blood-testis barrier does not fully develop until puberty (Camatini *et al.*, 1981). There is one main testosterone surge during human male development, namely from 4 to 6 weeks of gestation. Leydig cells undergo a rapid period of growth around the 3rd-4th month after birth (Codesal *et al.*, 1990; Mendis-Handagama and Ariyaratne, 2001), which is related to a transient increase in activity of the hypothalamic-pituitary-gonadal axis (Mann and Fraser, 1996; Prince, 2001).

5.5.3. Summary

The development of the female reproductive system in infants below 16 weeks of age is relatively quiescent. For the development of the male reproductive organ the testis, however, this is a sensitive period. The early postnatal testis exhibits a high proportion of immature Sertoli cells (Mendis-Handagama and Ariyaratne, 2001), which proliferate from birth until 6 months of age when the adult number of Sertoli cells is achieved (Lemasters *et al.*, 2000). Effects on the development of the reproductive organs generally become apparent later in life.

5.6. Development of the endocrine system

5.6.1. The hypothalamic-pituitary-gonadal (HPG) axis

In humans, the hypothalamic-pituitary-gonadal (HPG) axis develops *in utero* by the end of the first trimester (Mann and Fraser, 1996; Grumbach, 2002). Thus, the components of the HPG axis are present at birth. In the first 6 months after birth there are transient increases in FSH (females) and LH (males), followed by a period of diminished activity that lasts until puberty (Grumbach, 2002). There is some evidence that the HPG axis may be disrupted by chemicals what results in altered puberty. Perinatal exposure to chemicals such as DDT (Ouyang *et al.*, 2005), polybrominated biphenyls (Blanck *et al.*, 2000) and persistent halogenated organochemicals (Den Hond and Schoeters, 2006) has been associated with either accelerated puberty in girls (Louis *et al.*, 2008) or delayed puberty in boys (Louis *et al.*, 2008; Korrick *et al.*, 2011). Although precocious puberty has been associated with exposure to several chemicals the underlying mechanism remains unknown.

Minipuberty is the name given to the activation of the hypothalamic-pituitary-gonadal axis. It is described in detail and summarised here because it coincides with the period of concern for this risk assessment (Kuiri-Hänninen *et al.*, 2014). From mid-gestation, the HPG axis is active. Near to term, placental hormones cause its activity to be suppressed. Once the umbilical cord is cut at birth, the placental hormones no longer reach the early infant's body, and reactivation of the HPG axis increases circulating gonadotrophin levels. Gonadotrophin levels are still high during the first 3 months of life but decrease towards the age of 6 months (exceptionally FSH levels in girls remain high until 3-4 years). After this, the HPG axis remains inactive until puberty. The early postnatal availability of gonadotropins mediates gonadal development. Male babies show testosterone levels peaking at 1-3 months of age then declining following LH levels, and this is important for proper development of male genitalia. Girl babies' high gonadotrophin levels promote maturation of ovarian follicles and raised oestradiol levels.

Minipuberty is an under-researched area and knowledge of the mechanisms that suppress the HPG axis until puberty is limited. Such data gaps contribute to uncertainty in risk assessment. However, it is clear that the first post-natal months of life provide targets for endocrine active toxins that may have different endocrine mediated outcomes from those later in development and in adulthood.

5.6.2. The fetal and infant adrenal glands

Development of the adrenal glands starts by day 25 of gestation. The fetal adrenal glands function in cooperation with the placenta. By the 4th month of gestation they are four times the size of the kidney but by birth, following a rapid decline of the fetal adrenal cortex, they are only 1/3 the size of the kidney. Through apoptosis, the fetal adrenal cortex disappears almost completely by the end of the first year. At 1 year, a healthy human adrenal gland weighs approximately 1 g, but then increases to a final weight of 4-5 g when it is fully developed at 4-5 years (Ishimoto and Jaffe, 2011). These rapid changes from the fetal to the final form of the adrenal cortex occur in the time window of interest for this risk assessment. Premature babies, stillborn and some neonates that have experienced intrauterine stress have microscopic cystic changes in adrenal glands an observation that adds uncertainty to the risk assessment of substances found in food intended for preterm and term infants.

5.6.3. Adrenarche and the hypothalamic-pituitary-adrenal (HPA) axis

At adrenarchy, the preparation for puberty, there is an increase in adrenal androgen production by the zona fasciculata of the adrenal gland. This is biochemically detectable as a rise in dehydroepiandrosterone sulphate (DHEA-S) commencing at approximately six years of age in both sexes. It is a response to adrenocorticotropic hormone (ACTH) that causes a greater rise of 17-hydroxypregnenolone and dehydroepiandrosterone (DHEA) than the simultaneous rise of cortisol. The unique pattern of steroidogenic enzymes that accompanies these changes includes expression of sulfotransferase 2A1 (a catalyst of the formation of DHEA-S) which is the major adrenarchal steroid.

The onset of puberty varies across populations and sexes and may be influenced by nutritional status. Breast budding is normal in Black and Mexican girls as early as seven years (Rosenfield *et al.*, 2009) but good data for the onset of male puberty is still unavailable (Bordini and Rosenfield, 2011). Premature or precocious puberty is defined as onset 2 to 2.5 standard deviations (SD) earlier than population norms. Precocious puberty is attributable to androgen and/or oestrogen effects. These may be centrally mediated through the hypothalamic-pituitary-gonadal axis, or in 'peripheral' precocious puberty from an autonomous peripheral origin such as the adrenal glands, gonads, germline or neural crest derived tumours. It may also have an exogenous basis. In boys with precocious puberty, where both testes are enlarged this may be in response to luteinizing hormone [LH]. This condition is HPG related or 'central' precocious puberty. It could also be the result of a human chorionic gonadotropin (hCG)-secreting tumour. Where both testes are pre-pubertal in size, the most likely source of the androgens is adrenal. The exogenous causes include topical exposure to lavender and tea-tree oil. Here the effects are thought to depend on the anti-androgenic effects of these compounds which caused gynaecomastia in pre-pubertal boys (Henley *et al.*, 2007). Similarly, oestradiol in an Evamist spray, a menopausal symptom relief prescription treatment, may cause precocious puberty in girls,

gynaecomastia in boys and may affect mammary glands in companion animals when inadvertent skin contact is made.[11]

5.6.4. The fetal and infant parathyroid and thyroid glands

The structure and development of the parathyroid glands, of which four are usually found in humans and most mammals, are well described in a recent publication (Kalinin *et al.*, 2013). The glands develop prenatally and are essential for skeletal growth during early postnatal development.

The thyroid plays a key role in neurological development, metabolism regulation, bone remodelling and other essential functions (Boas *et al.*, 2012). Disruptions in thyroid signalling can have widespread effects on infant development (Zimmermann, 2011).

5.6.5. Summary

Infants below 16 weeks of age have specific endocrine profiles that are different from those in adults. This suggests that such differences may require particular attention for the risk assessment of substances present in food intended for infants below 16 weeks of age. Effects at any early developmental stage may be reflected in deficits at later time points, indicating that the window of exposure, the window of maximum sensitivity and the window of effect require a lifespan approach to generate a fully protective risk assessment. This is because the disruption of the programming role of hormones during postnatal development can potentially cause adverse effects that do not become evident until later in life. Although our scientific knowledge in this area is developing rapidly, concerted research and development efforts are still needed to fill existing knowledge gaps (Solecki *et al.*, 2017).

6. Considerations of testing strategy for substances present in food intended for infants below 16 weeks of age

Testing strategies for substances present in food intended for infants need to take into consideration the differences in development stages of the relevant organ systems and the related critical windows of maximum sensitivity between humans and laboratory animals used in safety testing. Information on comparative differences in the post-natal development of organ systems and physiological processes between humans and commonly used experimental animal models can be found in the literature (see e.g. (WHO, 2006; Office for Risk Assessment, 2008; Beck *et al.*, 2016; Kuper *et al.*, 2016).

6.1. Information provided by standard testing of substances present in food

The safety evaluation of substances that may be present (intentionally or not) in the foods consumed by infants below the age of 16 weeks should follow the general principles set for the safety evaluation processes used for older infants and adults and can make use of information already obtained in such studies. The testing programmes for the safety evaluation of substances present in food used for the general population will depend on the applicable guidances[12] and legislations.[13] Typically, these studies will provide information on ADME, sub-chronic and chronic toxicity, genotoxicity, carcinogenicity and reproductive toxicity.

[11] Http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm220185.htm

[12] See e.g. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760. [60 pp.] doi:10.2903/j.efsa.2012.2760;
EC (European Commission), 2001. Guidance of the Scientific Committee on Food (SCF) for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation. SCF/CS/PLEN/GEN/100 Final. 19 December 2001.
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. doi:10.2903/j.efsa.2016.4594.

[13] See e.g. COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

6.2. Additional information needed when testing substances present in food intended for infants below 16 weeks of age

In view of the potential greater sensitivity during the first few weeks of life, the EFSA SC considers that additional studies may be necessary for the risk assessment of substances intended to be consumed by this population. These are detailed below.

When standard toxicological studies do not show adverse effects and the ADME studies show that a substance is not absorbed, only a repeated dose study with direct oral administration to neonatal animals [preferably the (mini)pig] is needed. This latter study should include analysis of possible local effects on the gastrointestinal tract and on a possible reduction in the bioavailability of nutrients (minerals and vitamins) that are normally contained in food for infants.

When standard toxicological studies do not show adverse effects and the ADME studies show that a substance or its metabolite(s) is (are) absorbed, the approach as described below should be followed.

An Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) is warranted. Following the EOGRTS guidelines, rats are the species of choice and the pups are (possibly) exposed *in utero*, during lactation and after weaning, until at least postnatal day 42. For the purpose of this guidance, three cohorts of F1-animals (as described in the OECD TG 443) should be included: a cohort to assess the reproductive and developmental endpoints, a cohort to assess the potential impact of a test substance on the developing nervous system, and a cohort to assess the potential impact on the developing immune system. As exposure through mother's milk in experimental studies is usually substantially lower than the exposure through feed, the resulting doses in the neonatal animals may be insufficient for hazard characterisation. Therefore, for the purpose of this guidance, it is advised that direct dosing of the neonatal animals should be considered as soon as possible after birth.

There may be circumstances under which deviation from the above-mentioned studies may be justified. In such cases, a scientific justification should be provided. When a two-generation reproductive toxicity study (OECD TG 416) is available instead of an EOGRTS, the former could be complemented with appropriate postnatal development studies addressing e.g. neurotoxicity and immunotoxicity. In some cases, additional studies in neonatal animals in a second non-rodent species [preferably the (mini)pig], specific *in vitro* assays or mechanistic studies may be needed.

Other information

Human data (epidemiological, clinical studies and case reports, where available) should be included in the evaluation. The general principles underpinning the risk assessment performed according to the guidance or recommendations established by the different EFSA panels and units should be applied.

6.3. (Mini)pigs as model for toxicity testing of substances present in food intended for infants below 16 weeks of age

(Mini)pigs are frequently used as an animal model in safety and efficacy research of chemicals and pharmaceuticals as they closely resemble humans in anatomy, physiology and biochemistry and because of the practical features of toxicity testing in (mini)pigs [e.g. (Bode *et al.*, 2010)]. In particular, the gastrointestinal tract, the cardiovascular system, the skin, the urogenital system, metabolic aspects and the immune system of (mini)pigs are considered generally more like humans than those of other non-rodent species [e.g. (Helm *et al.*, 2007; Guilloteau *et al.*, 2010; Barrow, 2012; Heinritz *et al.*, 2013; Odle *et al.*, 2014)]. As in pigs no trans-placental transfer of antibodies exists, (pre)term piglets need to stay for the first 24-48 hours with their mother for obtaining passive immunological protection by antibodies via mother's colostral milk (Farmer and Quesnel, 2009; Penninks *et al.*, 2012), or by intra-arterial injection within the first 24 hours of mother's plasma to obtain immunological protection (Hansen *et al.*, 2016). In addition, direct oral administration of substances, including bottle-feeding, can be performed in this model. Therefore, the (pre)term (mini)piglet can be considered as a relevant animal model for the safety evaluation of food additives, pesticide residues and contaminants present in food (i.e. via pig-adapted infant formula) on general toxicity parameters (i.e. growth, food/water consumption, haematology and clinical pathology, toxicokinetics, organ weights and histopathology evaluations, etc.) and postnatal maturation and development of various organ systems, such as the gastrointestinal tract (Van Ginneken, 2012; Sangild *et al.*, 2013), the metabolic and renal capacities (McAnulty, 2012; Preusse and Tingleff

Skaanild, 2012), the immune- and the reproductive systems (Haley, 2012; McAnulty *et al.*, 2012), and the nervous system (Lind *et al.*, 2007).

