Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: August 31, 2023

Citation: Barmaz S, Castle L, et al., 2023. Principles of the Assessment of Food Additives Used in Food for Infants and Toddlers, Medical Research Archives, [online] 11(8). https://doi.org/10.18103/mra.v11i8.4127

DOI
https://doi.org/10.18103/mra.v11i8.4127

ISSN: 2375-1924

RESEARCH ARTICLE

Principles of the Assessment of Food Additives Used in Food for Infants and Toddlers

Stefania Barmaz, Laurence Castle, Birgit Dusemund, Peter Fürst, Christina Kyrkou, Agnieszka Mech, Alicja Mortensen, Ana Maria Rincon, Camilla Smeraldi, Alexandra Tard, Dominique Turck, Dina Waalkens-Berendsen, Detlef Wölfle, Ursula Gundert-Remy*

***Correspondence author:** ursula.gundert-remy@charite.de

ABSTRACT

Food additives are substances added to food to maintain or improve its safety, freshness, taste, texture, or appearance. Until recently, the assessment of food additives taken up with the normal food was performed only for the population above an age of 12 weeks. With the better knowledge of the physiological specificities in the age group below 12 weeks of age and considering that milk formulae can be the unique dietary source for infants up to 16 weeks, special assessments are performed for food additives already on the market for this age group. This publication explains the background and relevant guidelines for the assessment, including special guidelines for the age group below 16 weeks, and the different sources of information used. The principles followed to assess food additives are described. The requirements for the assessment of food additives used in infants' food includes testing in special animal models if human data are absent. The amount of food additives the infants are exposed to is estimated based on an exposure assessment. The aim of the assessment is to compare the estimated intake of the FA with milk formulae with safe concentrations taken from clinical studies in infants, special animal models and/or by comparing the exposure by the milk formulae with the content of breast milk whatever is appropriate. Two examples (locust bean gum, lecithin) illustrate the application of the principles. Of special interest is the concentration of impurities in the food additive with a toxic potential, such as lead, arsenic, cadmium, mercury, relevant for all food additives, or glycidyl esters, 3-monochloropropane diol, erucic acid, and trans-fatty acids, relevant for mono- and diglycerides of fatty acids. The assessment of food additives intended to be used in food for infants below 16 weeks of age revealed that in most cases the maximum permitted regulatory levels of the food additive would result in an exposure which does not raise health concerns, besides lecithins and locust bean gum. However, the content of impurities with a toxic potential at the regulatory permitted levels is of concern for all food additives which indicates the need for lowering those levels.

Keywords: Food additives, infant formula, impurities, safety assessment

Introduction

Food additives (FAs) are substances added to food to maintain or improve its safety, freshness, taste, texture, or appearance. FAs may have adverse health effects if consumed in too high amounts. As early as 1958 the Joint FAO/WHO Experts Committee of FAs (JECFA) coined the term" Acceptable Daily Intake (ADI)" to characterize an amount of FA which can be daily consumed without adverse impact on health[1].

It is international agreement that FAs need to be investigated for potential harmful effects on human health before their use[2]. In the EU, FAs must be evaluated before they can be authorised for use by the European Commission (EC) and placed on the market[3].

For a new FA to be authorised for use, or for changes to the conditions of use of already permitted FAs, a dossier must be compiled by an applicant, typically from the Food Industry, and submitted to the EC, which in turn will ask the European Food Safety Authority (EFSA), which is responsible for this evaluation, for a safety assessment.

In the case of FAs that were already permitted on the market in the EU before 20 January 2009[4], EFSA was requested by the EC to re-evaluate their safety, taking into account all available evidence, available not only from the original dossiers, but also from published literature, and other information sources, provided by interested parties (food business operators, manufacturers of the FAs, researchers) and also searched and collected by EFSA.

The assessment of the data is performed by a group of scientists, who are not employed by EFSA, but independent and who perform this task as members of expert working groups and panels. Administrative and scientific support is given by EFSA staff. In conjunction with EFSA staff the evaluation will result in an opinion which will be published and will provide the basis for regulatory decisions by EC.

The general approach for the evaluation of the safety of FAs usually leads to an estimation of the amount of a substance which can be reasonably assumed to be without appreciable harmful effects when ingested chronically via the diet. The typical health-based guidance value (HBGV) is an ADI for the consumption of food containing a specific food additive. Another approach is an assessment using the margin of exposure (MOE) approach (see below).

However, the susceptibility of infant and children to FAs has been a topic which has been discussed for a long time. It has been argued that infants of early age are more susceptible to adverse effects of substances than older children and adults because of their age-specific physiology. In line with this concept, at its 1971 meeting, JECFA expressed the opinion that children should not be exposed to FAs before the age of 12 weeks and that the ADI does not apply to children below this age[5]. In 1983, in the European Union (EU), the Scientific Committee on Food (SCF), also endorsed the principle that technological additives should not be used in food for infants and young children[6]. In 1992, the SCF confirmed this view[7]. However, the SCF considered certain FAs acceptable in food for infants from a toxicological point of view[8]. In the last years, in order to consider specific physiological aspects of this young age group and to give guidance on how to perform risk assessment for this group, a scientific guidance was developed[9]. The availability of this guidance document allowed to start the re-evaluation of the safety of those FAs permitted for use in foods intended for infants below 16 weeks of age, that had been already re-evaluated for their use in food forthe general population but were still missing the safety assessment for the youngest population.

Against this background, this publication is intended to explain (i) which data are requested and assessed, and (ii) which aspects are taken in consideration in the actual safety assessments for FAs used in food for infants below 16 weeks of age.

FAs authorised for use in formula for infants for which the assessment has been finalised are presented in Table 1.

Table 1: Food additives used in formula for infants below 16 weeks and for which the assessment has been finalised and their technological function

Substance name	**E number**	**Food additive technological purpose**
Acacia gum	E 414	emulsifier, stabilizer, thickener
Ascorbyl palmitate	E 304(i)	antioxidant
Lecithins	E 322	emulsifier
Locust bean gum	E 410	thickener, stabilizer, emulsifier, gelling agent
Mono- and di-glycerides of fatty acids	E 471	emulsifier
Pectin, and amidated pectin	E 440(i) E 440(ii)	gelling agent, thickener, stabilizer, emulsifier
Starch sodium octenyl succinate	E 1450	thickener, stabilizer, binder, emulsifier

General Principles of risk assessment of substances

In general, the risk assessment of FAs comprises four steps, i.e. hazard identification, hazard characterisation, exposure assessment and risk characterisation[10,11].

In the hazard identification step potential adverse effects are evaluated based on studies in animals or humans requested by existing EFSA guidance for the assessment of FAs[11,12]. The studies should provide data on toxicokinetics and toxicity data (on genotoxicity, subchronic, chronic toxicity, carcinogenicity, developmental and reproductive toxicity studies). Specific studies/information needed for the assessment of FAs for infants and young children are described below.

In the hazard characterisation step the adverse effects are analysed to establish a dose at which the effect is not yet observed (no-observed-adverse-effect level, NOAEL) as a reference point (RP) expressed in mg per kg body weight per day. In the more modern bench mark dose (BMD) approach dose-response curves are fitted to the dose-response data using mathematical models, allowing to make extended use of the dose-response data and to quantify their uncertainties[13,14,15]. In this approach a response rate for the adversity of the effect, being either a continuous (i.e. liver enzymes) or a discrete variable (i.e. vomiting), the Benchmark response rate (BMR), has to be set, indicating an effect level which is just outside of the normal variation. The dose at which the BMR is obtained is called Benchmark dose (BMD). The lower confidence limit of the BMD, i.e. the BMDL can be used as a RP.

