



Who will carry out the tests that would be necessary for proper safety evaluation of food emulsifiers?

Katalin F. Csáki*, Éva Sebestyén

National Food Chain Safety Office, Directorate for Food Safety Risk Assessment, PO BOX 407, H-1537 Budapest, Hungary



ARTICLE INFO

Article history:

Received 24 October 2018

Received in revised form 12 February 2019

Accepted 8 April 2019

Available online 9 April 2019

Keywords:

Food emulsifiers
Surfactants
Intestinal barriers
Crohn's diseases
Microbiota
Risk assessment
EFSA

ABSTRACT

Surfactant food emulsifiers are among the most extensively used food additives. Like all authorized food additives, emulsifiers have been evaluated by risk assessors, who consider them as safe. However, there are growing concerns among scientists about their possible harmful effects on intestinal barriers and microbiota. It is also suggested that emulsifier consumption might be one of the main causes of the rising incidence of a number of diseases, such as allergic diseases, celiac disease, type I diabetes and Crohn's disease. Moreover, it has recently been suggested that emulsifier consumption might contribute to the development of metabolic syndrome and can promote colitis-associated colorectal cancer also. This paper provides an overview of the current scientific knowledge on possible effects of surfactant emulsifiers on intestinal barriers and also of regulatory risk assessment approaches. Our main objective is to reveal the reasons for the discrepancies between the opinions of risk assessors and the scientific world. We would like to draw the attention of the academic world to the need of specific *in vivo* and/or clinical studies for each emulsifier in order to help risk assessors make adequate evaluations and rule out with certainty that authorized food emulsifiers pose a safety concern to consumers' health.

© 2019 "Society information". Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Most food emulsifiers are surface active (surfactant) substances, meaning these are amphipathic molecules that consist of both a hydrophobic and a hydrophilic part. Owing to their structure, they tend to accumulate at interfaces making them suitable to stabilize emulsions. In fact, the name "emulsifier" refers to the technological function of a food additive and thus represents a wide range of food additives capable of stabilizing emulsions, thus in addition to surfactants, a lot of macromolecules (e.g. modified celluloses or algae derivatives) are emulsifiers as well. In this paper we discuss only surfactant type food emulsifiers and use the terms "surfactant" and "emulsifier" synonymously.

There are almost 30 types of surfactant food emulsifiers authorized in the European Union (Table 1). These are used in staple foods

such as bakery products or dairies and even infant formulas [1]. This leads to a very high dietary intake of most emulsifiers in developed countries.

Recently, a number of studies have suggested that food emulsifiers can have a causative role in rising incidents of numerous diseases linked to impaired intestinal barrier functions and/or changed intestinal microbiota (e.g. allergic, autoimmune diseases and Inflammatory Bowel Diseases) [1,2]. Moreover, they can also increase the absorption of contaminants from foods [3,4].

As the intestinal lumen contains a huge amount of harmful substances (e.g. bacteria, toxins, allergens), an essential function of the gastrointestinal tract is to keep those agents away from the inner body (barrier function). If the barrier function is disturbed, the door is open to the development of numerous diseases [5].

It has long been known from pharmaceutical science that surfactants can increase the permeability of intestines in a number of ways [6]. Surfactants can impair the barrier function of the mucus layer by decreasing its hydrophobicity [1,7] and by breaking non-covalent bonds of the mucus gel [1,8] allowing macromolecules and nanoparticles and even pathogens to pass through the mucus. Furthermore, surfactants can increase the fluidity and permeability of the epithelial cell membranes facilitating the absorption of hydrophobic molecules and also the penetration of bacteria. More-

* Corresponding author.

E-mail address: Freckskanek@nebih.gov.hu (K. F. Csáki).

Peer review under responsibility of KeAi Communications Co., Ltd.



Production and hosting by Elsevier

Table 1
Surfactant food emulsifiers used in EU and their HLB values [60,92].