As pre-term piglets delivered at 90% gestation suffer from many of the same disorders and physiological problems as moderately immature human infants, they may be a relevant model to study the safety evaluation of early dietary interventions with food additives, pesticide residues and contaminants present in the food of the preterm piglets and on e.g. microbiota-related complications (Barrow, 2012).

Vaginally delivered neonatal piglets are described as a model to investigate influences of substances added to infant formulae on the development of the infant microbiota among other parameters of intestinal functions [e.g. (Alizadeh *et al.*, 2015; Hoeflinger *et al.*, 2015)]. More realistic testing conditions are generated using inter-species transplantation of gut microbiota from the human adult and infant gastrointestinal tract to that of piglets (Pang *et al.*, 2007; Zhang *et al.*, 2013; Wang and Donovan, 2015).

6.4. Testing of the developing immune system

Despite the need for developmental immunotoxicity testing, there are no validated or widely accepted methods for evaluating effects on the developing immune system, but there is general consensus that when possible, methods to assess developmental immunotoxicity testing (DIT) should be included in existing developmental and reproductive toxicology (DART) protocols (Holsapple *et al.*, 2005; Ladics *et al.*, 2005; Cooper *et al.*, 2006; Dietert and Holsapple, 2007; Burns-Naas *et al.*, 2008; DeWitt *et al.*, 2012).

When testing the developing immune system, it is important to account for the fact that there are different endpoints in the development of the immune system and in their maturation rates (US FDA, 2006). Integration of parameters reflecting the immune system in reproductive or developmental guidelines is minimal to date and it is limited to the EOGRTS guideline (OECD TG 443, 2011). This guideline is designed to provide an evaluation of the pre- and postnatal effects of chemicals on development as well as a thorough evaluation of systemic toxicity in pregnant and lactating females, and young and adult offspring, and includes additional endpoints for DIT as well as developmental neurotoxicity (DNT). The DIT default parameters include the standard immune pathology parameters and as this might not always be sensitive enough (Bunn and Dietert, 2001), an immune functional assay is also included in this EOGRTS guideline. The functional assay included is a T cell dependent antibody response (TDAR) with Sheep Red Blood Cells (SRBC) as particulate antigen or Keyhole Limpet Haemocyanin (KLH) as a protein antigen. Although not included in the EOGRTS guideline additional functional assays might be considered (Bunn and Dietert, 2001).

6.5. Testing of the developing nervous system

When testing the developmental nervous system to assess the safety of substances added to foods for infants the developmental neurotoxicity study under OECD TG 426 or as part of EOGRTS can be used. Direct oral dosing of the pups should be considered. It was noted[14] that the current data requirements for developmental neurotoxicity testing may not always be sufficient to identify all potentially hazardous compounds and that additional information may be provided by appropriate *in vitro* testing batteries (EFSA Panel on Plant Protection Products and their Residues (PPR), 2013).

6.6. *In vitro* assays

There is a scarcity of validated lower tier *in vitro* screening tests that are applicable to the first 16 weeks of post-natal human development. There are opportunities to develop such tests which would be relevant for example to measurement of uptake across the early gut and the brain barriers at early life stages. Likewise, there is a need for appropriately triggered developmental neurotoxicity tests. A recommendation follows that the existing test systems should be further developed to provide *in vitro* tools that can efficiently pre-screen substances as part of a comprehensive risk assessment approach for substances in food for infants below 16 weeks of age.

[14] https://www.efsa.europa.eu/en/events/event/161018b

7. Approach for the risk assessment of substances present in food intended for infants below 16 weeks of age

In general, it is assumed that effects observed in adults are also expected to occur in infants, which is supported by the available evidence. The reverse may not always be the case (e.g. the developing brain may be specifically affected or disruption of the programming role of hormones during postnatal development may cause adverse effects that do not become evident until later in life). Most differences between infants and adults are of a quantitative nature, i.e. the effects may occur at lower or higher doses than in adults. Appropriate developmental studies may reveal such effects for specific targets in that age group.

It is often assumed that the young infant is generally more sensitive than the adult is. This may not always be true, and the available evidence should be considered in the context of the assessment to conclude whether this assumption is supported or should be refuted.

Because considerable development is taking place in early infancy (particularly in preterm infants) and because milk/formula consumption per kg body weight is high, and hence exposure, is greatest in the first month of life, the period of potentially greatest sensitivity is the first few weeks. Moreover, this population is heterogeneous because the postnatal age may not be representative of the actual development of the newborn, specifically in pre-term born infants.

A decision tree to decide on the approach for the risk assessment of substances present in food intended for infants below 16 weeks of age is presented in Figure 7 below.

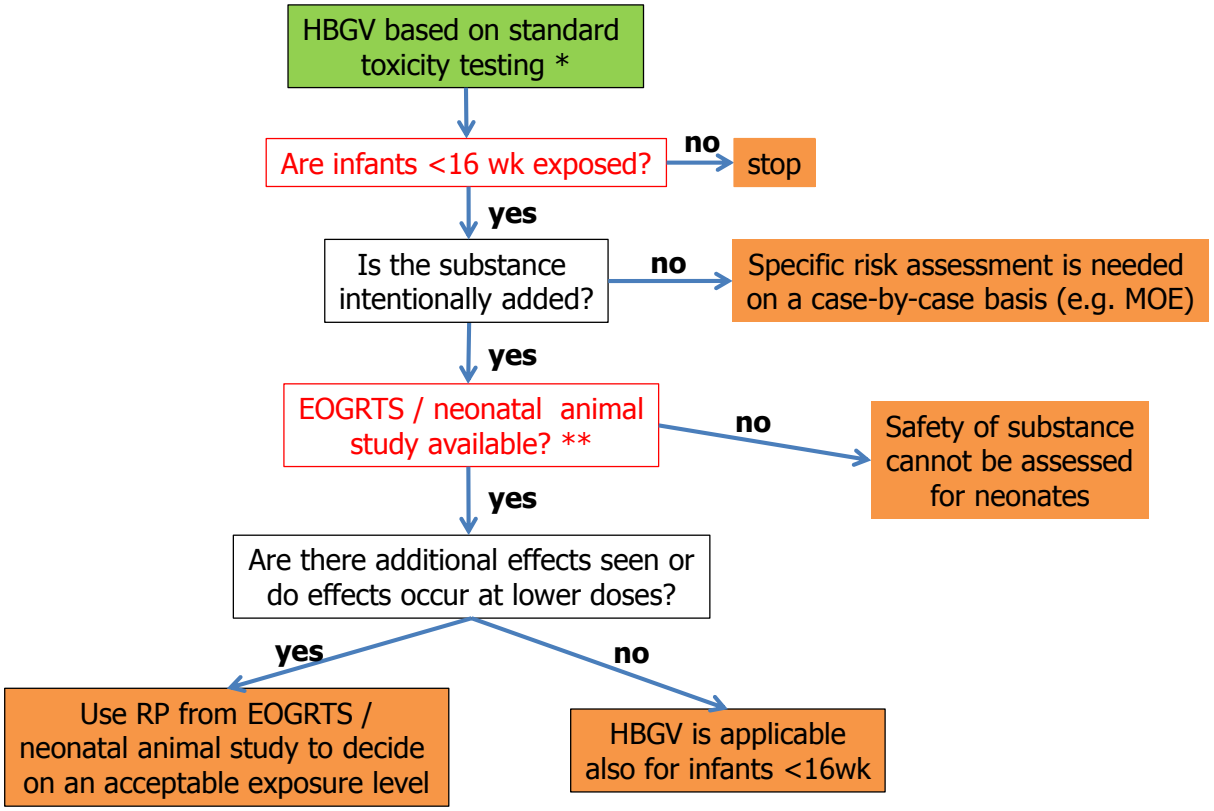


Figure 7. Decision tree approach for the risk assessment of substances present in food intended for infants below 16 weeks of age.

RP, Reference Point

* This decision tree assumes that a standard chemical risk assessment has already been performed on the substance of interest. Note that standard toxicity testing varies in the different areas of EFSA's remit

** Extended one-generation reproductive toxicity study (EOGRTS) if the substance is systemically available, neonatal animal study if the substance is not absorbed from the gastrointestinal tract and is not systemically available. There may be circumstances under which deviation from the above-mentioned studies may be justified (see Section 6).

7.1. Considerations for the risk assessment of substances not intentionally added to food intended for infants below 16 weeks of age

The presence of contaminants in food for infants should be avoided as much as possible.

Ideally, toxicity data from neonatal animal studies should be part of the body of information needed for the risk assessment for substances not intentionally added to food intended for infants below 16 weeks of age. However, such data might not always be available. The following section describes how knowledge on toxicokinetic and toxicodynamic considerations can be applied to support the risk assessment of unavoidable contaminants when data on their toxicity in infants are not available but some data from standard toxicological studies in animals are present.

Our understanding of the maturation of the gastrointestinal tract and the brain barriers has changed; these are now no longer considered as immature or leaky but as functional. Hence, the gut and the brain barriers can be considered to protect the young infant at a level that may be comparable to that seen in older infants and adults. However, it remains clear that developing organs such as the brain and the reproductive and immune systems may be more sensitive to harm in the infant population below 16 weeks of age as compared to the rest of the population.

The impact of the differences in toxicokinetic and toxicodynamic properties of substances in food between infants under the age of 16 weeks and older infants or adults and potential strategies on how to consider them in a risk assessment context are presented below.

Considerable knowledge of the ontogeny of the excretory pathways, specifically the metabolising enzymes, permits now to predict the toxicokinetic behaviour of substances if their metabolism in the adult is known. Even if their metabolic fate is not known in detail, the overall potential impact of the toxicokinetic variability in infants can be addressed. To account for the variability in toxicokinetics in the human population a kinetic sub-factor of 3.2 is applied as a default value for the derivation of a HBGV. This factor is in general appropriate. However, the differences between the adult population and the infants, in particular the very young ones, are in many cases greater than the factor of 3.2 and a larger factor may be necessary (see Section 5 and Appendix C).

Most differences between infants and adults are of a quantitative nature, and therefore, lower or higher doses of a chemical may be needed to cause adverse effects in infants as compared to adults. However, our current knowledge of the toxicodynamic variability in infants does not allow us to predict the magnitude of the difference in sensitivity between young infants and older infants or adults. Furthermore, whilst adverse effects observed in adults are also expected to occur in infants, the lack of observed adverse effects in adults (under standard test conditions) does not necessarily rule out a potential specific toxicity response in young infants to the same substance. Organs with critical developmental stages during early infancy such as the developing brain and the immune system may be affected specifically. Appropriate developmental studies, as described above, may not be available.

Taking into consideration that our current understanding of the toxicodynamic variability in infants is insufficient to make general quantitative and qualitative predictions of adverse effects in infants, the EFSA SC concludes that the uncertainty surrounding the toxicodynamic variability in infants did not allow identifying appropriate additional toxicodynamic default uncertainty factors (UFs). However, additional considerations on a case-by-case basis are needed to decide whether there is a health concern. In case studies on neonatal animals are available, these are expected to address the variability discussed above.

8. Conclusions

The EFSA SC notes that during the period from birth up to 16 weeks, infants are expected to be exclusively fed on breast milk and/or infant formula. The EFSA SC views this period as the time where HBGVs for the general population do not apply without further considerations.

In its approach to develop this guidance, the EFSA SC has taken into account the following:

- for the exposure assessment it is taken that in non-breastfed infants, formula is expected to be the only source of nutrition for the first 16 weeks of life;
- the overall toxicological profile of the substance identified through the standard toxicological tests, including critical effects;
- toxicokinetic data, especially:
    - the absorption of the substance from the GI tract
    - impaired renal excretion
    - metabolism in the neonatal organism compared with the adult;
- knowledge of organ development (critical windows) in human infants;
- the effects of exposure of neonatal animals at equivalent life stages of human infants;
- the relevance for the human infant of the neonatal animal models used and of the reported effects in toxicological testing;
- whether additional relevant effects are revealed or whether the effects occurred at lower doses in the neonatal animals, by comparison with standard toxicological studies.

The EFSA SC concludes the following:

1. High infant formula consumption per body weight is derived from 95th percentile consumption and the first weeks of life is the time of the highest relative consumption on a body weight basis. Therefore, when performing an exposure assessment, the EFSA SC proposes to use the highest consumption figures reported for the period of 14 to 27 days of life, i.e. 260 mL/kg bw per day.

2. A decision tree approach is proposed that enables a case-by-case risk assessment of substances present in food intended for infants below 16 weeks of age.