For the RP it is expected that this dose would not induce adverse effects in the respective study/studies. The RP is used to establish a health-based guidance value (HBGV), e.g. ADI. The ADI is an estimate of the amount of an FA that can be safely consumed daily over a lifetime without adverse health effects. If a RP was derived from animal studies it is necessary to apply factors[16,17] to adjust for interspecies differences and the variability among humans for deriving the ADI. The factors are composed of interspecies differences in toxicokinetic and toxicodynamic properties between the experimental animals and humans (if no specific information is available for the differences a default factor of 10 is applied) as well as the variability in the human population (if no specific information is available a default factor of 10 is applied). Thus, the overall default assessment (or uncertainty) factor is 100. If RPs are derived from human studies only factors for the variability among humans are needed. Chemical-specific adjustment factors (CSAFs) can be derived if knowledge on the toxicokinetics or toxicodynamics of the FA is available, allowing to increase or decrease the default uncertainty factors[16].

The exposure assessment is explained below for the relevant subgroup of infants below 16 weeks of age. It estimates the amount of the FA to which the infants are exposed. Because of the possibility that toxicologically relevant compounds may be present in FAs, as components, impurities or contaminants, the exposure assessment to those will also be described below.

The risk characterisation integrates the outcomes of hazard characterisation, i.e. ADI and exposure

assessment. Alternatively, when the data do not allow deriving an ADI, a margin of exposure (MOE) approach can be used[11]. The MOE denotes the factor between the estimated human exposure to the FA and the RP. Applying the same considerations as for the derivation of an ADI it is decided whether the MOE is acceptable. An exposure below the ADI and a large MOE indicate that the use of the FA at the exposure level can be considered to be safe.

A critical element in hazard identification is the evaluation of genotoxicity[10,18,19]. In case a direct genotoxic mechanism cannot be excluded no safe level can be derived, based on the assumption that for this effect no threshold dose exists which is different to toxicity other than genotoxicity for which a dose without adverse effects can be assumed.

As a general rule, toxicity studies should be conducted according to current OECD guidelines for testing of chemicals[20]. For FAs with variable manufacturing methods/profiles, e.g. chemical mixtures, tested in toxicological and human studies it is requested that they are identical to or representative for the FA in question. In addition, the studies should be appraised for their relevance for humans and the reliability of the findings. This can be done according to a structured set of criteria (NTP-OHAT[21]or the SciRAP tool[22]). While results of human studies have a higher relevance than the results of animal studies, unfortunately in many cases their reliability has been found to be lower compared to well controlled animal studies. This would lead to a higher impact of relevant findings from animal studies on the derivation of a RP for hazard characterisation.

Structure and Content of Opinions

The European Food Safety Authority (EFSA) published opinions on the safety assessments of FAs are organised in a fixed structure. They start with formulating the problem and give an overview on the outcome of previous evaluations/re-evaluations by EFSA. New data submitted by the interested business operators (industry) or obtained by literature searches are described. The main text contains evaluations and resulting conclusions on the safety focusing on uses of the additive in foods for infants. The structure follows the logic of the risk assessment of FAs in general as laid down in the 'Guidance for submission for food additive evaluations EFSA Panel on Food Additives and Nutrient Sources added to Food'[11].

The opinion starts with describing chemical and physico-chemical aspects and the identity of the FA (e.g. chemical name and formula, chemical composition of mixtures, botanical origin for plant-derived additives, physico-chemical parameters). A full physico-chemical characterisation of the FA is an important basic requirement for the assessment of its safety. Special interest is devoted to the purity of the substance, in particular to impurities which may be present e.g. toxic elements such as lead, arsenic, mercury, and cadmium, organic compounds (see below). Minimum requirement for purity as well as maximum limits for impurities are defined in regulatory specifications (e.g. lead not more than 2 mg/kg)[23]. Technical data submitted for the food additive from interested business operators are evaluated. The information is assessed for potential specific risks, related to impurities, and by the possible presence of nano-sized materials. Information on the manufacturing process is used to identify reaction intermediates, precursors and reagents that could remain in the additive and may present a hazard. Data on stability and reaction and fate in food are evaluated, as hazardous degradation products of the additive may be formed during storage of the additive or of food containing the additive.

The exposure assessment estimating the amount of the FA per kg body weight, consumed per day, is an indispensable element of risk assessment. The basis for the exposure assessment is built by legal frameworks and by information from industry. A regulation exists on the categories of food in which the particular FA can be used and on maximum levels up to which it is permitted to be added to the foods[3]. Information and data on uses and use levels of the FA for specific food categories are submitted by industry. As explained below different scenarios are used to estimate the dietary exposure.

Biological and toxicological data enable the identification and characterisation of the hazard, which is the second component needed for risk assessment. The safety assessment for infants and young children is based on standard toxicological testing as described above, supplemented by information from specific animal models mirroring the physiology of the early life stage, clinical studies, case reports and data from post-marketing surveillance, following existing guidance documents for the assessment of FA, specifically in this age groups. (Guidance for submission for food additive evaluations[11]; and Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age[12]). From the

data the information is extracted whether there are adverse effects observed in animals or humans and at which doses the adverse effects are observed. For the risk assessment the most relevant information is the dose at which no adverse effect was observed and from which a safe dose for infants and young children can be derived.

The discussion section deals with all aspects of the FA, its purity and impurities, the exposure and the toxicological data and clinical data in a holistic view. The risk due to impurities is evaluated and a risk assessment of the FA is performed by comparing the exposure and the safe dose.

In the conclusion section the consequences of the risk assessment are presented. If appropriate, recommendations for risk reducing measures are given, such as amendments to the regulation, e.g. reduction of the permitted maximum levels of toxic elements or reduction of the maximum permitted use level of the FA.

Specific aspects of risk assessment for infants

In 1997, an International Life Sciences Institute (ILSI) Europe workshop was held dealing with the applicability of the ADI to infants and children. The participants considered the differences in susceptibility to chemicals between infants and children compared to adults, whether the testing methods used at that time were adequate to cover the differences, whether the differences in food intake of infants and children and adults should be a point of concern and whether there was a need for a special safety factor for infants and children. In particular, it was discussed whether the test methods of chemicals intended for food use were adequate to reveal delayed functional toxicity later in life[24]. The participants considered that "an optimal test protocol would be a two-generation study, covering in utero exposure, the suckling period, 'creep feeding', weaning and rapid juvenile growth, and where the F1 generation was used for evaluation of chronic toxicity/ carcinogenicity" [24]. However, already in 1998, the SCF noted that such a study only covered the intake through a regular food or through the mother's milk until weaning and did not mimic an exposure where an infant receives only an infant formula, and that the newborn rat was not developmentally parallel to the newborn human. The SCF considered performing chronic studies starting in newborn piglets raised solely on mother's milk replacement, where the FA in question was added at different dose levels as a better alternative to the two-generation study in rats with *in utero* exposure[24], later on, the JECFA came with the opinion that studies with a direct oral administration to neonatal animals are needed for the evaluation of additives to be used in infant formulae[25]. An approach to the risk assessment of FA to food for infants aged less than 12-16weeks was discussed in the scientific literature in 2017[26]. In the same year EFSA published a guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age[12].

The EFSA guidance presents considerations regarding the physiology of young infant, the immaturity of xenobiotic metabolism and renal excretion. It contains the requirements for data, including requirements for animal data and for human data, the approach for exposure estimation and risk assessment of substances in food intended for this population. The guidance[12] states that standard toxicological studies are to be assessed, such as ADME (absorption, distribution, metabolism and excretion), sub-chronic and chronic toxicity, genotoxicity, carcinogenicity and reproductive toxicity studies. It is the general assumption that effects observed in adult animals will also be observed in pups and young animals.