Name	E number	HLB	Note
Lecithins	E 322	3–4	high exposure
Hydrolysed lecithins (lysolecithins)		10–12	
Polyoxyethylene sorbitan monolaurate (polysorbate 20)	E 432	16.7	
Polyoxyethylene sorbitan monooleate (polysorbate 80)	E 433	15.4	
Polyoxyethylene sorbitan monopalmitate (polysorbate 40)	E 434	15.6	high exposure of high consumers. Main contributors: fine bakery products, food supplements, desserts [11]
Polyoxyethylene sorbitan monostearate (polysorbate 60)	E 435	14.4	
Polyoxyethylene sorbitan tristearate (polysorbate 65)	E 436	10–11	
Sodium, potassium and calcium salts of fatty acids	E 470a	16–18	
Mono- and diglycerides of fatty acids	E 471	3–4	high exposure, widespread usage including the most frequently consumed products such as bread and rolls and infant formula
Acetic acid esters of mono- and diglycerides of fatty acids	E 472a	2–3	
Lactic acid esters of mono- and diglycerides of fatty acids	E 472b	3–4	
Citric acid esters of mono- and diglycerides of fatty acids (CITREM)	E 472c	10–12	widespread usage including the most frequently consumed products such as bread, rolls and special infant formulas
Tartaric acid esters of mono- and diglycerides of fatty acids	E 472d		
Mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids (DATEM)	E 472e	8–10	widespread usage including the most frequently consumed products such as bread, rolls
Mixed acetic and tartaric acid esters of mono- and diglycerides of fatty acids	E 472f		
Sucrose esters of fatty acids	E473	3–16	widespread usage including the most frequently consumed products such as fine bakery products and special infant formulas
Sucroglycerides	E474		
Polyglycerol esters of fatty acids	E475	5–13	usage examples: fine bakery products, chewing gum
Polyglycerol polyricinoleate	E476	1.5	usage examples: fat emulsions, ice cream, chocolate products
Propane-1,2-diol esters of fatty acids	E477	2–3	
Sodium stearyl-2-lactylate	E481	10–12 (according to other data: 21)	very high exposure. Used also in bread and rolls, flavored fermented milk products, fine bakery products
Calcium stearyl-2-lactylate	E482	5–6	
Stearyl tartrate	E483		
Sorbitan monostearate	E491	3–6	
Sorbitan tristearate	E492	2.1	
Sorbitan monolaurate	E493	8.6	usage examples: flavored fermented milk products, fat emulsions, chocolate products, non-alcoholic beverages [16]
Sorbitan monooleate	E494	4.3	
Sorbitan monopalmitate	E495	6.7	
Sucrose acetate isobutyrate	E444	1	
Glycerol ester of wood rosin	E445	no data	

over, surfactants can increase the pore size of tight junctions which make it possible to absorb macromolecules (e.g. peptides, proteins). On top of all, some surfactants can inhibit certain transport proteins (p-glycoprotein, breast cancer resistance protein) found in epithelial cells membranes causing increased absorption of the substrates of these proteins (e.g. pharmaceutical drugs or even environmental toxins such as phthalates, aflatoxin B1, benzo-(a)pyrene, a number of bacterial toxins, heterocyclic amines). It has been recently discovered that surfactant emulsifiers can also alter intestinal microbiota, increasing its proinflammatory and mucolytic potential [9].

The absorption enhancing property of surfactants has been used for decades to increase the bioavailability of drugs in pharmaceuticals. However, even the pharmaceutical literature warns of the risk that non-specific absorption enhancers – like surfactants – in pharmaceuticals can also enhance the absorption of undesirable substances (e.g. contaminants) present in the lumen [10]. In pharmaceutical formulations some risk can be acceptable considering a reasonable risk/benefit ratio. Since the application of surfactants in medicines may pose a risk with regard to their non-specific absorption enhancer property, their everyday use in foods should be even more reconsidered.

Like in the case of all authorized food additives, the safety of food emulsifiers is evaluated under the authorization procedure. Additionally, they have recently undergone re-evaluation processes in Europe [11–19]. Thus, the question may be raised: “How is it possible, that these surface active food additives are still considered as safe for human consumption and subsequently are still on the market?”

In order to resolve the controversies we take a closer look at the risk evaluation approach and also the existing and missing studies concerning the effects of emulsifiers on intestinal barriers. In this paper we will focus on the European practice, although the situation is similar worldwide. We would like to draw the academic world's attention to the urgent need of specific toxicity data on this issue in order to provide sufficient data for proper risk evaluation. We would also like to raise food industry researchers' awareness of the need of harmless alternatives to synthetic surfactant food additives.

2. Studies on the impact of surfactants on intestinal barriers

In this section we consider the studies that have found that surfactants impair intestinal barriers or enhance absorption of harmful substances or act as co-carcinogenic agents. We are focusing only on the surfactants which are now authorized as food additives and we are only looking at studies that are relevant in terms of exposure through oral route.

2.1. Early studies

The earliest studies investigated the co-carcinogenic potential of surfactants. As early as in the 1950s, Wong and coworkers published that polysorbate 80, when fed together with a known carcinogen (a polycyclic aromatic hydrocarbon: methylcholanthrene) significantly potentiates the local as well as distant carcinogenic activity in mice [20]. As polysorbate alone did not show any carcinogenic activity, authors concluded that polysor-

bate is co-carcinogen. Three other studies in the 1970s also showed increased carcinogenic activity of another known carcinogen (N-methyl-N'-nitro-N-nitrosoguanidine, MNNG) with several surfactants (polysorbates, sorbitan monolaurate, glyceryl monostearate, and sucrose monopalmitate) in rats [21–23]. These studies showed that rat groups fed with MNNG together with the surfactants developed undifferentiated adenocarcinomas and several sarcomas. Undifferentiated adenocarcinomas showed a high degree of anaplasia, and showed lymphatic invasion and direct spread to the omentum and liver. The mesenchymal tumors showed metastases and invasive growth. On the other hand, none of the rats in group fed MNNG alone (without surfactants) had undifferentiated adenocarcinomas or sarcomas, and neither lymphatic invasion nor metastasis was found, and they only exhibited well-differentiated adenocarcinoma.