9. Recommendations

The EFSA SC recommends that:

1. Contemporary data on high food consumption during the first weeks of life after birth are generated.
2. Accurate data on exposure to substances present in food during this time period are generated.
3. When evaluating substances not intentionally added to food (e.g. contaminants), information from neonatal animal studies should be made available to reduce uncertainty.
4. There is a need to develop validated *in vitro* tests applicable to the first 16 weeks of post-natal human development that could provide tools to efficiently pre-screen substances as part of a comprehensive risk assessment and to improve understanding of the molecular events underlying adverse outcomes.
5. When evaluating substances intentionally added to food, post-marketing studies monitoring potential adverse effects (e.g. effects on the gastrointestinal tract) should be made available to reduce uncertainty.

1120 **References (please see end of document)**

Abbreviations

ADI	Acceptable daily intake
ADME	Absorption, distribution, metabolism and excretion
ARfD	Acute reference dose
AUC	Area under the curve
BBB	Blood brain barrier
Bw	Body weight
CNS	Central nervous system
EFSA	European Food Safety Authority
EMA	European Medicines Agency
EOGRTS	Extended One Generation Reproductive Toxicity Study
HBGV	Health-based guidance value
JECFA	Joint FAO-WHO Expert Committee Report on Food Additives
RP	Reference point
SCF	Scientific Committee on Food
TDI	Tolerable daily intake
UF	Uncertainty factor
WHO	World Health Organisation

Appendix A – Overview of EU Legislation (Food Sector) covering food for infants (and young children)

Glossary

- Infants - Children under the age of 12 months (as defined in Regulation (EU) No 609/2013)
- Young children - Children aged between 1 and 3 years (as defined in Regulation (EU) No 609/2013)

FOOD ADDITIVES Annex II to Regulation (EC) No 1333/2008 on food additives: **Specific uses of food additives in food for infants and young children** (food category 13.1 of Annex II) and specifically from birth onwards and/or for infants below 16 weeks → additives presently authorised in food categories '13.1.1 Infant formulae' and 13.1.5.1 and 13.1.5.2 (as follows)
Infant formulae as defined in Regulation (EU) No 609/2013* Food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding
Follow-on formulae as defined in Regulation (EU) No 609/2013* Food intended for use by infants when appropriate complementary feeding is introduced and which constitutes the principal liquid element in a progressively diversified diet of such infants
**Commission delegated Regulation (EU) 2016/127 was adopted on 25 September 2015 and will start to apply on 22 February 2020. Until that date, the rules of Directive 2006/141/EC remain applicable.*
Processed cereal-based foods and baby foods for infants and young children as defined in Regulation (EU) No 609/2013** Food intended to fulfil the particular requirements of infants in good health while they are being weaned and by young children in good health as a supplement to their diet and/or for their progressive adaptation to ordinary food ***Until the finalisation of the delegated act, the rules of Directive 2006/125/EC remain applicable.*
Other foods for young children
(13.1.5) Dietary **foods for infants and young children for special medical purposes** as defined in Regulation (EU) No 609/2013*** **and special formulae for infants** *** *Commission delegated Regulation (EU) 2016/128 was adopted on 25 September 2015 and will start to apply on 22 February 2019. Until that date, the rules of Directive 1999/21/EC remain applicable.*
(13.1.5.1) Dietary **foods for infants for special medical purposes** and special formulae for infants **Examples** of specific uses of food additives: From four months onwards in special food products with adapted composition, required for metabolic disorders and for general tube-feeding From 12 months onwards in specialised diets intended for young children who have cow's milk intolerance or inborn errors of metabolism From birth onwards: - in products for reduction of gastro-oesophageal reflux - in products in liquid formulae containing hydrolysed proteins, peptides or amino acids

- for use in products based on amino acids or peptides for use with patients who have problems with impairment of the gastrointestinal tract, protein mal-absorption or inborn errors of metabolism - in products used in case of gastrointestinal disorders - in products for the dietary management of metabolic disorders - in specialised diets, particularly those devoid of proteins
(13.1.5.2) Dietary **foods for babies and young children for special medical purposes** as defined in Directive 1999/21/EC **Examples** of specific uses of food additives: From four months onwards in special food products with adapted composition, required for metabolic disorders and for general tube-feeding From 12 months onwards in specialised diets intended for young children who have cow's milk intolerance or inborn errors of metabolism From birth onwards: - in products for reduction of gastro-oesophageal reflux - in products in liquid formulae containing hydrolysed proteins, peptides or amino acids - for use in products based on amino acids or peptides for use with patients who have problems with impairment of the gastrointestinal tract, protein mal-absorption or inborn errors of metabolism - in products used in case of gastrointestinal disorders - in products for the dietary management of metabolic disorders - in specialised diets, particularly those devoid of proteins
PESTICIDE RESIDUES Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control General requirement: Article 11(1)(b) - specific requirements on pesticides for infant formula, follow-on formula, processed cereal-based food, baby food and food for special medical purposes developed to satisfy the nutritional requirements of infants and young children should be updated regularly and include, *inter alia,* provisions to restrict the use of pesticides as much as possible
Directive 2006/141/EC **Infant formulae** shall not contain residues of individual pesticides at levels exceeding 0.01 mg/kg of the product as proposed ready for consumption or as reconstituted according to the instructions of the manufacturer. Lower MRLs apply for specific pesticides listed in Annexes VIII and IX of Directive 2006/141/EC (the same rules are laid down in Commission delegated Regulation (EU) 2016/127 that will start to apply on 22 February 2020. Until that date, the rules of Directive 2006/141/EC remain applicable). **Follow-on formulae** (same rules as for Infant formulae) Directive 2006/125/EC **Processed cereal-based foods** (same rules as for Infant formulae and Follow-on formulae) (Until the finalisation of the delegated act, the rules of Directive 2006/125/EC remain applicable) **Baby foods** (same rules as for Processed cereal-based foods) **Delegated Regulation (EU) 2016/128**: food for special medical purposes intended for infants and young children shall comply with the same rules laid down for formulae and baby foods (from 22 February 2019).
CONTAMINANTS Regulation (EEC) No 315/93 laying down Union procedures for contaminants in food Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs

Maximum levels for certain contaminants in foodstuffs: - Nitrate, Mycotoxins, Metals, Polycyclic aromatic hydrocarbons and Tropane alkaloids in Processed cereal-based foods and baby foods for infants and young children - Mycotoxins, Metals, Polycyclic aromatic hydrocarbons, Melamine and Inherent plant toxins in Infant formulae and follow-on formulae - Mycotoxins, Metals, Polycyclic aromatic hydrocarbons in foods for special medical purposes intended specifically for infants - Metals in Drinks for infants and young children labelled and sold as such, in Rice destined for the production of food for infants and young children - Dioxins and PCBs in Foods for infants and young children
FOOD CONTACT MATERIALS Regulation (EU) No 1935/2004 on materials and articles intended to come into contact with food >> sets out the general principles of safety and inertness for all Food Contact Materials (FCMs) Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food Maximum levels of SML/specific migration limit for certain substances used in FCM for food for infants and other restrictions: - Epoxidised soybean oil in PVC gaskets used to seal glass jars containing infant formulae and follow-on formulae or processed cereal- based foods and baby foods for infants and young children - Bisphenol A (BPA) not to be used for the manufacture of polycarbonate infant feeding bottles

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Appendix B – Key milestones in human development

Developmental milestone	*Gestational age (weeks GA) and post-natal (weeks PN)*	*Start time (Fertilisation age in days: FA)*	*Completion time (Fertilisation age in days: FA)*
Fertilisation	2 weeks GA	0	1
Extreme Preterm[1]	< 28 weeks GA	140	180
Very Preterm	< 32 weeks GA	180	210
Moderate preterm	< 37 weeks GA	210	245
Perinatal Period	22 weeks GA - 1 week PN	155	280
Normal Term Birth[2]	40 weeks GA 0 weeks PN	270	1
Neonate/Newborn infant	0-4 weeks PN	270	300
Post term pregnancy	0-4 weeks PN	270	298
Postnatal/postpartum/puerperium	0-6 weeks PN	270	310
Exclusive Breast Feeding Recommended	0-26 weeks PN	270	450
Infant	0-52 weeks PN	270	635

[1] Limit of viability is considered to be 22 weeks GA or birthweight < 500 g.
[2] Less than 5% cent of births occur at precisely 40 weeks of gestational age. Some 50 % are born within 1 week of this: 80 % are born within 2 weeks. (H. Kieler; O. Axelsson; S. Nilsson; U. Waldenström (1995). 'The length of human pregnancy as calculated by ultrasonographic measurement of the fetal biparietal diameter'. Ultrasound in Obstetrics & Gynecology. **6**: 353–357). The variation has to be considered as introducing uncertainty into the risk assessment for toxicity at different time windows of development.

Explanatory notes

1. The timing of birth is variable and several systems have been used to time human developmental events during pregnancy and after birth. These include age before or from the time of birth, gestational age, menstrual age, ovulation age and fertilisation age (FA). The latter is in common use by developmental biologist and biomedical scientists. FA was chosen as it offers a relatively accurate starting reference point and can be used to define events and development windows on a single axis that can be used to compare easily the timing of important events in human development that occur both during pregnancy and after birth whether that birth is premature, at normal term, or postmature.

2. Definitions by different bodies have varied in their application of the term Infant. The one for EU regulatory purposes which classes infants as in the age range from the time of normal term birth to 1 year of age was used.

3. The term exclusive breastfeeding (EBF) indicates that the infant receives breast milk (including expressed breast milk or breast milk from a wet nurse) and allows the infant to receive oral rehydration solution, drops, syrups (vitamins, minerals, medicines), but nothing else.

4. EBF is recommended by WHO and many EU member states. It should be carried out from birth to 6 months of age (270-450 days FA) but not after 500 days FA.

5. There are several definitions of the perinatal period: in this guidance the definition from WHO 'The perinatal period commences at 22 completed weeks of gestation and ends 7 completed days after birth' i.e. 155-280 days FA is endorsed.

6. The usage by WHO in employing the term moderate preterm (210-245 days FA) rather than the general term preterm will be adhered to. This is in contrast to very preterm (180-210 days FA) and extremely preterm (140-180 days FA).

Reference:

WHO Library Cataloguing-in-Publication Data Indicators for assessing infant and young child feeding practices: conclusions of a consensus meeting held 6–8 November 2007 in Washington D.C., USA.

World Health Organization. Dept. of Child and Adolescent Health and Development. 1.Infant nutrition. 2.Breast feeding. 3.Bottle feeding. 4.Feeding behaviour. 5.Indicators. ISBN 978 92 4 159666 4 (NLM classification: WS 120)

Appendix C – Decision tree approach to address the difference in toxicokinetics between infants below 16 weeks of age and older population for the risk assessment of substances not intentionally added to food for infants below 16 weeks of age