For FAs in food for infants below 16 weeks of age, an Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443)[27] 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. In this study exposure of the neonatal animals is through mother's milk and is rather low; hence, the resulting doses in the neonatal animals are generally too low for an appropriate hazard characterization. Therefore, direct dosing of the neonatal animals with appropriate doses should be considered as soon as possible after birth. If standard toxicological studies do not show adverse effects in adult animals and it is shown that a substance is not absorbed, a special study in neonatal animals will be the study which is requested. This study is a repeated dose study with direct oral administration to neonatal animals (e.g. in piglets). Local effects on the gastrointestinal tract and the bioavailability of nutrients (minerals and vitamins) should be investigated in this study.

Human data (clinical and epidemiological studies, including post marketing nutrivigilance data, and case reports) should be part of the evaluation. If high quality human data are available, the assessment might be based solely on those data.

Specific animal studies

As pointed out, if in the standard toxicological studies toxicological effects have not been observed, special animal studies are needed in order to cover the specific sensitivity of the early postnatal period. According to the EFSA guidance[12] the study should take into consideration the differences in developmental stages of the relevant organ systems between humans and laboratory animals used in safety testing and the related critical windows of maximal sensitivity.

If additional studies in neonatal animals are needed, for this study type the piglet is the preferred species.

Piglet models, including neonatal mini piglets or neonatal farm piglets, are preferred because this animal model closely resembles humans in anatomy, physiology and biochemistry and because of the practical features of toxicity testing in piglet models (e.g.[28]). The developmental stage of several organ systems of the piglet from birth to 3- 4 weeks is quite comparable to the development in the human infant from birth to 16 weeks of age. The gastrointestinal tract, neurological system, the cardiovascular system, the skin, the urogenital system, metabolic aspects and the immune system of pigs are considered generally more human-like than those of other non-rodent species [29,30,31,32,33,34]. As in pigs no transplacental transfer of antibodies exists, (pre)term piglets need to stay for the first 24–48 h with their mother for obtaining passive immunological protection by antibodies via mother's colostral milk[35,36], or by intra-arterial injection within the first 24 h of mother's plasma to obtain immunological protection[37]. Another advantage of the piglet model is that direct oral administration of substances, including bottle-feeding, can be performed.

Piglets, postnatal age of 3-4 days, will be fed with pig-adapted infant formula for at least 21 days. A study will contain a control and three or more dose groups containing a low, mid and high concentration of the FA. The number of animals per group should be at least 6 males and 6 females. General toxicity parameters (i.e. growth, food/water consumption, haematology and clinical pathology, toxicokinetics, organ weights and histopathology examinations, etc.) should be evaluated. Furthermore, post-natal maturation and development of various organ systems, such as the gastrointestinal tract[38,39], the metabolic and renal capacities[40,41], the immune- and the reproductive systems[42,43], and the nervous system[44]) can be studied.

Which type of study is needed for the hazard assessment of FA in infant formula depends on the absorption of the FA. If the FA is absorbed from the gastrointestinal tract a reproductive toxicology study is required for the assessment. In several cases reliable data of an already performed two-generation reproductive toxicity according to OECD test guideline 416[45] study may be available. In case these data may not be at hand, an EOGRTS according to OECD test guideline 443[27] in rats should be performed. Three cohorts of F1-animals (as described in the OECD test guideline 443) should be included: (i). to assess the reproductive and developmental endpoints, (ii). to assess the potential impact of a test substance on the developing nervous system, and (iii). to assess the potential impact on the developing immune system.

If absorption from the gastrointestinal tract of the FA is low a reproductive toxicology study is not required. Then[46-48], a study in neonatal animals (e.g. piglets) should be performed to test the toxicity of substances. Analysis of possible local effects on the gastrointestinal tract and on a possible reduction in the bioavailability of nutrients (minerals and vitamins), normally contained in food for infants, should be included.

The additional testing in a special animal model increases the sensitivity of the testing approach. In several assessments the results of the piglet study were decisive for setting a safe level of use in infants.

Human Data

CLINICAL STUDIES

The results of interventional randomized controlled clinical studies performed in the target population, i.e. infants and young children, would provide the most important information on the dose-dependent effect of FAs on human health. Unfortunately, no guideline has been developed for designing, conducting, recording and reporting clinical studies specifically for FAs by regulatory authorities until now. However, the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement, published simultaneously in nine internationally acknowledged journals, describes the reporting of parallel group randomised trials. This guideline was updated in 2022[49]. The CONSORT Harms 2004 statement, updated in 2022, is a guideline specifically devoted to the reporting of adverse effects in randomized trials[50]. Another expert

group published in 2022 a specific guidance for the conduct and reporting of clinical trials of breast milk substitutes[51]. This guidance underlines that the trial protocol should include a valid and well recognized definition of common and anticipated adverse effects and that the method for evaluating, categorizing and reporting adverse effects should be independent of individuals or institutions with a potential commercial interest in the outcome of the trial. Despite these guidance documents the vast majority of clinical studies studying FAs, including those in infants and young children, do not fulfill the criteria for high quality studies as was found out when assessing the FAs authorized for food for infants. Detailed results of evaluations can be found in the published opinions of FAs in the age group below 16 weeks (e.g. [46, 48, 52]) and no conclusion can be drawn on the safety of the use of the FAs from those studies. Of note, none of them was specifically designed for the assessment of adverse effects that could be related to the use of FAs. The main flaws of the studies consist in lacking an appropriate, concomitantly treated control group, as well as a small sample size, unspecific primary endpoint for the safety assessment, and unclear exposure. Most studies were considered having a high risk of bias.

The Good Clinical Practice (GCP) for the evaluation of medicinal products (International Conference of Harmonization (ICH) topic 6) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects[53]. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, and that the clinical trial data are credible. The Guideline provides a unified standard for the European Union, Japan and the United States to facilitate the mutual acceptance of clinical data by the regulatory authorities. The quality of data from clinical studies for FAs would improve if the principles of this guideline would be applied which is of importance because these data have an impact on the safety and well-being of human subjects.

CASE REPORTS

While randomized controlled trials are considered the gold standard in clinical research, they are conducted in controlled settings and cannot provide information on rare adverse events, such as allergic reactions. Case reports have their place here in a real-life setting with longer follow-up time. They may have an impact on the safety assessment if the association between the intake of the FA and the adverse effect can be assessed as causally related or probably causally related. A group of clinicians, researchers, and journal editors has developed recommendations for the accurate reporting of information in case reports that resulted in the CARE (CAseREport) statement and checklist[54].

POST MARKETING SURVEILLANCE

Post marketing surveillance data are data collected by food business operators and/or official bodies which are based on reports of observed adverse effects after a drug, a medical device or a food/ingredient has been placed on the market. Such reports can be made by health care providers (mainly physicians and pharmacists), manufacturers, distributors and individuals. Because they are not collected systematically a quantitative relationship between reported adverse effects and the number of exposed subjects can only be roughly estimated from other sources (health insurance data, numbers of items sold). Similarly to case reports they can provide information on rare adverse effects.

Exposure assessment

The term dietary exposure used in the context of risk assessment means the estimation of the amount of a substance consumed by a person in the diet. The substance may be intentionally added, e.g. food additive or unintentionally present, e.g. food contaminant. The health impact the substance may have, depends on the amount consumed expressed as a dose (in mg/kg body weight per day) and the dose which has shown adverse effects in animal or human. Exposure estimates combine data on concentrations of a substance present in food with data on the quantity of those foods consumed (food consumption data).

Different methods exist to combine consumption data with concentration data of substances and the selection of the method depends on the population group of interest and the degree of accuracy required, and most important the availability of information.

Food consumption data encompass solid foods, beverages, including drinking water, and supplements. Food consumption data is collected through food consumption surveys, mainly at an individual level but can also be collected at a household level. Individual dietary surveys are the most accurate method for getting the food consumption per age groups.