The impacts of surfactants on intestinal barriers were investigated already in the 1980's. Tagesson et al. [24] in 1984 warned that surface-active food additives might impair the function of the mucosal barrier and increase the permeability of the gut to potentially toxic and pathogenic substances. In this paper polysorbate 60 and polysorbate 80 were studied on rat intestinal mucosa and found mucosal damage and increased permeability. It should be noted that this paper warned more than 30 years ago that increased absorption of macromolecules may facilitate the development of celiac disease, inflammatory bowel diseases and food allergy. Let us quote verbatim from their conclusion: "*it is possible that certain food additives may facilitate the intestinal absorption of potentially toxic and pathogenic compounds. This possibility should not be overlooked, since alterations in intestinal permeability may underlie a variety of diseases, not only in the gastro-intestinal tract itself but at distant sites such as the liver and joints.*" Unfortunately, too little attention has been paid to this very important suggestion at that time.

2.2. Pharmaceutical studies

Despite the fact that the possible role of intestinal barrier damage in pathogenesis of numerous diseases has been known for over 30 years, the possible harmful effects of surfactant food additives on intestinal barriers passed into oblivion for about 20 years. However, the continuously evolving pharmaceutical science provides useful data for food safety aspects also, as a lot of surfactants are excipients in pharmaceutical preparations.

Surfactants have multiple functions in medicines such as modulating solubility of the active ingredients, stabilizing emulsions, increasing the stability of ingredients and also increasing the absorption of active ingredients, in other words they are absorption enhancers. Accordingly, the pharmaceutical science has intensively studied the effects of surfactant on intestinal mucosa. Surfactants can increase the permeability of intestinal mucosa through both paracellular and transcellular mechanisms simultaneously [25,26] although the efficacy is largely dependent upon their specific properties. Water solubility is a key factor in general in absorption enhancement efficacy of surfactants, namely the more water-soluble (high hydrophilic-lipophilic balance, HLB) a surfactant is, the more effective enhancer it is both transcellularly and paracellularly [27,28]. In addition, other factors (e.g. size and shape of the alkyl chain and the polar group) also influence their absorption-enhancing ability [29]. Surfactants may cause increased mucosal permeability in such small concentrations that no lysis or micellar solubilisation occurs [28]. Some surfactants can inhibit p-glycoprotein and/or breast cancer resistance protein, although this is not a general characteristic for each surfactants.

In the pharmaceutical literature we can find studies connected with surfactant absorption enhancers which are also used as food additives [1]. Such substance is the most investigated food emulsi-

fier polysorbate 80 [30–33], but among others sucrose esters of fatty acids [27], polyglycerol esters of fatty acids and monoglycerides of fatty acids [34] are also included.

Surfactant impact on intestinal mucus is a relatively less investigated issue. Oberle et al. [35] studied the effects of polysorbate 80 on intestinal mucosa by a single-pass in situ perfusion model in male rats. They found that polysorbate 80 induced an increase in mucus release. Bernkop-Schnürch et al. [36] showed that polysorbate 20 decreased the viscosity of porcine mucus gel in vitro. These results are explained with the ability of surfactants to break non-covalent bonds within the mucus network leading to a network with larger pore size. These discoveries raise the question of whether surfactants can make mucus gel penetrable to intestinal bacteria facilitating their invasion, since the commensal intestinal microbiota is limited to the outer loose mucus layer but the inner layer is devoid of bacteria [37]. If the inner mucus layer becomes also permeable to bacteria the way is open for their invasion. This mechanism could be especially relevant to Crohn's disease development as the permeable inner mucus layer to bacteria is considered to be a critical etiological factor in IBD. However, as we will see later, additional mechanisms (connected with microbiota) also exacerbate the mucus loosening effect.

It is important to note that although the above mentioned investigations are related to enhanced absorption of drug molecules by surfactants, there is every reason to expect that these surfactants will also increase the absorption of other substances (e.g. antigens, toxic substances or pathogens), since surfactants are non-specific absorption enhancers. For example, knowing that polysorbates or sucrose esters effectively increased the pore size of tight junctions to enhance the absorption of macromolecular drugs [30], we can seriously believe that these very same surfactants in foods also enhance the absorption of macromolecular antigens, allergens with similar molecular size. Similarly, we cannot believe seriously that by inhibiting p-glycoprotein (e.g. monoglycerides [38], polysorbates [39], polyoxyethylene (40) stearate [40]) surfactants will only increase the absorption of p-glycoprotein substrate drugs but not p-glycoprotein substrate food contaminants (e.g. numerous pesticides). In the same way, when a surfactant (e.g. polysorbates, sorbitan monolaurate [41]) increases drug absorption by inhibiting breast cancer resistance protein (BCRP), it suggests that it can also increase absorption of BCRP substrate food contaminants (e.g. aflatoxin B1, heterocyclic amines, 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine, benzo[a]pyrene [42]) when applied as food additive.