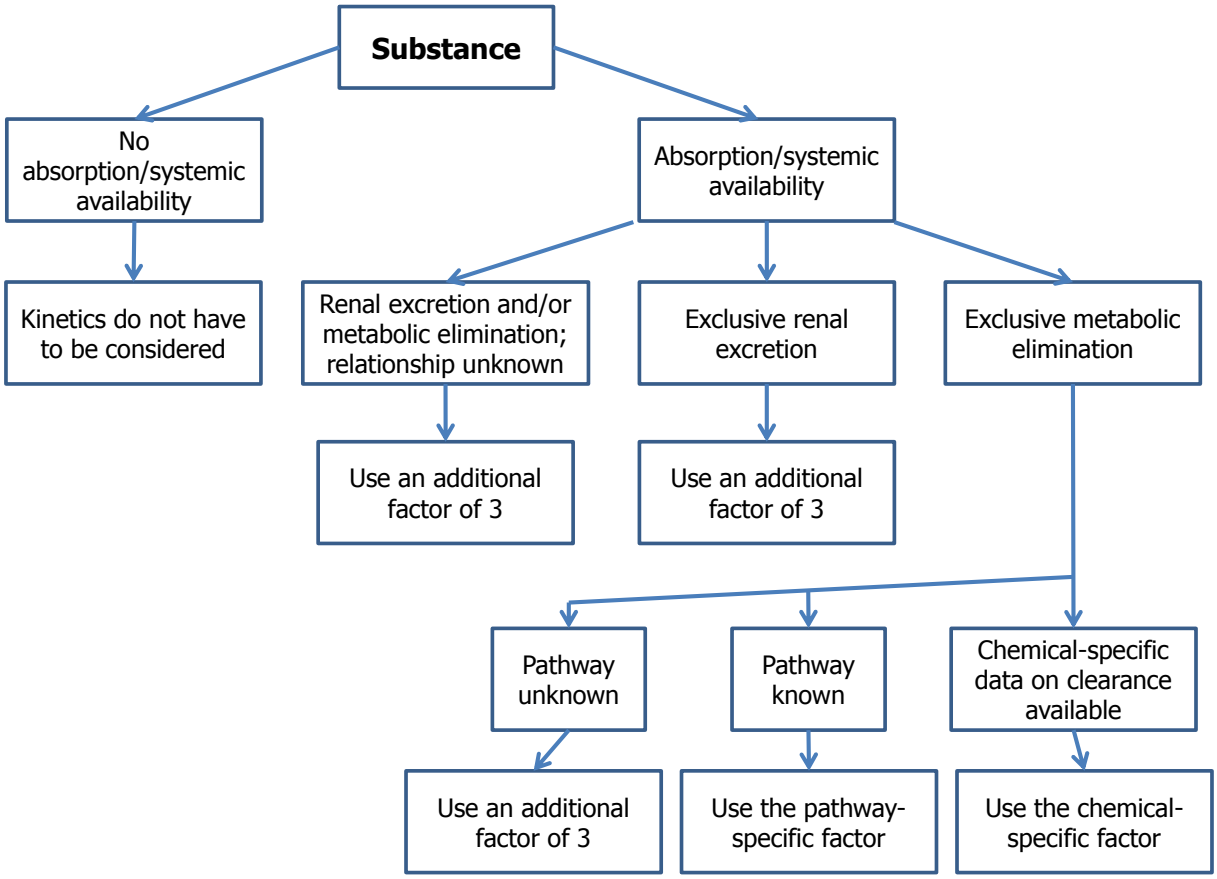

Figure C. The above decision tree applies exclusively to the risk assessment of substances **not** intentionally added to food for infants below 16 weeks of age. A default toxicokinetic factor of 3.2 is typically used to account for the inter-individual human variability within the general population. As discussed in Section 5, the differences between the adult population and the infants, in particular the very young ones, are in many cases greater than the default factor of 3.2 and hence necessitates an additional factor of 3 in the case where no data are available on the excretion or metabolism of a substance not intentionally added to food. In such case, an aggregate human UF of 30 needs to be applied to account for the increased inter-individual toxicokinetic and toxicodynamic variability (3 x 3.2 for TK and 3.2 for TD) that includes infants below 16 weeks of age. In case the pathway of metabolism is known, pathway-specific or chemical-specific factors should be added to the default factor of 3.2 to account for the increased inter-individual toxicokinetic variability between the adult population and infants below 16 weeks of age.

REFERENCES

Abraham K, Mielke H, Huisinga W and Gundert-Remy U, 2005. Elevated internal exposure of children in simulated acute inhalation of volatile organic compounds: effects of concentration and duration. Archives of Toxicology, 79, 63-73. 10.1007/s00204-004-0599-3

Adinolfi M, 1985. The development of the human blood-CSF-brain barrier. Developmental Medicine and Child Neurology, 27, 532-537.

Adinolfi M, Beck SE, Haddad SA and Seller MJ, 1976. Permeability of blood-cerebrospinal fluid barrier to plasma-proteins during fetal and perinatal life. Nature, 259, 140-141. 10.1038/259140a0

Adkins B, 2007. Heterogeneity in the CD4 T cell compartment and the variability of neonatal immune responsiveness. Curr Immunol Rev, 3, 151-159. 10.2174/157339507781483496

Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, Domellof M, Embleton ND, Fusch C, Genzel-Boroviczeny O, Goulet O, Kalhan SC, Kolacek S, Koletzko B, Lapillonne A, Mihatsch W, Moreno L, Neu J, Poindexter B, Puntis J, Putet G, Rigo J, Riskin A, Salle B, Sauer P, Shamir R, Szajewska H, Thureen P, Turck D, van Goudoever JB, Ziegler EE and ESPGHAN Committee on Nutrition, 2010. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr, 50, 85-91. 10.1097/MPG.0b013e3181adaee0

Alcorn J and McNamara PJ, 2002. Ontogeny of hepatic and renal systemic clearance pathways in infants - Part II. Clinical Pharmacokinetics, 41, 1077-1094. 10.2165/00003088-200241130-00005

Alegre ML, Mannon RB and Mannon PJ, 2014. The microbiota, the immune system and the allograft. American Journal of Transplantation, 14, 1236-1248. 10.1111/ajt.12760

Alizadeh A, Braber S, Akbari P, Garssen J and Fink-Gremmels J, 2015. Deoxynivalenol impairs weight gain and affects markers of gut health after low-dose, short-term exposure of growing pigs. Toxins, 7, 2071-2095. 10.3390/toxins7062071

Alvarez JI and Teale JM, 2006. Breakdown of the blood brain barrier and blood-cerebro spinal fluid barrier is associated with differential leukocyte migration in distinct compartments of the CNS during the course of murine NCC. Journal of Neuroimmunology, 173, 45-55. 10.1016/j.jneuroim.2005.11.020

Armand M, Hamosh M, Mehta NR, Angelus PA, Philpott JR, Henderson TR, Dwyer NK, Lairon D and Hamosh P, 1996. Effect of human milk or formula on gastric function and fat digestion in the premature infant. Pediatric Research, 40, 429-437. 10.1203/00006450-199609000-00011

Avery GB, Randolph JG and Weaver T, 1966. Gastric acidity in first days of life. Pediatrics, 37, 1005-1007.

Barrow PC, 2012. Use of the swine paediatric model. In: Doberman AM, and Lewis EM, editors. Pediatric Non-Clinical Drug Testing: Principles, Requirements, and Practice. Hoboken, NJ, USA: John Wiley & Sons, Inc. p 213-230.

Batchelor HK, Fotaki N and Klein S, 2014. Paediatric oral biopharmaceutics: Key considerations and current challenges. Advanced Drug Delivery Reviews, 73, 102-126. 10.1016/j.addr.2013.10.006

Batchelor HK and Marriott JF, 2015. Paediatric pharmacokinetics: key considerations. British Journal of Clinical Pharmacology, 79, 395-404. 10.1111/bcp.12267

Beck MJ, Padgett EL, Bowman CJ, Wilson DT, Kaufman LE, Varsho BJ, Stump DG, Nemec MD and Holson JF, 2016. Nonclinical Juvenile Toxicity Testing. In: Hood RD, editor. Developmental and Reproductive Toxicology: A Practical Approach. 3rd edition ed. Boca Raton, FL, USA: CRC Press. p 302-345.

Beckman DA and Feuston M, 2003. Landmarks in the development of the female reproductive system. Birth Defects Research Part B-Developmental and Reproductive Toxicology, 68, 137-143. 10.1002/bdrb.10016

Belderbos M, Levy O and Bont L, 2009. Neonatal innate immunity in allergy development. Current Opinion in Pediatrics, 21, 762-769. 10.1097/MOP.0b013e3283325e3a

Bengmark S, 2013. Gut microbiota, immune development and function. Pharmacological Research, 69, 87-113. 10.1016/j.phrs.2012.09.002

Berseth CL, 1989. Gestational evolution of small-intestine motility in preterm and term infants. Journal of Pediatrics, 115, 646-651. 10.1016/s0022-3476(89)80302-6

Berseth CL, 1996. Gastrointestinal motility in the neonate. Clinics in Perinatology, 23, 179-190.

Betz AL, Firth JA and Goldstein GW, 1980. Polarity of the blood-brain-barrier - distribution of enzymes between the luminal and antiluminal membranes of brain capillary endothelial-cells. Brain Research, 192, 17-28. 10.1016/0006-8993(80)91004-5
Betz AL and Goldstein GW, 1978. Polarity of blood-brain-barrier - neutral amino-acid transport into isolated brain capillaries. Science, 202, 225-227. 10.1126/science.211586
Blanck HM, Marcus M, Tolbert PE, Rubin C, Henderson AK, Hertzberg VS, Zhang RH and Cameron L, 2000. Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. Epidemiology, 11, 641-647. 10.1097/00001648-200011000-00005
Boas M, Feldt-Rasmussen U and Main KM, 2012. Thyroid effects of endocrine disrupting chemicals. Molecular and Cellular Endocrinology, 355, 240-248. 10.1016/j.mce.2011.09.005
Bode G, Clausing P, Gervais F, Loegsted J, Luft J, Nogues V, Sims J and Project R, 2010. The utility of the minipig as an animal model in regulatory toxicology. Journal of Pharmacological and Toxicological Methods, 62, 196-220. 10.1016/j.vascn.2010.05.009
Bode S, Dreyer T and Greisen G, 2004. Gastric emptying and small intestinal transit time in preterm infants: a scintigraphic method. J Pediatr Gastroenterol Nutr, 39, 378-382.
Bordini B and Rosenfield RL, 2011. Normal pubertal development: Part II: Clinical aspects of puberty. Pediatrics in Review, 32, 281-292.
Bourlieu C, Menard O, Bouzerzour K, Mandalari G, Macierzanka A, Mackie AR and Dupont D, 2014. Specificity of infant digestive conditions: Some clues for developing relevanti in vitro models. Critical Reviews in Food Science and Nutrition, 54, 1427-1457. 10.1080/10408398.2011.640757
Bowles A, Keane J, Ernest T, Clapham D and Tuleu C, 2010. Specific aspects of gastro-intestinal transit in children for drug delivery design. International Journal of Pharmaceutics, 395, 37-43. 10.1016/j.ijpharm.2010.04.048
Brouwer KLR, Aleksunes LM, Brandys B, Giacoia GP, Knipp G, Lukacova V, Meibohm B, Nigam SK, Rieder M, de Wildt SN and Pediat Transporter Working G, 2015. Human ontogeny of drug transporters: Review and recommendations of the pediatric transporter working group. Clinical Pharmacology & Therapeutics, 98, 266-287. 10.1002/cpt.176
Brown RC, Mark KS, Egleton RD and Davis TP, 2004. Protection against hypoxia-induced blood-brain barrier disruption: changes in intracellular calcium. American Journal of Physiology-Cell Physiology, 286, C1045-C1052. 10.1152/ajpcell.00360.2003
Brugman S, Perdijk O, van Neerven RJJ and Savelkoul HFJ, 2015. Mucosal immune development in early life: Setting the stage. Archivum Immunologiae Et Therapiae Experimentalis, 63, 251-268. 10.1007/s00005-015-0329-y
Bunn TL and Dietert RR, 2001. Developmental immunotoxicology assessment in the rat: Age, gender, and strain comparisons after exposure to lead. Toxicology Methods, 11, 41-58.
Burns-Naas LA, Hastings KL, Ladics GS, Makris SL, Parker GA and Holsapple MP, 2008. What's so special about the developing immune system? International Journal of Toxicology, 27, 223-254. 10.1080/10915810801978110
Butt AM, Jones HC and Abbott NJ, 1990. Electrical-resistance across the blood-brain-barrier in anesthetized rats - a developmental-study. Journal of Physiology-London, 429, 47-62.
Camatini M, Franchi E and DeCurtis I, 1981. Differentiation of inter-sertoli junctions in human testis. Cell Biology International Reports, 5, 109-109.
Catassi C, Bonucci A, Coppa GV, Carlucci A and Giorgi PL, 1995. Intestinal permeability changes during the first month - effect of natural versus artificial feeding. J Pediatr Gastroenterol Nutr, 21, 383-386. 10.1097/00005176-199511000-00003
Chen SHM, Stins MF, Huang SH, Chen YH, Kwon-Chung KJ, Chang Y, Kim KS, Suzuki K and Jong AY, 2003. Cryptococcus neoformans induces alterations in the cytoskeleton of human brain microvascular endothelial cells. Journal of Medical Microbiology, 52, 961-970. 10.1002/jmm.0.05230-0
Clewell HJ, Teeguarden J, McDonald T, Sarangapani R, Lawrence G, Covington T, Gentry R and Shipp A, 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Critical Reviews in Toxicology, 32, 329-389. 10.1080/20024091064264
Codesal J, Regadera J, Nistal M, Regaderasejas J and Paniagua R, 1990. Involution of human fetal Leydig cells: An immunohistochemical, ultrastructural and quantitative study. Journal of Anatomy, 172, 103-114.