When performing the estimation of dietary exposure, consumption among individuals varies, as does the concentration level of the substance in foods. This leads to a distribution of exposure. Individuals consuming large quantities of foods containing high concentrations of substances have a higher exposure to the substances and might be at health risk[10]. Those subjects are called high consumers and among the distribution of the consumption the 95th percentiles is often used to characterize their consumption.

In the context of this publication the exposure assessment of the specific group of infants below 16 weeks of age is in the focus. Their consumption behaviour is characterised by the fact that their diet is not diverse as they would consume maternal milk if breastfeed, or infant formulae if not. Thus, infant formulae is the only food to be considered for assessing exposure to food additives and toxic elements (e.g. arsenic, cadmium, lead, mercury) or organic impurities (e.g. 3-monochlorpropandiol, 3-MCPD) that these food additives may contain.

The amounts of food consumed considered for the exposure assessment of infants below 16 weeks of age are based on the dietary surveys, performed in the respective age group[55-57] and available in the EFSA Comprehensive Database. For the risk assessment of substances present in food for healthy non-breastfed term infants during the first 16 weeks, consumption is defined to be of 200 mL/kg bw per day at the mean and of 260 mL/kg bw per day at the high-level consumption (based on 95th percentile of consumption in boys 14 to 21 days old).

The concentrations in the infant formula are taken from several sources. Maximum levels of food additives authorised in foods for infants below 16 weeks of age are defined in the Regulation[3] on food additives. For the purpose of this publication these levels are termed maximum permitted levels (MPLs). The second source of concentration data is coming from industry producing infant formula, which reported the levels of use of the FA in their products. The exposure is then calculated by multiplying the MPLs or the concentrations reported by industry by the mean volume of consumption (200 mL/kg bw per day) and the high-level consumption (260 mL/kg bw per day).
In this publication the resulting exposure is given mainly using the MPLs.

The procedure of calculating the exposure is not only performed for the food additive but also for toxic elements and organic impurities.

Approach Used for the Risk Assessment of Impurities

Like all substances, FAs are never 100% pure. Specifications relating to the identity and purity criteria for FAs are given in Regulation (EC) No 231/2012. In order to be able to assess whether the presence of impurities poses a health risk, information on their concentration in the FA is needed. Data on the concentration of impurities of concern in the FA has to be provided by interested business operators (IBOs) using reliable analytical methods and preferably accompanied by certificates of analysis. The submitted data are evaluated with a focus on adequate quality and sensitivity of the applied analytical method as well as of the representativeness of the results.

To estimate the potential exposure to the impurities of concern, the assumption is made that they are not chemically degraded or physically lost from the food by processing the food, e.g.by processes such as thermal breakdown or volatilisation. Dietary exposure to an impurity in the FA can then be calculated simply *pro-rata* to the estimate of exposure for the FA itself. As an illustrative example, if the estimated exposure to the FA is (say) 1000 mg/kg bw/day and the impurity is 0.01% w/w in the FA, then the consequential exposure to the impurity from using the FA would be 0.1 mg/kg bw/day. The concentration levels used in these calculations are the specification limits for the impurity in the FA (if established in Regulation (EC) No 231/2012) and/or the concentration data submitted by the IBOs, often applying a factor of 5 or 10 to provide some 'headroom' to account for representativeness, homogeneity and analytical measurement uncertainty of the provided data.

The resulting estimate of potential exposure to the impurity is then compared with the target value that would not give rise to concern for toxicity. The health-based guidance values (HBGVs) and reference points (RPs) for substances of concern have been established in previous opinions by EFSA.

ORGANIC IMPURITIES

Organic impurities may be present in a FA because of their presence already in the raw materials or because they may be formed as unwanted by-products in the manufacturing process. Substances of special toxicological interest are, among others, 3-MCPD and 3-MCPD-esters, glycidyl esters, trans-fatty acids and erucic acid. In the following, the basis for the reference points of the substances of interest is explained.

For 3-MCPD and 3-MCPD-esters (expressed as total 3-MCPD), a tolerable daily intake (TDI) of 2µg/kg bw per day is established[58]. This HBGV is based on increased incidence of kidney tubular hyperplasia. BMD analysis using model averaging resulted in a BMDL10 of 0.20 mg/kg bw per day in male rats, which was selected as the reference point for renal effects. This reference point was considered to derive a group TDI of 2 µg/kg bw per day for 3-MCPD and 3-MCPD fatty acid esters and was considered protective also for effects on male fertility[59].

Glycidyl-esters have shown to elicit tumours in long term studies in rodents. To characterise their tumourigenic toxicity a T25 value was derived from the toxicological data. This value is the chronic dose in mg/kg bw per day, which will give 25% of the animal tumours at a specific tissue site, after specific correction for the spontaneous incidence within the standard life time of that species. A T25 of 10.2 mg/kg bw per day was established for peritoneal mesothelioma in male rats and is used as the reference point. An MOE of 25,000 and above is considered of low health concern[58].

For erucic acid, the EFSA Contaminants Panel has established a tolerable daily intake (TDI) of 7.0 mg/kg bw per day for erucic acid based on a no observed adverse effect level of 700 mg/kg bw per day for lipidosis in young rats and newborn piglets[60]. The heart is considered as the principal target organ for toxic effects after exposure to erucic acid. Myocardial lipidosis was identified by EFSA as the critical effect for chronic exposure to erucic acid. This effect is reversible and transient during prolonged exposure.

Some organic impurities, including 3-MCPD and glycidyl esters, are also regulated in the final infant formula[61]. In these cases, the Panel calculates the level of the organic impurity in the final product due to use of the FA and compares the result with the corresponding legal limits in the final infant formula. The outcome of the risk assessment helps inform whether there could be a possible health concern if these impurities would be present at the considered legal limit values in the FA.

TOXIC ELEMENTS

FAs may be susceptible to contamination by toxic elements. Of general interest are lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As). Other elements such as aluminium (Al) and nickel (Ni)) may be of interest for some FAs. Factors which lead to contamination might be that the FA is produced from environmentally contaminated plant materials (cultivated or obtained from the wild). Other potential sources of contamination of food additives are reagents and processing aids used for manufacturing and the contact materials used, from which there may be metal pick-up.

As described above, the HBGVs and RPs for substances of concern, including the toxic elements, have been established in previous opinions by EFSA. For arsenic the reference point is based on a range of benchmark dose lower confidence limit (BMDL01) values between 0.3 and 8 µg/kg bw/day identified for cancers of the lung, skin and bladder, as well as skin lesions from human epidemiological studies[62]. In general, the target MOE should be at least 10,000 if a reference point is based on carcinogenicity in animal studies[63]. However, as the BMDL for arsenic is derived from human studies, an interspecies extrapolation factor (i.e. 10) is not needed and so an MOE of 1,000 would be sufficient. Notwithstanding this, potential exposure to arsenic as an impurity in FAs is frequently calculated to be too high (see later) with MOE values well below the target of 1000. The reference point for lead is based on a study demonstrating perturbation of intellectual development in children with the critical response size of 1 point reduction in intelligence quotient (IQ) which is related to a 4.5% increase in the risk of failure to graduate from high school and can be associated with a decrease of later productivity of about 2%[64]. The tolerated weekly intake for mercury was set using kidney weight changes in male rats as the pivotal effect and the application of the default factor of 100 to the RP[65]. The derivation of the RP for cadmium is based on the dose-response relationship between urinary cadmium and urinary beta-2-microglobulin as the biomarker of tubular damage in humans[66].

Taking into account the innovation in production processes used to make FA, the developments in analytical methods with lower limits of detection and quantification, along with developments in our knowledge of the toxicity of these elements and other potential sources of exposure, there can be a need to consider revision of FA specifications (see discussion).