We might well ask, of course, how those surfactants impact on intestinal barriers which are not yet examined. The answer is not known yet.

2.3. Recent studies

Since the 2000s the food safety concerns have been in the spotlight again. By that period the mechanism of surfactants impacting intestinal mucosa was known and the 40 years of intensive usage of food emulsifiers has created the opportunity to investigate epidemiological impacts.

From epidemiological data it has become increasingly clear by now that the incidence of autoimmune diseases is increasing over the last several decades world-wide. A growing number of studies have suggested that consumption of surfactant emulsifiers in foods promote Crohn's disease, allergic and autoimmune diseases and, moreover, the consumption of these additives might be the main cause of the rising incidence of these diseases in developed countries [1,2,43].

The role of impaired barrier function in the pathomechanisms of a number of diseases is now becoming more apparent also.

It was hypothesized as early as the 1980's that the intestinal barrier dysfunction is not only a consequence of various diseases (e.g. celiac disease, food allergy, inflammatory bowel diseases) but it also plays a causative role in their development [24,44]. A growing number of scientific evidence now supports these assumptions [45,46]. It is now generally accepted that in the case of autoimmune diseases intestinal barrier dysfunction is one of the key pre-existing conditions (in addition to genetic susceptibility and antigen exposure) [47,48]. Scientific evidence proves that increased intestinal permeability precedes numerous diseases concerned, for example in the case of type 1 diabetes [49,50], celiac disease [50] or relapse in Crohn's disease [51–53]. Intestinal barrier dysfunction and altered microbiota is associated with a number of additional diseases, including nonalcoholic fatty liver disease [54], colon cancer [55], metabolic syndrome [56], irritable bowel syndrome [57] and even various mental disorders [58,59].

As previously indicated, surfactants can increase the pore size of tight junctions when applied in pharmaceuticals. The question therefore arises whether these surfactants in foods also enhance the absorption of harmful macromolecules (e.g. antigens, allergens). This issue has been in the focus of some recent studies.

One group of the most investigated food emulsifiers is sucrose esters of fatty acids. These additives consist of a mixture of mono- di- and triesters of sucrose edible fatty acids. Depending on its degree of esterification and chain length of the consisting fatty acids, sucrose esters can have a very wide range of hydrophilic-lipophilic balance (HLB = 1–18). This wide variation in water solubility makes sucrose esters suitable for a variety of applications [60,61]. These substances are used for example in fine bakery products, flavored drinks, dairy-based drinks and special infant formulas. Owing to the intensive usage of sucrose esters, their dietary exposure is very high in Europe, not only for high consumers but the mean intake also exceeds even the ADI (40 mg/bw/day) established by the European Food Safety Authority (EFSA) for many population groups [62]. Moreover, as it will be shown later, the ADI level does not protect against barrier impairment. In pharmaceuticals the sucrose esters levels that are used to enhance the absorption of macromolecular drugs through tight junctions are far lower than the ADI level [1,63].

Mine et al. studied the effect of sucrose monoester fatty acids (a highly water soluble sucrose ester) on intestinal epithelial Caco-2 cells [64]. They found that sucrose esters significantly increased the permeability of tight junctions and the paracellular uptake of ovomucoid (a major allergenic egg protein) antigens even in low concentrations which are relevant to special infant formulas.

Recently, Glynn et al. investigated the effect of sucrose esters on intestinal permeability both separately and when co-administered with some surface active food contaminants (natural toxins chaconine and solanine and an environmental pollutant perfluorooctane sulfonate) *in vitro* [65]. Their results indicated that sucrose esters compromise tight junction integrity in concentrations far below than used in food applications. The authors point out that surfactant mixtures may act additively on the integrity of tight junctions, which should be considered in risk assessment of emulsifier authorization processes.

Weangsripanaval et al. investigated the effects of corn oil and sucrose ester on the absorption of a major soybean allergen (oil-body-associated protein Gly m Bd 30 K) in mice [66]. It was shown that the soybean allergen was absorbed from the gastrointestinal tract into the blood in some degree. By co-administering corn oil with the soybean allergen, the absorption was significantly increased. Similarly, sucrose ester also increased the allergen absorption to an extent comparable to corn oil. The combination of 30% corn oil and 3% sucrose fatty acid ester increased the absorption of the soybean allergen to an even greater extent than corn oil or sucrose ester alone. As the investigated soybean allergen is

a hydrophobic substance, the increased bioavailability in presence of oil is not surprising. It is however noteworthy that absorption enhancing effects of natural food components (oil) and synthetic surfactant cumulated, causing significantly higher allergen absorption.