Cong XM, Xu WL, Janton S, Henderson WA, Matson A, McGrath JM, Maas K and Graf J, 2016. Gut microbiome developmental patterns in early life of preterm infants: Impacts of feeding and gender. Plos One, 11. 10.1371/journal.pone.0152751

Cooper RL, Lamb JC, Barlow SM, Bentley K, Brady AM, Doerrer NG, Eisenbrandt DL, Fenner-Crisp PA, Hines RN, Irvine LFH, Kimmel CA, Koeter H, Li AA, Makris SL, Sheets LP, Speijers GJA and Whitby KE, 2006. A tiered approach to life stages testing for agricultural chemical safety assessment. Critical Reviews in Toxicology, 36, 69-98. 10.1080/10408440500541367

Cortes D, Muller J and Skakkebaek NE, 1987. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. International Journal of Andrology, 10, 589-596. 10.1111/j.1365-2605.1987.tb00358.x

Cresteil T, Beaune P, Kremers P, Celier C, Guengerich FP and Leroux JP, 1985. Immunoquantification of epoxide hydrolase and cytochrome P450 isozymes in fetal and adult human-liver microsomes. European Journal of Biochemistry, 151, 345-350. 10.1111/j.1432-1033.1985.tb09107.x

Culot M, Lundquist S, Vanuxeem D, Nion S, Landry C, Delplace Y, Dehouck MP, Berezowski V, Fenart L and Cecchelli R, 2008. An in vitro blood-brain barrier model for high throughput (HTS) toxicological screening. Toxicology in Vitro, 22, 799-811. 10.1016/j.tiv.2007.12.016

Dallas DC, Guerrero A, Khaldi N, Borghese R, Bhandari A, Underwood MA, Lebrilla CB, German JB and Barile D, 2014. A peptidomic analysis of human milk digestion in the infant stomach reveals protein-specific degradation patterns. Journal of Nutrition, 144, 815-820. 10.3945/jn.113.185793

Daneman R, Zhou L, Agalliu D, Cahoy JD, Kaushal A and Barres BA, 2010. The mouse blood-brain barrier transcriptome: A new resource for understanding the development and function of brain endothelial cells. Plos One, 5. 10.1371/journal.pone.0013741

De Cock RFW, Allegaert K, Vanhaesebrouck S, de Hoon J, Verbesselt R, Danhof M and Knibbe CAJ, 2014. Low but inducible contribution of renal elimination to clearance of propylene glycol in preterm and term neonates. Therapeutic Drug Monitoring, 36, 278-287. 10.1097/ftd.0000000000000003

De Cock RFW, Knibbe CAJ, Kulo A, de Hoon J, Verbesselt R, Danhof M and Allegaert K, 2013. Developmental pharmacokinetics of propylene glycol in preterm and term neonates. British Journal of Clinical Pharmacology, 75, 162-171. 10.1111/j.1365-2125.2012.04312.x

de Silva D, Geromi M, Halken S, Host A, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Cardona V, Dubois AEJ, Poulsen LK, Van Ree R, Vlieg-Boerstra B, Agache I, Grimshaw K, O'Mahony L, Venter C, Arshad SH, Sheikh A and Anaphylaxis EFA, 2014. Primary prevention of food allergy in children and adults: systematic review. Allergy, 69, 581-589. 10.1111/all.12334

de Wildt SN, 2011. Profound changes in drug metabolism enzymes and possible effects on drug therapy in neonates and children. Expert Opinion on Drug Metabolism & Toxicology, 7, 935-948. 10.1517/17425255.2011.577739

de Wildt SN, Tibboel D and Leeder JS, 2014. Drug metabolism for the paediatrician. Archives of Disease in Childhood, 99, 1137-1142. 10.1136/archdischild-2013-305212

Dehouck MP, Meresse S, Delorme P, Fruchart JC and Cecchelli R, 1990. An easier, reproducible, and mass-production method to study the blood-brain-barrier in vitro. Journal of Neurochemistry, 54, 1798-1801. 10.1111/j.1471-4159.1990.tb01236.x

Delves PJ, Martin SJ, Burton DR and Roitt IM, 2017. Roitt's Essential Immunology. 576 p.

Den Hond E and Schoeters G, 2006. Endocrine disrupters and human puberty. International Journal of Andrology, 29, 264-270. 10.1111/j.1365-2605.2005.00561.x

DeVizia B, Ciccimarra F, Decicco N and Auricchio S, 1975. Digestibility of starches in infants and children. Journal of Pediatrics, 86, 50-55. 10.1016/s0022-3476(75)80703-7

DeWitt JC, Peden-Adams MM, Keil DE and Dietert RR, 2012. Developmental Immunotoxicity (DIT): Assays for evaluating effects of exogenous agents on development of the immune system. Current Protocols in Toxicology, 51, 18.15.11–18.15.14.

DeWoskin RS and Thompson CM, 2008. Renal clearance parameters for PBPK model analysis of early lifestage differences in the disposition of environmental toxicants. Regulatory Toxicology and Pharmacology, 51, 66-86. 10.1016/j.yrtph.2008.02.005

Di Mauro A, Neu J, Riezzo G, Raimondi F, Martinelli D, Francavilla R and Indrio F, 2013. Gastrointestinal function development and microbiota. Italian Journal of Pediatrics, 39. 10.1186/1824-7288-39-15

Dietert RR, 2009a. Developmental immunotoxicity (DIT), postnatal immune dysfunction and childhood leukemia. Blood Cells Molecules and Diseases, 42, 108-112. 10.1016/j.bcmd.2008.10.005
Dietert RR, 2009b. Developmental Immunotoxicology: Focus on Health Risks. Chemical Research in Toxicology, 22, 17-23. 10.1021/tx800198m
Dietert RR, 2011. Role of developmental immunotoxicity and immune dysfunction in chronic disease and cancer. Reproductive Toxicology, 31, 319-326. 10.1016/j.reprotox.2010.09.006
Dietert RR and Holsapple MP, 2007. Methodologies for developmental immunotoxicity (DIT) testing. Methods, 41, 123-131. 10.1016/j.ymeth.2006.06.018
Dietert RR and Piepenbrink MS, 2006. Perinatal immunotoxicity: Why adult exposure assessment fails to predict risk. Environmental Health Perspectives, 114, 477-483. 10.1289/ehp.8566
Donovan SM, Wang M, Li M, Friedberg I, Schwartz SL and Chapkin RS, 2012. Host-microbe interactions in the neonatal intestine: Role of human milk oligosaccharides. Advances in Nutrition, 3, 450S-455S. 10.3945/an.112.001859
Doring B and Petzinger E, 2014. Phase 0 and phase III transport in various organs: Combined concept of phases in xenobiotic transport and metabolism. Drug Metabolism Reviews, 46, 261-282. 10.3109/03602532.2014.882353
Dorne J, Walton K and Renwick AG, 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. Food and Chemical Toxicology, 43, 203-216. 10.1016/j.fct.2004.05.011
Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, Brough HA, Phippard D, Basting M, Feeney M, Turcanu V, Sever ML, Lorenzo MG, Plaut M, Lack G and Team LS, 2015. Randomized trial of peanut consumption in infants at risk for peanut allergy. New England Journal of Medicine, 372, 803-813. 10.1056/NEJMoa1414850
Duanmu ZB, Weckle A, Koukouritaki SB, Hines RN, Falany JL, Falany CN, Kocarek TA and Runge-Morris M, 2006. Developmental expression of aryl, estrogen, and hydroxysteroid sulfotransferases in pre- and postnatal human liver. Journal of Pharmacology and Experimental Therapeutics, 316, 1310-1317. 10.1124/jpet.105.093633
Dutta S, Singh B, Chessell L, Wilson J, Janes M, McDonald K, Shahid S, Gardner VA, Hjartarson A, Purcha M, Watson J, de Boer C, Gaal B and Fusch C, 2015. Guidelines for feeding very low birth weight infants. Nutrients, 7, 423-442. 10.3390/nu7010423
Edginton AN, Schmitt W, Voith B and Willmann S, 2006. A mechanistic approach for the scaling of clearance in children. Clinical Pharmacokinetics, 45, 683-704. 10.2165/00003088-200645070-00004
EFSA, 2014. Modern methodologies and tools for human hazard assessment of chemicals. EFSA Journal, 12, 87. doi:10.2903/j.efsa.2014.3638
EFSA Panel on Contaminants in the Food Chain (CONTAM), 2010. Scientific Opinion on Lead in Food. EFSA Journal, 8, 151. doi:10.2903/j.efsa.2010.1570
EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2009. Scientific Opinion on the appropriate age for introduction of complementary feeding of infants. EFSA Journal, 7, 38. doi:10.2903/j.efsa.2009.1423
EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2013. Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. EFSA Journal, 11, 103. doi:10.2903/j.efsa.2013.3408
EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2014. Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal, 12, 106. doi:10.2903/j.efsa.2014.3760
EFSA Panel on Plant Protection Products and their Residues (PPR), 2013. Scientific Opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid. EFSA Journal, 11, 51. doi:10.2903/j.efsa.2013.3471
EFSA Scientific Committee, 2015. Scientific Opinion: Guidance on the review, revision and development of EFSA's Cross-cutting Guidance Documents. EFSA Journal, 13, 11. doi:10.2903/j.efsa.2015.4080
Ehrnebo M, Agurell S, Jalling B and Boreus LO, 1971. Age differences in drug binding by plasma proteins - studies on human foetuses, neonates and adults. European Journal of Clinical Pharmacology, 3, 189-193. 10.1007/bf00565004
Ek CJ, Dziegielewska KM, Habgood MD and Saunders NR, 2012. Barriers in the developing brain and neurotoxicology. Neurotoxicology, 33, 586-604. 10.1016/j.neuro.2011.12.009

Elashoff JD, Reedy TJ and Meyer JH, 1982. Analysis of gastric-emptying data. Gastroenterology, 83, 1306-1312.
Ennis SR, Ren XD and Betz AL, 1996. Mechanisms of sodium transport at the blood-brain barrier studied with in situ perfusion of rat brain. Journal of Neurochemistry, 66, 756-763.
European Medicines Agency, 2009. Guideline on the investigation of medicinal products in the term and preterm neonate (Committee for medicinal products for human use (CHMP) and paediatric committee (PDCO)).
FAO/WHO, 1972. Evaluation of food additives: some enzymes, modified starches, and certain other substances: toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants. Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva.
FAO/WHO, 2014. Safety evaluation of certain food additives. Seventy-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva.
Farmer C and Quesnel H, 2009. Nutritional, hormonal, and environmental effects on colostrum in sows. Journal of Animal Science, 87, 56-65. 10.2527/jas.2008-1203
Fomon SJ, 1993. Nutrition of Normal Infants: Mosby. 488 p.
Fomon SJ, Filer LJ, Thomas LN, Ziegler EE and Leonard MT, 1971. Food consumption and growth of normal infants fed milk-based formulas. Acta Paediatrica Scandinavica, 1-36.
Fredholm BB, Rane A and Persson B, 1975. Diphenylhydantoin binding to proteins in plasma and its dependence on free fatty-acid and bilirubin concentration in dogs and newborn-infants. Pediatric Research, 9, 26-30. 10.1203/00006450-197509010-00005
Friis-Hansen B, 1983. Water distribution in the fetus and newborn-infant. Acta Paediatrica Scandinavica, 7-11.
Ganshorn A and Kurz H, 1968. Differences between protein bonding in newborns and children and their significance for pharmacological effects. Naunyn-Schmiedebergs Archiv Fur Pharmakologie, 260, 117-118. 10.1007/bf00537925
Garberg P, Ball M, Borg N, Cecchelli R, Fenart L, Hurst RD, Lindmark T, Mabondzo A, Nilsson JE, Raub TJ, Stanimirovic D, Terasaki T, Oberg JO and Osterberg T, 2005. In vitro models for the blood-brain barrier. Toxicology in Vitro, 19, 299-334. 10.1016/j.tiv.2004.06.011
Gasparoni A, Ciardelli L, Avanzini A, Castellazzi AM, Carini R, Rondini G and Chirico G, 2003. Age-related changes in intracellular Th1/Th2 cytokine production, immunoproliferative T lymphocyte response and natural killer cell activity in newborns, children and adults. Biology of the Neonate, 84, 297-303. 10.1159/000073638
Gattineni J and Baum M, 2015. Developmental changes in renal tubular transport-an overview. Pediatric Nephrology, 30, 2085-2098. 10.1007/s00467-013-2666-6
Ginsberg G, Hattis D and Sonawane B, 2004. Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. Toxicology and Applied Pharmacology, 198, 164-183. 10.1016/j.taap.2003.10.010
Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S and Goble R, 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicological Sciences, 66, 185-200. 10.1093/toxsci/66.2.185
Goldmann EE, 1913. Vitalfärbung am Zentralnervensystem: Beitrag zur Physio-Pathologie des Plexus Chorioideus und der Hirnhäute. Abhandlungen der Preussischen Akademie der Wissenschaften Physikalisch-Mathematische Classe, 1, 1-60.
Grand RJ, Watkins JB and Torti FM, 1976. Development of human gastrointestinal-tract - Review. Gastroenterology, 70, 790-810.
Gray CL, 2015. Allergy prevention: Something old, something new, something borrowed, something blue. Current Allergy & Clinical Immunology, 28, 166-175.
Greer FR, Sicherer SH, Burks AW and Comm N, 2008. Effects of early nutritional interventions on the development of atopic disease in infants and children: The role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics, 121, 183-191. 10.1542/peds.2007-3022
Gröntoft O, 1954. Intracranial haemorrhage and blood-brain barrier problems in the new-born: A pathologico-anatomical and experimental investigation. Acta pathologica et microbiologica Scandinavica Supplement, 100, 8-109.
Grumbach MM, 2002. The neuroendocrinology of human puberty revisited. Hormone Research, 57, 2-14. 10.1159/000058094

Guilloteau P, Zabielski R, Hammon HM and Metges CC, 2010. Nutritional programming of gastrointestinal tract development. Is the pig a good model for man? Nutrition Research Reviews, 23, 4-22. 10.1017/s0954422410000077

Gupta M and Brans YW, 1978. Gastric retention in neonates. Pediatrics, 62, 26-29.