Outcome of the Assessments

FOOD ADDITIVE ASSESSMENTS

In the HBGV approach (e.g., ADI) an adjustment of the dose of the RP is to be made if the RP was

obtained from an animal study considering interspecies differences and the variability in the human population. If the RP is derived from a study in humans, no adjustment for species differences is needed. For the risk assessment the exposure is compared with the ADI and the exposure should be lower than the ADI. When applying the MOE approach, we calculate the factor which is between exposure and RP and assess whether the factor is large enough for considering that the exposure is safe.

When assessing FA used in food for infants, we applied an additional method for the risk assessment, for those FA which are naturally occurring substances and are also in the human milk. We compared the concentration of the substance in human milk with the concentration in infant formula. If both concentrations were similar the exposure from this FA was considered safe.
In order to explain the reasoning in the assessment two examples are given.

Case 1: Reference point derived from a special animal study and use of MOE approach
Locust bean gum (LBG, E 410))
Relevant studies: The clinical studies had severe methodological limitations; a reference point could not be derived from them. In a piglet study with doses of 1,050, 1,500, or 2,400 mg LBG/kg bw per day a reduced blood zinc level was observed in all dose groups. Dose-response modelling using the BMD approach[14], gave a BMDL of 1,400 mg/kg bw per day when a reduction of more than 20% of zinc concentration in blood compared to the controls was set as the BMR. The selection of the BMR of 20% for zinc was deduced from an epidemiological study performed in children[67].

Approach: A margin of exposure (MoE) approach was applied to assess the safety of LBG. In the absence of further mechanistic information for the adverse effect (reduced zinc blood level), the default value for the MoE is 100 by convention. However, the mechanism for the reduced zinc blood concentration is due to a reduced zinc absorption in the gastrointestinal tract due to the binding of zinc to LBG as shown in experiments and is the same for animals and humans. Toxicodynamic differences and intra-human variability are not to be considered. Because LBG is not absorbed, kinetic differences and variability have not to be considered. Hence, the default factor of 100 can be replaced by a substance specific and mechanism-based factor of 1.

Outcome: Comparing the BMDL of 1,400 mg/kg bw per day with the exposure in infants (mean: 869 mg/kg bw per day, high: 1,130 mg/kg bw per day)[48] the MoE was above 1, indicating no concern. However, for maximum regulatory level and the maximum use levels reported by industry the exposure estimates are higher (2000 mg/kg bw per day and 2600 mg/kg bw per day)[48] and the MOS is below 1 indicating that maximum use levels should be lowered.

Case 2: Comparison of the concentration of the main component in the infant formula with its content in breast milk
Lecithins (E 322)
Relevant studies: Indications for impaired brain development were found by feeding soya lecithins during the gestation, lactation and the post-weaning period of mice and rats. The main safety concern with respect to lecithin exposure came from studies of[68,69,70,71] with choline as the substance of concern. Because of limitations the results of the studies were implausible. Specific studies in infants were not found in the literature.
Approach: We decided to compare the exposure to choline released from lecithins (E 322) in infant formula with its adequate intake (AI), derived from its content in human milk[72].

Outcome: From the information provided by industry, the mean level of choline released from lecithins used as the food additive (E 322) in infant formula is 12 mg/L[73] which is lower than the mean concentration of total choline in human milk (138 mg/L). Hence, this level does not raise concern at the current use levels including the maximum permitted regulatory levels for lecithins (E 322).

In Table 2 the outcome of the assessment for the FA is tabulated. The assessment did not reveal that the exposure is higher than a safe level with the exception of two FAs. It is noteworthy that for the risk characterization mainly non-standard methods have been applied (i.e. the exposure compared to breast milk content, physiological considerations, modified MOE).

Table 2: Outcome of all assessments for FA in formula for infants below 16 weeks of age

Food additive	Outcome	Reason for the outcome
Acacia gum (E 414)[74]	No reasons for health concern	MOE large enough (1,000 – 8,000*)
Ascorbyl palmitate (E 304i)[52]	No reasons for health concern	Ascorbyl palmitate fully hydrolyses pre-systemically to ascorbic acid and palmitate (normal constituents of food and the body)
Lecithins (E 332)[73]	No reasons for health concern	Content in infant formula in the same order of magnitude as in human breast milk
Locust bean gum (E 410)[48]	Reasons for health concern for high level consumers	Exposure too high compared to the modified MoE of 1**
Mono-and diglycerides of fatty acids (E 471)[75]	No reasons for health concern	Content in infant formula in the same order of magnitude as in human breast milk
Pectin (E 440i) and amidated Pectin (E 440ii)[47]	Reasons for health concern	Exposure too high compared to the modified MOE of 1**
Starch sodium octenyl succinate (E 1450)[46]	No reasons for health concern	The range of the exposure reported in the clinical studies was without indication of adverse effects

*Standard/Default MoE = 100 ** Modified MoE, MoE for the substance 1 because interspecies differences and variability among the population could be reasonably modified.

Organic impurities

Organic impurities were found when assessing mono- and diglycerides of fatty acids (E 471). A high content of 3-MCPD and glycidyl esters (GEs) as well as trans-fatty acids and erucic acid was found[75].

Toxic elements

The outcome of the assessment for toxic elements is given in Table 3, based on the regulatory maximum permitted level (MPL) exposure scenario and using concentration data coming from the current maximum limits in the EU specifications, as examples. It is notable that the MOE for arsenic is too low for all 6 FAs and for lead it is too low for 5 FAs. Similarly, the percentage exhaustion of the HBGV for Cd and Hg it is too high for 3 and 4 FAs respectively. This indicates that potential exposure to these toxic elements could be too high for those FAs. In all cases it was noted that the analytical data on toxic elements in production batches of the additive and/or proposals for revised specifications coming from IBOs, were all below and most often were well below the current specifications. Therefore, the lowering of current specifications for these toxic elements was not only desirable but seemed to be achievable by the business operators.

Two examples are described here to see these conclusions in context.

Starch sodium octenyl succinate (E 1450) is an emulsifier and it is obtained by chemical modification of starch. Use levels are high, with MPLs up to 20,000 mg/kg for infant formulae. Based on the calculations made, the current maximum limits set for Pb, Hg and As, are substantially too high and a maximum limit for Cd was considered necessary[48].

Pectin (E 440(i)) and Amidated Pectin (E 440(ii)) are thickeners and they are obtained by extraction of edible plant material, usually citrus fruits or apples. Use levels are high with MPLs up to 10,000 mg/kg for infant formulae. The calculations clearly indicated the need to decrease the current maximum limits for Pb, Cd, Hg and As, and to set limit values for Al[47].

Discussion

Food additives are constituents of many foods. It is not common knowledge in which way the safety of their use is assured in contrast to drugs for which it is known that a regulatory approval procedure exists. In contrast to drugs, where well performed clinical studies are the cornerstone for regulatory decisions, the regulatory requirements for FA are focusing on animal data and, unfortunately, high quality studies in the human target population, including infants and young children are neither required nor provided according to our data analysis. Thus, extrapolation of results from the animal data to the human situation remains a source of uncertainty in the safety assessment of FA.

Table 3: Margin of exposure for the regulatory maximum level exposure assessment of toxic elements in food for infants below 16 weeks of age

	MOE			
Food additive	Cadmium	Mercury	Arsenic	Lead
Ascorbylpalmitate (E 304i)	n.a.	0.5	39–1,026*	96
Lecithins (E 332)	n.a.	46	0.4–10*	0.96**
Locus bean gum (E 410)	730***	460****	0.04–1.03*	0.10**
Mono-and diglycerides of fatty acids (E 471)	364***	228****	0.08–2.1*	0.19**
Pectin (E 440i) and amidated Pectin (E 440ii)	728***	455****	0.04 – 1*	0.04**
Starch sodium octenyl succinate (E 1450) [(a)]	150***	92****	0.06 – 1.55*	0.05**

* MOE too low, should be at least 1,000, based on BMDL01 of 0.3 to 8 µg/kg bw per day in humans [62]** MOE too low, should be at least 1, based on a BMDL01 of 0.5 µg/kg bw per day in humans for lead[73]*** Exhaustion too high, based on a tolerable weekly intake (TWI) of 2.5 µg/kg bw[74].**** Exhaustion too high, based on a TWI of 4 µg/kg bw[75]. [(a)] Values recalculated from Table 8 of the published opinion, for levels of Hg, As and Pb at their current specification values for E 1450 and for Cd based on the FAF Panel considerations included in the opinion.