For example, in 2010 Roberts et al. [67] investigated the effect of polysorbates on the translocation of *E. coli* isolates from Crohn's disease patients *in vitro*. They found that 0.01% polysorbate 80 increased *E. coli* translocation through Caco2 monolayers by 59-fold, and at higher concentrations, increased translocation across M-cells was found. The used surfactant concentrations were relevant to the levels that might occur in the distal ileum after consumption of processed foods.

Scientific breakthroughs were published in this topic in Nature in 2015. Chassaing et al. (hereinafter Chassaing study) investigated the effects of two emulsifiers (polysorbate 80: a non-ionic surfactant and carboxy methyl cellulose, CMC: a polyelectrolyte) on intestinal microbiota and on colitis promoting potential *in vivo* [9]. They used a wild type and also two types of genetically susceptible (knockout) mice to intestinal inflammation and metabolic syndrome respectively. Multiple important scientific discoveries were made in this study.

The localization of bacteria within the mucus was measured by confocal microscopy. In the case of control animals there was a safe distance between the closest bacteria and epithelial cells (10 µm). In contrast, in the case of emulsifier treated mice, not only the average distance was reduced significantly but also some bacteria were in direct contact with epithelial cells. Measuring the intestinal permeability by FITC-labelled dextran method it was shown that emulsifier treatment increased gut permeability in both wild-type and knockout mice genetically susceptible to IBD. As the intestinal permeability of these knockout mice are originally much higher than of the wild type mice thus the increased permeability was really high in their case after emulsifier treatment.

It was shown that food emulsifiers (polysorbate 80 and also CMC) promote the extent and incidence of colitis in genetically susceptible animals but they do not induce colitis in wild-type ones. We believe that it is an *in vivo* evidence that food emulsifiers can trigger IBD in genetically predisposed individuals. Knowing that dietary intake of a number of food emulsifiers is very high even among average consumers in developed countries, this result gives reason to believe that food emulsifier consumption can be one of the most significant contributing factors in the rising incidences of Crohn's disease. As colitis was not induced in wild type animals, this study also explains why toxicity data in original authorization dossiers did not indicate any sign of Crohn's disease (or other genetically predisposed disease) promoting potency.

In wild type mice it was shown that polysorbate 80 and carboxy methyl cellulose in low concentrations induced low-grade inflammation, increased food consumption, increased body weights and caused metabolic syndrome. These effects were due to alteration of microbiota composition, increasing its proinflammatory and mucolytic potential. It was proven by experiments with germ-free mice as follows: On the one hand, emulsifiers did not induce either low-grade inflammation or metabolic syndrome in germ-free mice, on the other hand, faecal transplantation from emulsifier treated mice triggered these conditions.

Since the main aim of this review is to reveal the causes of the discrepancies between the opinions of risk assessors and of the academic world, we would like to make a few remarks on this point. EFSA re-evaluated polysorbates [11] after the Chassaing study was published. In the re-evaluation report this study was discussed, but it was not taken into account in the risk assessment on the ground that "The Panel considered that if such effects occurred with polysorbates, then an increase in body weights would have been expected in subchronic, chronic toxicity and carcinogenicity studies" (i.e. by

National Toxicology Program, hereinafter NTP study [68]). “No such increase has been observed, and therefore the relevance of the observed effects remains unclear”.

On this basis the entire study was neglected by EFSA, even the very important evidences of the potential of polysorbates to promote colitis and increase intestinal permeability. Therefore it is essential to reveal the possible causes of the differences between the Chassaing and NTP studies regarding the body weight gain.

Here we would like to make some comments connected with the body weight issue. It is worth considering whether the mentioned differences in body weight gains is a real contradiction or only a consequence of the different experimental designs or even possibly the difference between the analyses of their experimental data. As a preliminary point, it should be pointed out that the aims of the two studies were different. The NTP study is a standard toxicological study aiming to determine the no observed adverse effect level (NOAEL) in order to calculate acceptable daily intake (ADI) and also exclude unacceptable effects (such as genotoxicity, carcinogenicity, developmental toxicity) for regulatory purposes. In this manner the main purpose is to detect serious changes in animal health.

By contrast, the Chassaing study is a specific toxicity study aiming to prove or disprove the hypothesis that emulsifiers can alter intestinal microbiota, and promote inflammatory bowel disease and metabolic syndrome. In the Chassaing study the body weight gain was demonstrated by the relative body weight values (Figure 3a in the study) which are percentages of the initial body weight for each experimental animal. This method is suitable to present modest but significant effects. Seeing the raw body weight vs time data from the supplementary information of this article [69] (Figure 3b in supplementary information), it is apparent that there is no significant difference in raw body weight data between the control group and the polysorbate group. It does not mean that there is no difference between the weight gains of the two groups but rather it indicates that the variations in original body weights of the individual animals hide the effect in this representation. In contrast, in the NTP study report (Table 10. on page 36. of the study) the body weight data were averaged over one group (in other words the mean body weight of 10 animals) both in initial and in final cases. This data analysis method is unsuitable to detect such a small effect. As we cannot see the raw data of the NTP study, it is impossible to decide if there was any body weight gain due to polysorbate consumption or not.