Haley P, 2012. The immune system of pigs: Structure and Function. In: McAnulty PA, Dayan AD, Ganderup NC, and Hastings KL, editors. The minipig in biomedical research. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group. p 343-355.

Halken S, 2004. Prevention of allergic disease in childhood: clinical and epidemiological aspects of primary and secondary allergy prevention. Pediatric Allergy and Immunology, 15, 9-32. 10.1111/j.1399-3038.2004.0148b.x

Hansen CF, Thymann T, Andersen AD, Holst JJ, Hartmann B, Hilsted L, Langhorn L, Jelsing J and Sangild PT, 2016. Rapid gut growth but persistent delay in digestive function in the postnatal period of preterm pigs. American Journal of Physiology-Gastrointestinal and Liver Physiology, 310, G550-G560. 10.1152/ajpgi.00221.2015

Hartel C, Adam N, Strunk T, Temming P, Muller-Steinhardt M and Schultz C, 2005. Cytokine responses correlate differentially with age in infancy and early childhood. Clinical and Experimental Immunology, 142, 446-453. 10.1111/j.1365-2249.2005.02928.x

Hattis D, Ginsberg G, Sonawane B, Smolenski S, Russ A, Kozlak M and Goble R, 2003. Differences in pharmacokinetics between children and Adults - II. Children's variability in drug elimination half-lives and in some parameters needed for physiologically-based pharmacokinetic modeling. Risk Analysis, 23, 117-142. 10.1111/1539-6924.00295

He H, Y.L. N, Li JF, Meng XG, Yang WH, Chen YL, Wang SJ, Ma X, Kan QC and Zhang LR, 2016. Developmental regulation of CYP3A4 and CYP3A7 in Chinese Han population. Drug Metabolism and Pharmacokinetics, 31.

Heimann G, 1980. Enteral absorption and bioavailability in children in relation to age. European Journal of Clinical Pharmacology, 18, 43-50. 10.1007/bf00561477

Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B and Dewey KG, 1993. Energy and protein intakes of breast-fed and formula-fed infants during the 1st year of life and their association with growth velocity - The DARLING Study. American Journal of Clinical Nutrition, 58, 152-161.

Heinritz SN, Mosenthin R and Weiss E, 2013. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutrition Research Reviews, 26, 191-209. 10.1017/s0954422413000152

Helm RM, Golden C, McMahon M, Thampi P, Badger TM and Nagarajan S, 2007. Diet regulates the development of gut-associated lymphoid tissue in neonatal piglets. Neonatology, 91, 248-255. 10.1159/000098523

Henley DV, Lipson N, Korach KS and Bloch CA, 2007. Brief report - Prepubertal gynecomastia linked to lavender and tea tree oils. New England Journal of Medicine, 356, 479-485. 10.1056/NEJMoa064725

Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, Cass CE, Zhang L and Int Transporter C, 2013. Emerging transporters of clinical importance: An update from the international transporter consortium. Clinical Pharmacology & Therapeutics, 94, 52-63. 10.1038/clpt.2013.74

Hines RN, 2008. The ontogeny of drug metabolism enzymes and implications for adverse drug events. Pharmacology & Therapeutics, 118, 250-267. 10.1016/j.pharmthera.2008.02.005

Hirata Y, Matsuo T and Kokubu H, 1965. Digestion and absorption of milk protein in infant's intestine. Kobe Journal of Medical Sciences, 11, 103-109.

Hoeflinger JL, Kashtanov DO, Cox SB, Dowd SE, Jouni ZE, Donovan SM and Miller MJ, 2015. Characterization of the intestinal lactobacilli community following galactooligosaccharides and polydextrose supplementation in the neonatal piglet. Plos One, 10. 10.1371/journal.pone.0135494

Holladay SD and Smialowicz RJ, 2000. Development of the murine and human immune system: Differential effects of immunotoxicants depend on time of exposure. Environmental Health Perspectives, 108, 463-473. 10.2307/3454538

Holsapple MP, Burns-Naas LA, Hastings KL, Ladics GS, Lavin AL, Makris SL, Yang Y and Luster MI, 2005. A proposed testing framework for developmental immunotoxicology (DIT). Toxicological Sciences, 83, 18-24. 10.1093/toxsci/kfh299

Hrubee TC, Blaylock BL and Holladay SD, 2005. Developmental Immunotoxicology of Halogenated Aromatic Hydrocarbons and Polycyclic Aromatic Hydrocarbons. In: Holladay SD, editor. Developmental Immunotoxicology. p 139-152.
ICRP, 2002. Basic anatomical and physiological data for use in radiological protection reference values. ICRP Publication 89. Annals of the ICRP, 32.
Institute of Medicine (IOM), 1991. Nutrition during lactation. Washington DC.
International Programme on Chemical Safety (IPCS), 1987. Principles for the safety assessment of food additives and contaminants in food. Geneva: WHO.
International Programme on Chemical Safety (IPCS), 2006. Principles for evaluating health risks in children associated with exposure to chemicals. Geneva: WHO.
International Programme on Chemical Safety (IPCS), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food: Chapter 7 - Risk Characterization. Geneva: WHO.
Ishimoto H and Jaffe RB, 2011. Development and function of the human fetal adrenal cortex: A key component in the feto-placental unit. Endocrine Reviews, 32, 317-355. 10.1210/er.2010-0001
Ittmann PI, Amarnath R and Berseth CL, 1992. Maturation of antroduodenal motor-activity in preterm and term infants. Digestive Diseases and Sciences, 37, 14-19. 10.1007/bf01308336
Johansson PA, Dziegielewska KM, Liddelow SA and Saunders NR, 2008. The blood-CSF barrier explained: When development is not immaturity. Bioessays, 30, 237-248. 10.1002/bies.20718
Johnson TN, Rostami-Hodjegan A and Tucker GT, 2006. Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. Clinical Pharmacokinetics, 45, 931-956. 10.2165/00003088-200645090-00005
Kabat AM, Srinivasan N and Maloy KJ, 2014. Modulation of immune development and function by intestinal microbiota. Trends in Immunology, 35, 507-517. 10.1016/j.it.2014.07.010
Kabouridis PS and Pachnis V, 2015. Emerging roles of gut microbiota and the immune system in the development of the enteric nervous system. Journal of Clinical Investigation, 125, 956-964. 10.1172/jci76308
Kalinin AV, Pavlov AV, Alexandrov AV, Kotova IV, Patrunov YN and Pamputis SN, 2013. The Parathyroid Glands: Imaging and Surgery Heidelberg, New York, Dodrecht and London: Springer. 157 p.
Katz Y, Rajuan N, Goldberg MR, Eisenberg E, Heyman E, Cohen A and Leshno M, 2010. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. Journal of Allergy and Clinical Immunology, 126, 77-82. 10.1016/j.jaci.2010.04.020
Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS and Kauffman RE, 2003. Developmental pharmacology - Drug disposition, action, and therapy in infants and children. New England Journal of Medicine, 349, 1157-1167.
Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schoch G, 1998. Measured consumption of commercial infant food products in German infants: Results from the DONALD study. J Pediatr Gastroenterol Nutr, 27, 547-552. 10.1097/00005176-199811000-00011
Kimelberg HK, 2004. Water homeostasis in the brain: Basic concepts. Neuroscience, 129, 851-860. 10.1016/j.neuroscience.2004.07.033
Knibbe CAJ, Krekels EHJ, van den Anker JN, DeJongh J, Santen GWE, van Dijk M, Simons SHP, van Lingen RA, Jacqz-Aigrain EM, Danhof M and Tibboel D, 2009. Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. Clinical Pharmacokinetics, 48, 371-385.
Kollmann TR, Levy O, Montgomery RR and Goriely S, 2012. Innate immune function by Toll-like receptors: Distinct responses in newborns and the elderly. Immunity, 37, 771-783. 10.1016/j.immuni.2012.10.014
Koplin JJ, Osborne NJ, Wake M, Martin PE, Gurrin LC, Robinson MN, Tey D, Slaa M, Thiele L, Miles L, Anderson D, Tan T, Dang TD, Hill DJ, Lowe AJ, Matheson MC, Ponsonby AL, Tang MLK, Dharmage SC and Allen KJ, 2010. Can early introduction of egg prevent egg allergy in infants? A population-based study. Journal of Allergy and Clinical Immunology, 126, 807-813. 10.1016/j.jaci.2010.07.028
Korrick SA, Lee MM, Williams PL, Sergeyev O, Burns JS, Patterson DG, Turner WE, Needham LL, Altshul L, Revich B and Hauser R, 2011. Dioxin exposure and age of pubertal onset among Russian boys. Environmental Health Perspectives, 119, 1339-1344. 10.1289/ehp.1003102
Kotiranta-Ainamo A, Rautonen J and Rautonen N, 2004. Imbalanced cytokine secretion in newborns. Biology of the Neonate, 85, 55-60. 10.1159/000074959

Kramer MS and Kakuma R, 2001. The optimal duration of exclusive breastfeeding: A systematic review. Geneva: World Health Organisation. WHO/NHD/01.08 WHO/FCH/CAH/01.23

Krishnamoorthy N, Khare A, Oriss TB, Raundhal M, Morse C, Yarlagadda M, Wenzel SE, Moore ML, Peebles RS, Ray A and Ray P, 2012. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. Nature Medicine, 18, 1525-1530. 10.1038/nm.2896

Kuiri-Hänninen T, Sankilampi U and Dunkel L, 2014. Activation of the hypothalamic-pituitary-gonadal axis in infancy: Minipuberty. Hormone Research in Paediatrics, 82, 73-80. 10.1159/000362414

Kumar V, Abbas A, Fausto N and Aster JC, 2010. Diseases of Infancy and Childhood. Robbins & Cotran Pathologic Basis of Disease. Philadelphia, PA, USA: W. B. Saunders, Elsevier

Kuper CF, van Bilsen J, Cnossen H, Houben G, Garthoff J and Wolterbeek A, 2016. Development of immune organs and functioning in humans and test animals: Implications for immune intervention studies. Reproductive Toxicology, 64, 180-190. 10.1016/j.reprotox.2016.06.002

Ladics GS, Chapin RE, Hastings KL, Holsapple MP, Makris SL, Sheets LP, Woolhiser MR and Burns-Naas LA, 2005. Developmental toxicology evaluations - Issues with including neurotoxicology and immunotoxicology assessments in reproductive toxicology studies. Toxicological Sciences, 88, 24-29. 10.1093/toxsci/kfi299

Larsen JC and Pascal G, 1998. Workshop on the applicability of the ADI to infants and children: consensus summary. Food Additives and Contaminants, 15 (Supplement), 1-9.

Lebenthal E and Lee PC, 1980. Development of functional response in human exocrine pancreas. Pediatrics, 66, 556-560.