The susceptibility of infants and children to FAs has been a long-discussed topic, excluding this age group from the applicability of HBGV, such as ADIs. Hence, although FAs were used in food for infants, i.e. infant formula, no assessment was done to assure their safe use. The scientific guidance, which was developed lately, addressing the age-specific physiology of infants and the need for specific studies, formed the basis allowing assessing FAs in food for infants[9]. Until now, worldwide, no other regulatory body or group of scientists has performed assessments of FAs used in food for infants. Therefore, it was the aim of the authors to explain the principles behind the assessment and to briefly illustrate the outcome of assessments.

The substances used as FAs need to have a technical purpose in the food, e.g., infant formula. Hence, the assessment of the safety of a FA does not include the safety of the product. However, the assessment includes the assessment of impurities which is of importance. Concerning the impurities, it is to be considered that the HBGVs or the RPs for the MOE approach are conceptually selected for a lifelong exposure whereas the exposure duration for the infants is 16 weeks and exposure to infant formula may be lower thereafter. Nevertheless, a higher than technically achievable level is to be avoided, having in mind that a formula may contain more than one FA and that exposure by other foods, potentially containing FA or other food ingredients containing toxic elements will continue.

Conclusion

Our assessment revealed that the use of most of the FAs in food for infants below 16 weeks of age does not raise health concerns (Table 2). It has to be noted, however, that the assessments made were assessing the single FA whereas some infant formulae contain more than one single FA which may have combined effects, e.g. thickener (see Table 1). Combined assessment of FAs might be seen as necessary in the future.

An important result of our assessment is the outcome of the safety assessment for toxic elements contained in FAs. Because limit values for toxic elements can be set as specifications by regulation, we selected the current specification levels as examples for this publication. It is noteworthy that potential exposure to arsenic raises concerns for all FAs, concerns are also raised for a high proportion of FAs for lead, and for half of the FAs for cadmium and mercury (Table 3).

Hence, reduction of the limit values for toxic elements is needed from a health point of view. In the EU setting legal specifications this is the task of the European Commission working in conjunction with the Member States.

Disclaimer

The authors are members of the EFSA working group on the re-evaluation of food additives permitted in foods for infants below 16 weeks of age or EFSA staff members contributing to the work of the same working group.
The publication was drafted under the sole responsibility of the authors and is not regarded as an EFSA output. The positions and opinions presented are those of the authors alone and are not intended to represent the views of EFSA.

References

1. Scientific Committee on Food. Opinion of the Scientific Committee on Food on the applicability of the ADI (Acceptable Daily Intak) for food additives to infant (expressed on 17/09/1998). https://food.ec.europa.eu/system/files/2020-12/sci-com_scf_out13_en.pdf
2. World Health Organisation (WHO). Factsheets on food additives. World Health Organisation, Geneva. https://www.who.int/news-room/fact-sheets/detail/food-additives
3. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance). OJ L 354, 31.12.2008, p. 16–33.
4. Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives (Text with EEA relevance) OJ L 80, 26.3.2010, p. 19–27.
5. World Health Organisation (WHO) Principles for the safety assessment of food additives and contaminants in food. 1987. Environmental Health Criteria no. 70. World Health Organisation, Geneva.
6. Commission of the European Communities. First report of the Scientific Committee for Food on the essential requirements of infant formulae and follow-up milks based on cows' milk protein (14th series, opinion expressed 27 April 1983).
7. Commission of the European Communities. Opinion on certain additives for use in infant formulae, follow-on formulae and weaning foods. Report of the Scientific Committee for Food (32nd series, opinion expressed 11 December 1992).
8. Commission of the European Communities. Opinion on additives in nutrient preparation for use in infant formulae, follow-on formulae and weaning foods. (40th series, opinion expressed on 7 June 1996)
9. EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Bresson J-L, Dusemund B, Gundert-Remy U, Kersting M, Lambre C, Penninks A, Tritscher A, Waalkens-Berendsen I, Woutersen R, Arcella D, Court Marques D, Dorne J-L, Kass GEN and Mortensen A. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA J*. 2017;15(5):4849, 58pp. https://doi.org/10.2903/j.efsa.2017.4849
10. World Health Organisation (WHO). Principles and methods for the risk assessment of chemicals in food. Environmental health criteria, 2009 updated in 2020; 240. https://www.who.int/publications/i/item/9789241572408
11. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Guidance for submission for food additive evaluations. *EFSA J*. 2012;10(7):2760, 53pp. https://doi.org/10.2903/j.efsa.2012.2760
12. EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Bresson J-L, Dusemund B, Gundert-Remy U, Kersting M, Lambré C, Penninks A, Tritscher A, Waalkens-Berendsen I, Woutersen R, Arcella D, Court Marques D, Dorne J-L, Kass GEN and Mortensen A. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA J*.2017;15(5):4849, 58pp. https://doi.org/10.2903/j.efsa.2017.4849
13. EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen KH, More S, Mortensen A, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Aerts M, Bodin L, Davis A, Edler L, Gundert-Remy U, Sand S, Slob W, Bottex B, Abrahantes JC, Marques DC, Kass G and Schlatter JR. Update: Guidance on the use of the benchmark dose approach in risk assessment. *EFSA J*. 2017;15(1):4658, 41pp. https://doi.org/10.2903/j.efsa.2017.4658
14. EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C, Halldorsson TI, Hernández-Jerez AF, Bennekou SH, Koutsoumanis K, Lambré C, Machera K, Mennes W, Mullins E, Nielsen SS, Schrenk D, Turck D, Younes M, Aerts M, Edler L, Sand S, Wright M, Binaglia M, Bottex B, Cortinas Abrahantes J and Schlatter J. Guidance on the use of the benchmark dose approach in risk assessment. *EFSA J*. 2022;20(10):7584, 67pp. https://doi.org/10.2903/j.efsa.2022.7584
15. Haber LT, Dourson ML, Allen BC, Hertzberg RC, Parker A, Vincent MJ, Maier A, Boobis AR. Benchmark dose (BMD) modeling: current practice, issues, and challenges. *Crit Rev Toxicol*. 2018;48(5):387-415. https://doi.org/10.1080/10408444.2018.1430121