Another point to consider is that the experimental designs were not the same in the two studies. There were differences between the rodent feeds, namely in the Chassaing study the feed contained more fiber, the fat types were different (animal fat vs soy oil) and administration routes were also different (in the Chassaing study it was via drinking water, on the contrary in the NTP study it was mixed with feed). The differences between the experimental designs might also explain the differences in their results, since polysorbates can impact the absorption of nutrients depending on a number of factors (e.g. fat droplet size) [70,71]. Since the altered microbiota was the reason of the body weight changes in the Chassaing study, the fiber content can also be a relevant factor.

As surfactants can impact on body weight in a number of ways (e.g. through nutrient absorption, microbiota, causing diarrhea), it is also conceivable that the resulting effect in body weight is not a monotonic function of surfactant dosage. In this manner it is also possible that there are not enough measuring concentration points to detect the increasing part of the function in NTP study.

It would be important to clarify the supposed contradictions between body weight gain and the related clinical findings as described in the results of the Chassaing and NTP studies. It would also be an important step to get risk assessors to take into account

the very important evidences of the Chassaing study in further evaluations.

After the mentioned discoveries were published, increasing attention is being paid to the impact of food emulsifiers on intestinal microbiota. Jiang et al. studied the impact of glyceryl monolaurate (another emulsifier) on microbiota, and found that relatively low-dose (150 mg/kg) of emulsifier consumption promotes metabolic syndrome, gut microbiota dysbiosis and systemic low-grade inflammation in mice [72]. Their results showed that the body weight, weight gain, food intake, body fat percentage and epididymal fat in the emulsifier group significantly increased compared to those in the control group. Here we would like to draw the risk assessors' attention that the body weight gain was demonstrated by the relative body weight values which are percentages of the initial body weight for each animal.

The authors urge the reassessment of the safety of glyceryl monolaurate. We would like to make some brief remarks on this specific emulsifier. As we will see later, the legal denomination of an emulsifier can refer not to one specific chemical exclusively, but in several cases to a chemical group. The official name of the emulsifier in question is mono- and diglycerides of fatty acids (E 471). The commercial product is a mixture of mono-, di- and triglycerides and their exact content is not specified. So mono- and diglycerides of fatty acids can be glyceryl monolaurate and according to the European specification they are also synonyms [73]. Mono- and diglycerides of fatty acids are the most extensively used surfactant food emulsifiers permitted in numerous food categories (namely in 84 food categories [12]) including infant formula in 4000 mg/kg level. The main application area of monoglycerides is in bakery products (e.g. bread, sponge cakes) [60]. Consequently the dietary exposure is very high for this type of emulsifier.

In their subsequent studies Chassaing et al. [74,75] showed by a human microbiota model (M-SHIME) that polysorbate 80 and CMC directly alter microbiota, increasing its proinflammatory potential. It was also shown by colitis-associated colorectal cancer model that polysorbate 80 and CMC exacerbated tumor development [76]. This effect was associated with alteration of microbiota. These findings are of great importance to public health since colorectal cancer was the third most common cancer in 2018 according to WHO data [77]. We would also like to mention here that in real conditions, when carcinogenic contaminants are unavoidably present in foods, additional mechanisms can exacerbate tumor promoting potency of emulsifiers, namely by enhancing the absorption of carcinogens.

The complexity of the emulsifier effect on mucus gel was demonstrated by Lock et al. [78]. They studied the direct impact of emulsifiers (polysorbate and CMC) on mucus structure and on the diffusion of nanoparticles and *E. coli* through a mucus layer obtained from porcine small intestine. They found that the emulsifiers changed structural properties of mucus gel, and polysorbate increased *E. coli* speed in the mucus.

Another serious concern with food emulsifier consumption is connected with their effects on transport proteins (p-glycoprotein and BCRP). Is it possible that surfactant food emulsifiers significantly increase the absorption of a number of food contaminants by inhibiting the mentioned transport proteins? To find the answer to this question, one should look at the results of several *in vivo* studies recently published. Phthalates are environmental endocrine disruptor contaminants, which are substrates of p-glycoprotein. In the following studies food emulsifiers were investigated that had previously been proven to inhibit p-glycoprotein. Lu et al. demonstrated that polysorbate 80 significantly increased the absorption of di-(2-ethylhexyl) phthalate in rats [79]. In their subsequent studies the effect of glyceryl monostearate on the bioavailability of six phthalates by *in vivo* rat model was investigated and they also found significant absorption enhancing effect [80]. They have also shown a greater reduction in testosterone level and exacerbated damage

of testis and liver by co-administrating glyceryl monostearate in male rats [4,81].