Lee JS, Ward WO, Knapp G, Ren HZ, Vallanat B, Abbott B, Ho K, Karp SJ and Corton JC, 2012. Transcriptional ontogeny of the developing liver. Bmc Genomics, 13. 10.1186/1471-2164-13-33

Lee KR, Kawai N, Kim S, Sagher O and Hoff JT, 1997. Mechanisms of edema formation after intracerebral hemorrhage: Effects of thrombin on cerebral blood flow, blood-brain barrier permeability, and cell survival in a rat model. Journal of Neurosurgery, 86, 272-278. 10.3171/jns.1997.86.2.0272

Lemasters GK, Perreault SD, Hales BF, Hatch M, Hirshfield AN, Hughes CL, Kimmel GL, Lamb JC, Pryor JL, Rubin C and Seed JG, 2000. Workshop to identify critical windows of exposure for children's health: Reproductive health in children and adolescents work group summary. Environmental Health Perspectives, 108, 505-509. 10.2307/3454542

Liddelow SA, Dziegielewska KM, Ek CJ, Habgood MD, Bauer H, Bauer HC, Lindsay H, Wakefield MJ, Strazielle N, Kratzer I, Mollgard K, Ghersi-Egea JF and Saunders NR, 2013. Mechanisms that determine the internal environment of the developing brain: A transcriptomic, functional and ultrastructural approach. Plos One, 8. 10.1371/journal.pone.0065629

Liddelow SA, Dziegielewska KM, Mollgard K, Phoenix TN, Temple S, VandeBerg JL and Saunders NR, 2011a. SPARC/osteonectin, an endogenous mechanism for targeting albumin to the blood-cerebrospinal fluid interface during brain development. European Journal of Neuroscience, 34, 1062-1073. 10.1111/j.1460-9568.2011.07821.x

Liddelow SA, Dziegielewska KM, VandeBerg JL, Noor NM, Potter AM and Saunders NR, 2011b. Modification of protein transfer across blood/cerebrospinal fluid barrier in response to altered plasma protein composition during development. European Journal of Neuroscience, 33, 391-400. 10.1111/j.1460-9568.2010.07509.x

Lim ES, Wang D and Holtz LR, 2016. The bacterial microbiome and virome milestones of infant development. Trends in Microbiology, 24, 801-810. 10.1016/j.tim.2016.06.001

Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P and Hansen AK, 2007. The use of pigs in neuroscience: Modeling brain disorders. Neuroscience and Biobehavioral Reviews, 31, 728-751. 10.1016/j.neubiorev.2007.02.003

Lossinsky AS and Shivers RR, 2004. Structural pathways for macromolecular and cellular transport across the blood-brain barrier during inflammatory conditions. Review. Histology and Histopathology, 19, 535-564.

Louis GMB, Gray LE, Marcus M, Ojeda SR, Pescovitz OH, Witchel SF, Sippell W, Abbott DH, Soto A, Tyl RW, Bourguignon JP, Skakkebaek NE, Swan SH, Golub MS, Wabitsch M, Toppari J and Euling SY, 2008. Environmental factors and puberty timing: Expert panel research needs. Pediatrics, 121, S192-S207. 10.1542/peds.1813E

Luebke RW, Chen DH, Dietert R, Yang Y and Luster MI, 2006. Immune system maturity and sensitivity to chemical exposure. Journal of Toxicology and Environmental Health-Part a-Current Issues, 69, 811-825. 10.1080/15287390600591496
Mackic JB, Weiss MH, Miao W, Kirkman E, Ghiso J, Calero M, Bading J, Frangione B and Zlokovic BV, 1998. Cerebrovascular accumulation and increased blood-brain barrier permeability to circulating Alzheimer's amyloid beta peptide in aged squirrel monkey with cerebral amyloid angiopathy. Journal of Neurochemistry, 70, 210-215.
Madhusudan A, Vogel P and Knuesel I, 2013. Impact of prenatal immune system disturbances on brain development. Journal of Neuroimmune Pharmacology, 8, 79-86. 10.1007/s11481-012-9374-z
Mann A, Han H and Eyal S, 2016. Imaging transporters: Transforming diagnostic and therapeutic development. Clinical Pharmacology & Therapeutics, 100, 479-488. 10.1002/cpt.416
Mann DR and Fraser HM, 1996. The neonatal period: A critical interval in male primate development. Journal of Endocrinology, 149, 191-197. 10.1677/joe.0.1490191
Manson WG, Coward WA, Harding M and Weaver LT, 1999. Development of fat digestion in infancy. Archives of Disease in Childhood, 80, F183-F187.
Marques AH, O'Connor TG, Roth C, Susser E and Bjorke-Monsen AL, 2013. The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. Frontiers in Neuroscience, 7. 10.3389/fnins.2013.00120
McAnulty PA, 2012. The kidney. In: McAnulty PA, Dayan AD, Ganderup NC, and Hastings KL, editors. The minipig in biomedical research. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group. p 253-262.
McAnulty PA, Barrow P and Marsden E, 2012. Reproductive system including studies in juvenile mini pigs. In: McAnulty PA, Dayan AD, Ganderup NC, and Hastings KL, editors. The minipig in biomedical research. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group. p 263-282.
Menard D, Monfils S and Tremblay E, 1995. Ontogeny of human gastric lipase and pepsin activities. Gastroenterology, 108, 1650-1656. 10.1016/0016-5085(95)90125-6
Mendis-Handagama S and Ariyaratne HBS, 2001. Differentiation of the adult Leydig cell population in the postnatal testis. Biology of Reproduction, 65, 660-671.
Mielke H and Gundert-Remy U, 2009. Bisphenol A levels in blood depend on age and exposure. Toxicology Letters, 190, 32-40. 10.1016/j.toxlet.2009.06.861
Milla PJ and Bisset WM, 1988. The gastrointestinal tract. British Medical Bulletin, 44, 1010-1024.
Mooij MG, Nies AT, Knibbe CAJ, Schaeffeler E, Tibboel D, Schwab M and de Wildt SN, 2016. Development of human membrane transporters: Drug disposition and pharmacogenetics. Clinical Pharmacokinetics, 55, 507-524. 10.1007/s40262-015-0328-5
Morein B, Blomqvist G and Hu K, 2007. Immune responsiveness in the neonatal period. Journal of Comparative Pathology, 137, S27-S31. 10.1016/j.jcpa.2007.04.008
Moxey PC and Trier JS, 1979. Development of villus absorptive cells in the human-fetal small-intestine - morphological and morphometric study. Anatomical Record, 195, 462-483.
Muldoon LL, Alvarez JI, Begley DJ, Boado RJ, del Zoppo GJ, Doolittle ND, Engelhardt B, Hallenbeck JM, Lonser RR, Ohlfest JR, Prat A, Scarpa M, Smeyne RJ, Drewes LR and Neuwelt EA, 2013. Immunologic privilege in the central nervous system and the blood-brain barrier. Journal of Cerebral Blood Flow and Metabolism, 33, 13-21. 10.1038/jcbfm.2012.153
Muraro A, Hoffmann-Sommergruber K, Holzhauser T, Poulsen LK, Gowland MH, Akdis CA, Mills ENC, Papadopoulos N, Roberts G, Schnadt S, van Ree R, Sheikh A, Vieths S and Anaphylaxis EFA, 2014. EAACI Food Allergy and Anaphylaxis Guidelines. Protecting consumers with food allergies: understanding food consumption, meeting regulations and identifying unmet needs. Allergy, 69, 1464-1472. 10.1111/all.12453
Narushima I, Kita T, Kubo K, Yonetani Y, Momochi C, Yoshikawa I, Shimada K and Nakashima T, 1999. Contribution of endothelin-1 to disruption of blood-brain barrier permeability in dogs. Naunyn-Schmiedebergs Archives of Pharmacology, 360, 639-645. 10.1007/s002109900137
Neal-Kluever A, Aungst J, Gu Y, Hatwell K, Muldoon-Jacobs K, Liem A, Ogungbesan A and Shackelford M, 2014. Infant toxicology: State of the science and considerations in evaluation of safety. Food and Chemical Toxicology, 70, 68-83. 10.1016/j.fct.2014.05.003
Neubauer S and Hublin JJ, 2012. The evolution of human brain development. Evolutionary Biology, 39, 568-586. 10.1007/s11692-011-9156-1

Nizet V, Kim KS, Stins M, Jonas M, Chi EY, Nguyen D and Rubens CE, 1997. Invasion of brain microvascular endothelial cells by group B streptococci. Infection and Immunity, 65, 5074-5081.
Norman A, Ojamae O and Strandvik B, 1972. Bile-acids and pancreatic enzymes during absorption in newborn. Acta Paediatrica Scandinavica, 61, 571-576. 10.1111/j.1651-2227.1972.tb15947.x
Nwaru BI, Erkkola M, Ahonen S, Kaila M, Haapala AM, Kronberg-Kippila C, Salmelin R, Veijola R, Ilonen J, Simell O, Knip M and Virtanen SM, 2010. Age at the introduction of solid foods during the first year and allergic sensitization at age 5 years. Pediatrics, 125, 50-59. 10.1542/peds.2009-0813
O'Hara K, Wright IMR, Schneider JJ, Jones AL and Martin JH, 2015. Pharmacokinetics in neonatal prescribing: evidence base, paradigms and the future. British Journal of Clinical Pharmacology, 80, 1281-1288. 10.1111/bcp.12741
Obermeier B, Daneman R and Ransohoff RM, 2013. Development, maintenance and disruption of the blood-brain barrier. Nature Medicine, 19, 1584-1596. 10.1038/nm.3407
Odle J, Lin X, Jacobi SK, Kim SW and Stahl CH, 2014. The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. Annu Rev Anim Biosci, 2, 419-444. 10.1146/annurev-animal-022513-114158
Office for Risk Assessment, 2008. Children and chemical substances in the diet. The Hague, NL. 60 p.
Onyango AW, Receveur O and Esrey SA, 2002. The contribution of breast milk to toddler diets in western Kenya. Geneva: World Health Organization.
Ouyang F, Perry MJ, Venners SA, Chen C, Wang B, Yang F, Fang Z, Zang T, Wang L, Xu X and Wang X, 2005. Serum DDT, age at menarche, and abnormal menstrual cycle length. Occupational and Environmental Medicine, 62, 878-884. 10.1136/oem.2005.020248
Pang XY, Hua XG, Yang Q, Ding DH, Che CY, Cui L, Jia W, Bucheli P and Zhao LP, 2007. Inter-species transplantation of gut microbiota from human to pigs. Isme Journal, 1, 156-162. 10.1038/ismej.2007.23
Paolinelli R, Corada M, Ferrarini L, Devraj K, Artus C, Czupalla CJ, Rudini N, Maddaluno L, Papa E, Engelhardt B, Couraud PO, Liebner S and Dejana E, 2013. Wnt activation of immortalized brain endothelial cells as a tool for generating a standardized model of the blood brain barrier in vitro. Plos One, 8. 10.1371/journal.pone.0070233
Parigi SM, Eldh M, Larssen P, Gabrielsson S and Villablanca EJ, 2015. Breast milk and solid food shaping intestinal immunity. Front Immunol, 6, 415. 10.3389/fimmu.2015.00415
Pelkonen O, Kapitulnik J, Gundert-Remy U, Boobis AR and Stockis A, 2008. Local kinetics and dynamics of xenobiotics. Critical Reviews in Toxicology, 38, 697-720. 10.1080/10408440802194931
Penninks AH, Van Mierlo GJD, Kuper F, Snel CJ, Ganderup NC and Wolterbeek APM, 2012. Juvenile immunodevelopment in minipigs. In: Doberman AM, and Lewis EM, editors. Pediatric Non-Clinical Drug Testing: Principles, Requirements, and Practice. Hoboken, NJ, USA: John Wiley & Sons, Inc. p 231-254.
Perkin MR, Logan K, Marrs T, Radulovic S, Craven J, Flohr C, Lack G and Team EATS, 2016. Enquiring About Tolerance (EAT) study: Feasibility of an early allergenic food introduction regimen. Journal of Allergy and Clinical Immunology, 137, 1477-+. 10.1016/j.jaci.2015.12.1322
Pettengill MA, van Haree SD and Levy O, 2014. Soluble mediators regulating immunity in early life. Frontiers in Immunology, 5. 10.3389/fimmu.2014.00457
Poley JR, Dower JC, Owen CA and Stickler GB, 1964. Bile acids in infants + children. Journal of Laboratory and Clinical Medicine, 63, 838-846.
Poole JA, Barriga K, Leung DYM, Hoffman M, Eisenbarth GS, Rewers M and Norris JM, 2006. Timing of initial exposure to cereal grains and the risk of wheat allergy. Pediatrics, 117, 2175-2182. 10.1542/peds.2005-1803
Preusse C and Tingleff Skaanild M, 2012. Minipigs in Absorption, Distribution, Metabolism, and Excretion (ADME) studies. In: McAnulty PA, Dayan AD, Ganderup NC, and Hastings KL, editors. The minipig in biomedical research. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group. p 143-158.
Prince FP, 2001. The triphasic nature of Leydig cell development in humans, and comments on nomenclature. Journal of Endocrinology, 168, 213-216. 10.1677/joe.0.1680213
Renwick AG, Dorne JL and Walton K, 2000. An analysis of the need for an additional uncertainty factor for infants and children. Regulatory Toxicology and Pharmacology, 31, 286-296. 10.1006/rtph.2000.1394