16. International Programme on Chemical Safety & IPCS Workshop on Incorporating Uncertainty and Variability into Risk Assessment (2000: Berlin, Germany). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration-response assessment. 2005. World Health Organization. https://apps.who.int/iris/handle/10665/43294
17. EFSA Scientific Committee. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA J*. 2012;10(3):2579,32pp. https//doi.org:10.2903/j.efsa.2012.2579
18. EFSA Scientific Committee. Scientific Opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA J*. 2011;9(9):2379, 69pp. https://doi.org/10.2903/j.efsa.2011.2379
19. EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, Gürtler R, Hirsch-Ernst K, Mosesso P, Nielsen E, van Benthem J, Carfì M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J. Clarification of some aspects related to genotoxicity assessment. *EFSA J*. 2017;15(12):5113,25pp. https://doi.org/10.2903/j.efsa.2017.5113
20. OECD Test Guidelines for Chemicals, Section 4. OECD, Paris. https://www.oecd.org/chemicalsafety/testing/
21. National Toxicology Program – Oral Health Assessment Tool (NTP-OHAT). Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration. 2015.
22. Beronius A, Molander L, Rudén C and Hanberg A. Facilitating the use of non-standard in vivo studies in health risk assessment of chemicals: A proposal to improve evaluation criteria and reporting. *J Appl Toxicol*. 2014; 34:607–617. https://doi.org/10.1002/jat.2991
23. Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance. OJ L 83, 22.3.2012, p. 1–295
24. Larsen JC. Risk assessment of chemicals in European traditional foods. *Trends in Food Science & Technology*. 2006;17: 471-481. https://doi.org/10.1016/j.tifs.2006.04.007
25. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Safety Evaluation of certain food additives. Seventy-ninth report of the Joint FAO/WHO Expert Committee on food additives. Geneva. 2014. https://www.who.int/publications/i/item/9789241209908
26. Constable A, Mahedavan B, Pressman P, Garthoff JA, Meunier L, Schrenk D, Speijers G, O'Sullivan A, Hayes AW. An integrated approach to the safety assessment of food additives in early life. *Toxicology Research and Application*. 2017; 1: 1-26. https://doi.org/10.1177/2397847317707370
27. OECD Guideline for the testing of chemicals. Extended one-generation reproductive toxicity (TG 443) adopted 25 June 2018. https://doi.org/10.1787/9789264185371-en
28. Bode G, Clausing P, Gervais F, Loegsted J, Luft J, Nogues V, Sims J and Project R. The utility of the minipig as an animal model in regulatory toxicology. *J Pharmacol Toxicol* Methods. 2010;62:196–220. https://doi.org/10.1016/j.vascn.2010.05.009
29. Helm RM, Golden C, McMahon M, Thampi P, Badger TM, Nagarajan S. Diet regulates the development of gut-associated lymphoid tissue in neonatal piglets. *Neonatology*. 2007; 91:248–255. https://doi.org/10.1159/000098523
30. Guilloteau P, Zabielski R, Hammon HM, Metges CC. Nutritional programming of gastrointestinal tract development. Is the pig a good model for man? *Nutr Res Rev*. 2010; 23:4–22. https://doi.org/10.1017/s0954422410000077
31. Barrow PC. Use of the swine paediatric model. In: Doberman AM,Lewis EM (eds.). Pediatric Non-Clinical Drug Testing: Principles, Requirements, and Practice. John Wiley & Sons Inc, Hoboken, NJ, USA;2012: pp. 213–230.
32. Heinritz SN, Mosenthin R, Weiss E. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. *Nutr Res Rev*. 2013;26:191–209. https://doi.org/10.1017/s0954422413000152

33. Odle J, Lin X, Jacobi SK, Kim SW and Stahl CH. The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. *Annu Rev Anim Biosci*. 2014; 2:419–444. https://doi.org/10.1146/annurev-animal-022513-114158
34. Mudd AT, Dilger RN. Early-life nutrition and neurodevelopment: use of the piglet as a translational model. *Adv Nutr*. 2017;8:92-104. https://doi.org/10.3945/an.116.013243
35. Farmer C, Quesnel H. Nutritional, hormonal, and environmental effects on colostrum in sows. *J. Anim. Sci*. 2009; 87:56–65. https://doi.org/10.2527/jas.2008-1203
36. Penninks AH, Van Mierlo GJD, Kuper F, Snel CJ, Ganderup NC, Wolterbeek APM. Juvenile immunodevelopment in minipigs. 2012. In: Doberman AM and Lewis EM (eds.). Pediatric Non-Clinical Drug Testing: Principles, Requirements, and Practice.John Wiley & Sons Inc, Hoboken, NJ, USA: 231–254.
37. Hansen CF, Thymann T, Andersen AD et al. Rapid gut growth but persistent delay in digestive function in the postnatal period of preterm pigs. *Am J Physiol*. Gastrointest. 2016; 310, G550–G560. https://doi.org/10.1152/ajpgi.00221.2015
38. Van Ginneken C. Gastrointestinal tract. In: McAnulty PA, Dayan AD, Ganderup NC and Hastings KL (eds.). The Minipig in Biomedical Research. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA; 2012: pp. 211–236.
39. Sangild PT, Thymann T, Schmidt M, Stoll B, Burrin DG, Buddington RK. Invited Review: the preterm pig as a model in pediatric gastroenterology. *J Anim Sci*. 2013; 91:4713–4729. https://doi.org/10.2527/jas.2013-6359
40. McAnulty PA. The kidney. In: McAnulty PA, Dayan AD, Ganderup NC and Hastings KL (eds.). The Minipig in Biomedical Research. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA;2012: 253–262.
41. Preusse C and Tingleff Skaanild M. Minipigs in Absorption, Distribution, Metabolism, and Excretion (ADME) studies. In: McAnulty PA, Dayan AD, Ganderup NC and Hastings KL (eds.). The Minipig in Biomedical Research. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA;2012: 143–158.
42. Haley P. The immune system of pigs: Structure and Function. In: McAnulty PA, Dayan AD, Ganderup NC and Hastings KL (eds.). The Minipig In Biomedical Research. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA;2012: 343–355
43. McAnulty PA, Barrow P, Marsden E. Reproductive system including studies in juvenile mini pigs. In: McAnulty PA, Dayan AD, Ganderup NC and Hastings KL (eds.). The minipig in biomedical research. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA;2012: 263–282.
44. Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P and Hansen AK, 2007. The use of pigs in neuroscience: Modeling brain disorders. *Neurosci Biobehav Rev*. 2007;31:728–751. https://doi.org/10.1016/j.neubiorev.2007.02.003
45. OECD Guideline for the testing of chemicals. Two-generation reproduction toxicity study (TG 416) adopted 22 January 2001.
46. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Dusemund B, Mortensen A, Turck D, Barmaz S, Rincon AM, Smeraldi C, Tard A, Vianello G and Gundert-Remy U, 2020. Opinion on the re-evaluation of starch sodium octenyl succinate (E 1450) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups. *EFSA J*. 2020;18(8):5874, 60pp. https://doi.org/10.2903/j.efsa.2020.5874
47. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Dusemund B, Mortensen A, Turck D, Barmaz S, Tard A, Vianello G and Gundert-Remy U, 2021. Opinion on the re-evaluation of pectin (E 440i) and amidated pectin (E 440ii) as food additives in foods for infants below 16 weeks of age and follow-up of their re-evaluation as food additives for uses in foods for all population groups. *EFSA J*. 2021;19(1):6387, 57pp. https://doi.org/10.2903/j.efsa.2021.6387
48. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Degen G, Engel K-H, Fowler PJ, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I,

Wright M, Wölfle D, Dusemund B, Mortensen A, Turck D, Barmaz S, Mech A, Rincon AM, Tard A, Vianello G and Gundert-Remy U, 2022. Re-evaluation of locust bean gum (E 410) as a food additive in foods for infants below 16 weeks of age and follow-up of its re-evaluation as a food additive for uses in foods for all population groups. *EFSA J*. 2023;21(2):7775, 70pp. https://doi.org/10.2903/j.efsa.2023.7775