In view of the above it can therefore be concluded that surfactant food emulsifiers can impact on intestinal barrier in various ways simultaneously in one direction, namely to disrupt intestinal barriers.

Now let us examine why these harmful agents are still present in our foods, namely review the risk assessment practice in Europe.

3. Safety evaluation of food emulsifiers

Each food additive, as part of the authorization procedure, must be assessed before they are placed on the European market. Before 2002 the risk assessments were carried out by the Scientific Committee on Food (SCF) in Europe, and then EFSA became responsible for it. The toxicological data requirement for authorization of a food additive is described in a guidance published in 2012 [82]. The original authorization processes of emulsifiers were carried out decades ago. Recently EFSA has re-evaluated most food emulsifiers according to a re-evaluation program. The mentioned guidance is also relevant for re-evaluations of food additives. The main difference between the original evaluation and the re-evaluation is that in the case of incumbent additives there is no single applicant to provide toxicological data. Instead, EFSA launches a public call for data relevant to safety assessment. In a re-evaluation process toxicity data from the original opinion and dossier, information submitted to the call for data and also the latest scientific publications must be considered.

The mentioned guidance introduces a tiered approach for toxicity testing requirements of food additives. For each additive testing of absorption, genotoxicity in vitro and extended 90-day toxicity data are required (Tier 1). If additives which are absorbed demonstrate toxicity or genotoxicity in Tier 1 tests, need to be tested to generate more extensive data (Tier 2). It has to be noted that Tier 1 and Tier 2 tests are unable to detect the impairments in barrier functions. These effects could be detected only by specific toxicity data. Specific toxicity data requirements are in Tier 3. However, according to the guidance, Tier 3 testing should be performed on a case-by-case basis when Tier 2 tests show that specific endpoints are needed.

How can we interpret this requirement? At first sight one might assume that the common absorption enhancer characteristic of surfactants gives sufficient reason for additional tests. One would also think that the actual usage of a certain emulsifier as absorption enhancer in pharmaceuticals also gives sufficient reason for additional tests. One would also think that the fact that independent in vivo scientific studies show that a certain emulsifier causes intestinal inflammation and colitis in genetically susceptible animals also gives sufficient reason for additional tests. Furthermore, the fact that independent scientific studies show that certain emulsifiers significantly increase the absorption of phthalates could easily give the impression that there is sufficient reason to require additional tests. However, these are false assumptions.

None of these conditions provide sufficient grounds for EFSA to require specific toxicity tests from industry. Specific toxicity tests will be required only if the general toxicity testing (Tier 1) from original application for authorization demonstrates its necessity. This explains why emulsifier re-evaluation reports connected with the effects on intestinal mucosa contain the following: *“even though some of these endpoints are not systematically included in toxicity studies performed according to toxicity testing guidelines, they would be investigated on a case by case basis if indicated by the results of the general toxicity testing as recommended in the guidance of the ANS Panel on food additive evaluation (EFSA, 2012)”* [11–19]. As there are no investigation requirements on intestinal permeabil-

ity or intestinal microbiota in Tier 1 it cannot be expected that the original toxicity studies will suggest these effects. Furthermore, most of the diseases related to impaired barrier function are multifactorial, namely there are more than one main pre-existing conditions that lead to their development. For example, in the case of autoimmune diseases, not only the increased intestinal permeability is the precondition, but also a genetic susceptibility and the presence of antigen in the lumen. As the original toxicity studies for applications were not carried out with genetically susceptible animals, it cannot be expected that these tests show any sign of inducing autoimmunity or Crohn's disease. As we have seen in the Chassaing study, colitis was not induced by emulsifier in wild type animals only exclusively in genetically predisposed knockout animals. These effects can only be demonstrated through specific toxicity testing.

It is of another serious concern that surfactants can significantly increase the absorption of food contaminants including potent carcinogens (e.g. PAHs) or endocrine disruptors (like phthalates). The potential co-carcinogenic or tumor promoter effects were not tested either in the original toxicity studies, as there are no toxicity study requirements with mixtures. Consequently, is it possible that effective co-carcinogens or tumor promoters are present in our foods on a daily basis? We cannot yet answer this question, since the opposite is not proven. Knowing that some food contaminants unavoidably present in foods and cancer is one of the leading causes of death in the developed world, more attention should be paid to this issue.

However, the situation described above shall not mean that risk assessors do not give particular attention to specific toxicity studies by the academic world. The mentioned re-evaluations indicate that additional studies would be needed for each emulsifier to show the relevance of recent studies. Similarly, in response to our submission [83] Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded in the evaluation of citric acid esters of mono- and diglycerides of fatty acids (CITREM) usage in infant formula that no in vivo studies were carried out with the special emulsifier, therefore it is not possible to conclude that CITREM itself will affect the intestinal barrier under in vivo conditions [84]. However, these uncertainties concerning possible harmful effects on intestinal barriers do not prevent risk assessors from concluding that there are no toxicological concerns about the use of these emulsifiers.