Renz H, Brandtzaeg P and Hornef M, 2012. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nature Reviews Immunology, 12, 9-23. 10.1038/nri3112

Roman C, Carriere F, Villeneuve P, Pina M, Millet V, Simeoni U and Sarles J, 2007. Quantitative and qualitative study of gastric lipolysis in premature infants: Do MCT-enriched infant formulas improve fat digestion? Pediatric Research, 61, 83-88. 10.1203/01.pdr.0000250199.24107.fb

Romano-Keeler J and Weitkamp JH, 2015. Maternal influences on fetal microbial colonization and immune development. Pediatric Research, 77, 189-195. 10.1038/pr.2014.163

Rosenfield RL, Lipton RB and Drum ML, 2009. Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index. Pediatrics, 123, 84-88. 10.1542/peds.2008-0146

Saghir SA, Khan SA and McCoy AT, 2012. Ontogeny of mammalian metabolizing enzymes in humans and animals used in toxicological studies. Critical Reviews in Toxicology, 42, 323-357. 10.3109/10408444.2012.674100

Saito F, Kuwata H, Oiki E, Koike M, Uchiyama Y, Honda K and Takeda K, 2008. Inefficient phagosome maturation in infant macrophages. Biochemical and Biophysical Research Communications, 375, 113-118. 10.1016/j.bbrc.2008.07.141

Salem F, Johnson TN, Abduljalil K, Tucker GT and Rostami-Hodjegan A, 2014. A re-evaluation and validation of ontogeny functions for cytochrome P450 1A2 and 3A4 based on in vivo data. Clinical Pharmacokinetics, 53, 625-636. 10.1007/s40262-014-0140-7

Salzman NH, 2014. The role of the microbiome in immune cell development. Annals of Allergy Asthma & Immunology, 113, 593-598. 10.1016/j.anai.2014.08.020

Sangild PT, Thymann T, Schmidt M, Stoll B, Burrin DG and Buddington RK, 2013. Invited Review: The preterm pig as a model in pediatric gastroenterology. Journal of Animal Science, 91, 4713-4729. 10.2527/jas.2013-6359

Sato T and Clevers H, 2013. Growing self-organizing mini-guts from a single intestinal stem cell: Mechanism and applications. Science, 340, 1190-1194. 10.1126/science.1234852

Sato Y, Nagata M, Kawamura A, Miyashita A and Usui T, 2012. Protein quantification of UDP-glucuronosyltransferases 1A1 and 2B7 in human liver microsomes by LC-MS/MS and correlation with glucuronidation activities. Xenobiotica, 42, 823-829. 10.3109/00498254.2012.665950

Saunders NR, Dreifuss JJ, Dziegielewska KM, Johansson PA, Habgood MD, Mollgard K and Bauer HC, 2014. The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history. Frontiers in Neuroscience, 8. 10.3389/fnins.2014.00404

Schmitz JR, J, 1977. La stéatorrhée du nouveau-né. Paris: Flammarion Medecine-Sciences.

Scientific Committee on Food (SCF), 1998. Opinion of the Scientific Committee on Food on the applicability of the ADI (Acceptable Daily Intake) for food additives to infants (expressed on 17/09/1998).

Senterre J, 1976. Contribution à l'étude de l'alimentation optimale du prématuré. Liège: Vaillant-Carmanne.

Sharma AA, Jen R, Butler A and Lavoie PM, 2012. The developing human preterm neonatal immune system: A case for more research in this area. Clinical Immunology, 145, 61-68. 10.1016/j.clim.2012.08.006

Shmerling DH, 1976. Development of digestive and absorptive function in human fetus. Nutrition and Metabolism, 20, 76-79.

Siegrist CA and Aspinall R, 2009. B-cell responses to vaccination at the extremes of age. Nature Reviews Immunology, 9, 185-194. 10.1038/nri2508

Smith DA, Di L and Kerns EH, 2010. The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. Nature Reviews Drug Discovery, 9, 929-939. 10.1038/nrd3287

Solecki R, Kortenkamp A, Bergman A, Chahoud I, Degen GH, Dietrich D, Greim H, Hakansson H, Hass U, Husoy T, Jacobs M, Jobling S, Mantovani A, Marx-Stoelting P, Piersma A, Ritz V, Slama R, Stahlmann R, van den Berg M, Zoeller RT and Boobis AR, 2017. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. Arch Toxicol, 91, 1001-1006. 10.1007/s00204-016-1866-9

Speth PAJ, Vree TB, Neilen NFM, Demulder PHM, Newell DR, Gore ME and Depauw BE, 1987. Propylene-glycol pharmacokinetics and effects after intravenous-infusion in humans. Therapeutic Drug Monitoring, 9, 255-258. 10.1097/00007691-198709000-00001

Stamatovic SM, Keep RF and Andjelkovic AV, 2008. Brain endothelial cell-cell junctions: How to "Open" the blood brain barrier. Current Neuropharmacology, 6, 179-192. 10.2174/157015908785777210

Suchy FJ, Balistreri WF, Heubi JE, Searcy JE and Levin RS, 1981. Physiologic cholestasis: Elevation of the primary serum bile-acid concentrations in normal infants. Gastroenterology, 80, 1037-1041.

Taylor SN, Basile LA, Ebeling M and Wagner CL, 2009. Intestinal permeability in preterm infants by feeding type: Mother's milk versus formula. Breastfeeding Medicine, 4, 13-17. 10.1089/bfm.2008.0114

Tey D, Allen KJ, Peters RL, Koplin JJ, Tang MLK, Gurrin LC, Ponsonby AL, Lowe AJ, Wake M, Dharmage SC and HealthNuts Study I, 2014. Population response to change in infant feeding guidelines for allergy prevention. Journal of Allergy and Clinical Immunology, 133, 476-484. 10.1016/j.jaci.2013.11.019

Tudehope D, Fewtrell M, Kashyap S and Udaeta E, 2013. Nutritional needs of the micropreterm infant. Journal of Pediatrics, 162, S72-S80. 10.1016/j.jpeds.2012.11.056

US FDA, 2006. Guidance for Industry: S8 Immunotoxicity Studies for Human Pharmaceuticals. Rockville, MD, USA: U.S. Department of Health and Human Services, Food and Drug Administration 1-13 p.

Valcke M and Krishnan K, 2013. Assessing the impact of child/adult differences in hepatic first-pass effect on the human kinetic adjustment factor for ingested toxicants. Regulatory Toxicology and Pharmacology, 65, 126-134. 10.1016/j.yrtph.2012.11.006

Valiathan R, Ashman M and Asthana D, 2016. Effects of ageing on the immune system: Infants to elderly. Scandinavian Journal of Immunology, 83, 255-266. 10.1111/sji.12413

van den Anker JN, Schoemaker RC, Hop WCJ, van der Heijden BJ, Weber A, Sauer PJJ, Neijens HJ and deGroot R, 1995. Ceftazidime pharmacokinetics in preterm infants: Effects of renal function and gestational age. Clinical Pharmacology & Therapeutics, 58, 650-659. 10.1016/0009-9236(95)90021-7

van den Anker JN, Schwab M and Kearns GL, 2011. Developmental pharmacokinetics. Handbook of Experimental Pharmacology. p 51-75.

Van Ginneken C, 2012. Gastrointestinal tract. In: McAnulty PA, Dayan AD, Ganderup NC, and Hastings KL, editors. The minipig in biomedical research. Boca Raton, FL, USA: CRC Press, Taylor and Francis Group. p 211-236.

van Hinsbergh VWM and Amerongen GPV, 2002. Intracellular signalling involved in modulating human endothelial barrier function. Journal of Anatomy, 200, 549-560. 10.1046/j.1469-7580.2002.00060.x

Velilla PA, Rugeles MT and Chougnet CA, 2006. Defective antigen-presenting cell function in human neonates. Clinical Immunology, 121, 251-259. 10.1016/j.clim.2006.08.010

von Berg A, Filipiak-Pittroff B, Schulz H, Hoffmann U, Link E, Sussmann M, Schnappinger M, Bruske I, Standl M, Kramer U, Hoffmann B, Heinrich J, Bauer CP, Koletzko S, Berdel D and Grp GIS, 2016. Allergic manifestation 15years after early intervention with hydrolyzed formulas - the GINI Study. Allergy, 71, 210-219. 10.1111/all.12790

Walker JC, Smolders M, Gemen EFA, Antonius TAJ, Leuvenink J and de Vries E, 2011. Development of lymphocyte subpopulations in preterm infants. Scandinavian Journal of Immunology, 73, 53-58. 10.1111/j.1365-3083.2010.02473.x

Wang M and Donovan SM, 2015. Human microbiota-associated swine: Current progress and future opportunities. Ilar Journal, 56, 63-73. 10.1093/ilar/ilv006

Watchko JF, 2016. Bilirubin-induced neurotoxicity in the preterm neonate. Clinics in Perinatology, 43, 297-+. 10.1016/j.clp.2016.01.007

Weaver LT, Austin S and Cole TJ, 1991. Small intestinal length - a factor essential for gut adaptation. Gut, 32, 1321-1323. 10.1136/gut.32.11.1321

Weaver LT, Laker MF and Nelson R, 1984. Intestinal permeability in the newborn. Archives of Disease in Childhood, 59, 236-241.

Wells JM and Spence JR, 2014. How to make an intestine. Development, 141, 752-760. 10.1242/dev.097386

Westerbeek EAM, van den Berg A, Lafeber HN, Fetter WPF and van Elburg RM, 2011. The effect of enteral supplementation of a prebiotic mixture of non-human milk galacto-, fructo- and acidic oligosaccharides on intestinal permeability in preterm infants. British Journal of Nutrition, 105, 268-274. 10.1017/s0007114510003405

WHO, 2001. The optimal duration of exclusive breastfeeding: report of an expert consultation. Geneva: World Health Organization. 6 p. WHO/NHD/01.09,WHO/FCH/CAH 01.24
WHO, 2006. Principles for evaluating health risks in children associated with exposure to chemicals.
WHO, 2016. Infant and young child feeding: World Health Organization.
Williamson S, Finucane E, Ellis H and Gamsu HR, 1978. Effect of heat-treatment of human milk on absorption of nitrogen, fat, sodium, calcium, and phosphorus by preterm infants. Archives of Disease in Childhood, 53, 555-563.
Windorfer A, Karitzky D, Gasteiger U and Stehr K, 1978. Investigations on salicylate protein-binding in newborns and infants. European Journal of Pediatrics, 127, 163-172. 10.1007/bf00442057
Ygberg S and Nilsson A, 2012. The developing immune system - from foetus to toddler. Acta Paediatrica, 101, 120-127. 10.1111/j.1651-2227.2011.02494.x
Yong SB, Wu CC, Wang L and Yang KD, 2013. Influence and mechanisms of maternal and infant diets on the development of childhood asthma. Pediatrics and Neonatology, 54, 5-11. 10.1016/j.pedneo.2012.12.009
Zabielski R, Godlewski MM and Guilloteau P, 2008. Control of development of gastrointestinal system in neonates. Journal of Physiology and Pharmacology, 59, 35-54.
Zaghouani H, Hoeman CM and Adkins B, 2009. Neonatal immunity: faulty T-helpers and the shortcomings of dendritic cells. Trends in Immunology, 30, 585-591. 10.1016/j.it.2009.09.002
Zangen S, Di Lorenzo C, Zangen T, Mertz H, Schwankovsky L and Hyman PE, 2001. Rapid maturation of gastric relaxation in newborn infants. Pediatric Research, 50, 629-632. 10.1203/00006450-200111000-00016
Zhang Q, Widmer G and Tzipori S, 2013. A pig model of the human gastrointestinal tract. Gut Microbes, 4, 193-200. 10.4161/gmic.23867
Zimmermann MB, 2011. The role of iodine in human growth and development. Seminars in Cell & Developmental Biology, 22, 645-652. 10.1016/j.semcdb.2011.07.009
Zoppi G, Andreotti G, Pajno-Ferrara F, Bellini P and Gaburro D, 1973. Development of specific responses of exocrine pancreas to pancreozymin and secretin stimulation in newborn infants. Pediatric Research, 7, 198-203. 10.1203/00006450-197304000-00023
Zoppi G, Andreotti G, Pajno-Ferrara F, Njai DM and Gaburro D, 1972. Exocrine pancreas function in premature and full term neonates. Pediatric Research, 6, 880-886. 10.1203/00006450-197212000-00005