49. Guidelines for reporting outcomes in trial reports. The CONSORT-Outcomes 2022 Extension. Butcher NJ, Monsour A, Mew EJ, Chan AW, Moher D, Mayo-Wilson E, Terwee CB, Chee-A-Tow A, MPH; Baba A, Gavin F, Grimshaw JM, Kelly LE, Saeed L, Thabane L, Askie L, Smith M, Farid-Kapadia M, Williamson PR, Szatmari P, Tugwell P, Golub RM, Monga S, Vohra S, MD; Marlin S, Ungar WJ, Offringa M. *JAMA*. 2022; 328: 2252-64. https://doi:10.1001/jama.2022.21022
50. Junqueira DR, Zorzela L, Golder S, Loke Y, Gagnier JJ, Julious SA, Li T, Mayo-Wilson E, Pham B, Phillips R, Santaguida P, Scherer RW, Gøtzsche PC, Moher D, Ioannidis JPA, Vohra S; CONSORT Harms Group. *J Clin Epidemiol*.2023 Apr 22: S0895-356(23)00090-2. https://doi.org/10.1016/j.jclinepi.2023.04.005. Online ahead of print.
51. Jarrold K; Helfer B, Eskander M, Crawley H, Trabulsi J, Caulfield LE, Duffy G, Garcia-Larsen V, Hayward D, Hyde M, Jeffries S, Knip M, Leonardi-Bee J, Loder E, Lodge CJ, Lowe AJ, McGuire W, Osborn D, Przyrembel H, Renfrew MJ, Trumbo P, Warner J, Schneeman B, Boyle RB. Guidance for the conduct and reporting of clinical trials of breast milk substitutes. *JAMA Pediatr*. 2022; 174: 874-81. https://doi.org10.1001/jamapediatrics.2020.0578
52. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Dusemund B, Mortensen A, Turck D, Barmaz S, Smeraldi C, Tard A, Vianello G, Rincon AM, Gundert-Remy U, 2020. Scientific Opinion on the re-evaluation of ascorbyl palmitate (E 304i) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups. *EFSA J*. 2020;18(6):6153: 1-37. https://doi.org/10.2903/j.efsa.2020.6153
53. EMA. Committee for Human Medicinal Products.Guideline for good clinical practice E6(R2), 2016. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-good-clinical-practice-e6r2-step-5 en.pdf accessed 19 April 2023
54. Riley DS, Barber MS, Kienle GS, Aronson JK, von Schoen-Angerer T, Tugwell P, Kiene H, Helfand M, Altman DG, Sox H, Werthmann PG, Moher D, Rison RA, Shamseer L, Koch CA, Sun GH, Hanaway P, Sudak NL, Kaszkin-Bettag M, Carpenter JM, Gagnier JJ. CARE guidelines for case reports: explanation and elaboration document. *J Clin Epidemiol*. 2017; 89: 218-35. https://doi.org/10.1016/j.jclinepi.2017.04.026
55. Fomon SJ. Nutrition of Normal Infants. 1993. Mosby, 488 pp.
56. Fomon SJ, Filer LJ, Thomas LN, Ziegler EE and Leonard MT. Food consumption and growth of normal infants fed milk-based formulas. *Acta Paediatr Scand Suppl*. 1971: 1–36.
57. Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schoch G. Measured consumption of commercial infant food products in German infants: results from the DONALD study. *JPGN*. 1998;2: 547–552. https://doi.org/10.1097/00005176-199811000-00011
58. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Scientific opinion on the risk for human health related to the presence of 3- and 2-monochloropropandiol (MCPD) and their fatty acids, and glycidyl fatty acids esters in food. *EFSA J*. 2016; 14 (5): 4426, 159pp. https://doi.org/10.2903/j.efsa.2016.4426
59. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Scientific Opinion on the update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. *EFSA J*. 2018;16(1):5083, 48pp. https://doi.org/10.2903/j.efsa.2018.5083
60. EFSA Panel on Contaminants in the Food Chain (CONTAM), Scientific Opinion on erucic acid in feed and food. *EFSA J*. 2016;14(11):4593, 173 pp. https//:doi.org/10.2903/j.efsa.2016.4593
61. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA

relevance).C/2023/35.OJ L 119, 5.5.2023, p. 103–1572023/915

62. EFSA CONTAM Panel (Panel on Contaminants in the Food Chain. Scientific opinion on Arsenic in food. *EFSA J*. 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980
63. EFSA Scientific Committee, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA J*. 2012;10(3):2579, 32 pp. https//doi.org/10.2903/j.efsa.2012.2579
64. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Mennes W, Moldeus P, Oskarsson A, Shah R, Waalkens-Berendsen I, Wölfle D, Dusemund B, Mortensen A, Turck D, Barmaz S, Smeraldi C, Tard A and Gundert-Remy U. Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive in foods for infants below 16 weeks of age and the follow-up of its re-evaluation as a food additive for uses in foods for all population groups. *EFSA J*. 2019;17(12):5922, 23 pp. https://doi.org/10.2903/j.efsa.2019.5922
65. Cole CR, Grant FK, Swaby-Ellis ED, Smith JL, Jacques A, Northrop-Clewes CA, Caldwell KL, Pfeiffer CM and ZieglerTR. Zinc and iron deficiency and their interrelations in low-income African American and Hispanic children in Atlanta. *Am J Clin Nutr*. 2010; 91: 1027–1034. https://doi.org/10.3945/ajcn.2009.28089
66. Gozzo S, Oliverio A. Effects of dietary phospholipids and odd-chain fatty acids on the behavioural maturation of mice. *Food Chem Toxicology*. 1982; 20: 153–157. https://doi.org/10.1016/s0278-6915(82)80240-8
67. Bell JM, Lundberg PK. Effects of a commercial soy lecithin preparation on development of sensorimotor behavior and brain biochemistry in the rat. *Dev Psychobiol*.1985; 18: 59–66. https://doi.org/10.1002/dev.420180105
68. Bell JM, Slotkin TA. Perinatal dietary supplementation with a commercial soy lecithin preparation: effects on behavior and brain biochemistry in the developing rat. *Dev Psychobiol*. 1985;18: 383–394. https://doi.org/10.1002/dev.420180504
69. Bell JM, Withmore WL, Cowdery T, Slotkin TA. Perinatal dietary supplementation with a soy lecithin preparation: effects on development of central catecholaminergic neurotransmitter systems. *Brain Res Bull*. 1986; 17: 189–195. https://doi.org/10.1016/0361-9230(86)90115-2
70. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). Scientific opinion on Dietary Reference Values for choline. *EFSA J*. 2016; 14(8):4484, 70 pp. https://doi.org/10.2903/j.efsa.2016.4484
71. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Woelfle D, Wright M, Dusemund B, Mortensen A, Turck D, Barmaz S, Tard A, Vianello G, Gundert-Remy U. Scientific Opinion on the re-evaluation of lecithins (E 322) as a food additive in foods for infants below 16 weeks of age and follow up of its re-evaluation as food additive for uses in foods for all population groups. *EFSA J*. 2020;18 (11):6266, 37 pp. https://doi.org/10.2903/j.efsa.2020.6266
72. EFSA FAF Panel (EFSA Panel on Food Additives and Flavourings), Younes M, Aquilina G, Castle L, Engel K-H, Fowler P, Frutos Fernandez MJ, Fürst P, Gürtler R, Husøy T, Manco M, Mennes W, Moldeus P, Passamonti S, Shah R, Waalkens-Berendsen I, Wölfle D, Wright M, Dusemund B, Mortensen A, Turck D, Barmaz S, Tard A, Vianello G and Gundert-Remy U. Scientific opinion on the re-evaluation of mono- and diglycerides of fatty acids (E 471) as food additive in foods for infants below 16 weeks of age and follow-up of their re-evaluation as food additives for uses in foods for all population groups. *EFSA J*. 2021;19(11):6885, 44 pp. https://doi.org/10.2903/j.efsa.2021.6885
73. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain). Scientific opinion on Lead in food. *EFSA J*. 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570
74. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain. Scientific opinion on Cadmium in food. *EFSA J*. 2009;7(10):1351, 199 pp. https://doi.org/10.2903/j.efsa.2009.1351
75. EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA J*. 2012;10(12):2985,15 pp. https://doi.org/10.2903/j.efsa.2012.2985