The mentioned effects on intestinal barriers of food emulsifiers came into view of EFSA in 2014 when the Emerging Risks Exchange Network of EFSA considered this topic as an emerging issue and the network suggested EFSA to monitor the scientific literature in connection with this topic [85].

In simple terms, risk assessors are awaiting scientific evidences from the academic world, but in the meantime those are considered to be safe.

Although, the re-evaluations of most food emulsifiers have been carried out by EFSA already, their safety should be reassessed once new scientific evidences show possible health concerns.

As long as the effects on intestinal barrier functions are not involved in the toxicological studies on the basis of which the ADI is determined, ADI is no guarantee as regards the safety exposure level of these additives.

4. Necessary studies: the way for solution

Taking into account the above, let us see what options we have to stop the rising occurrence of the mentioned diseases.

The European legislation states that a food additive may be included in the Community list if “it does not, on the basis of the scientific evidence available, pose a safety concern to the health of the consumer at the level of use proposed” [86].

In Europe EFSA is competent to decide whether or not there is safety concern. A food additive is removed from the Union list for safety reasons if EFSA is not able to confirm the safety of the additive (Precautionary Principle) or EFSA deems it unsafe.

As we have seen above, the original toxicological studies do not include endpoints connected with compromised intestinal barrier function or microbiota. These tests are unsuitable to reveal the emulsifiers' role in the development of the mentioned diseases. Whilst it is true that there are numerous very valuable scientific studies, most of the authorized emulsifiers have not been investigated yet at all to this aspect. It means that at this moment there are not enough scientific evidences to prove the barrier disrupting and disease promoting effects for each emulsifier. Although it is somewhat surprising, the final conclusions of the re-evaluation reports show that in the absence of necessary evidences, surfactant food emulsifiers are considered to be safe by risk assessors. However, they mention that additional studies would be needed to show the relevance of the studies that demonstrated the outlined effects of emulsifiers.

Consequently, surface active food additives will be present in foods in high quantities until new experimental data are available for all permitted emulsifiers.

On this basis the question may be raised: Who will carry out the tests that would be necessary for proper safety evaluation of food emulsifiers?

According to the official risk assessment approach discussed above, there is nobody to be obliged to carry out the necessary specific toxicity tests.

Meanwhile – possibly at least partly owing to the intensive usage of food emulsifiers – the incidence and prevalence of such devastating diseases as Crohn's or Type 1 diabetes rapidly rises [87]. It is no coincidence that the European Crohn's and Colitis Organisation (ECCO) urge testing the intestinal inflammation potential of food emulsifiers [88].

Thus, there is no other option than for the scientific world to carry out the necessary tests in order to stop the increase of incidences of the concerned diseases. It therefore seems that the burden of proof is not on producers but on the independent scientific community to provide new relevant scientific evidences to prove or disprove the harmfulness for each emulsifier for each mode of actions one by one.

As almost 30 different types of surfactant food emulsifiers (Table 1) are on the market, it requires a lot of experiments.

In addition, the situation is complicated by the fact that in numerous cases a food emulsifier's name and its E number can denote different materials. It means that there is a possibility that an emulsifier's name covers both a harmless and an intestinal disruptor food additive simultaneously. For example lecithins (E 322) denote both natural lecithins and their hydrolyzed derivatives. While natural lecithins can be even beneficial to intestinal health, their hydrolyzed derivatives (lysolecithins) having much higher HLB value can significantly increase the absorption of macromolecules [29]. Another example is sucrose esters of fatty acids whose composition can vary according to the ester composition and can have very different HLB values, and therefore different absorption enhancing activity [89].

In order to prioritize the testing of food emulsifiers, a number of aspects can be considered. It would be preferable to investigate emulsifiers for which dietary exposures are high or authorized in the most frequently consumed foods such as bread (e.g. sodium stearoyl-2-lactylate, DATEM, mono- and diglycerides of fatty acids) or infant formulas (CITREM, sucrose esters of fatty acids, mono- and diglycerides of fatty acids) and/or have high HLB values (see Table 1). Greater emphasis should be given to testing of emulsifiers permitted in infant formulas, as barrier dysfunction is a key factor in the pathogenesis of several early infancy autoimmune diseases

including necrotising enterocolitis and allergic gastro-enteropathy, and intestinal barrier dysfunction in infancy is also a risk factor for inflammatory and autoimmune diseases during adulthood [90]. It is particularly regrettable that water soluble surfactants are used in hypoallergenic infant formulas. This is because, these formulas contain partially and extensively hydrolyzed proteins leading to the absence of natural emulsifying macromolecules in these emulsions. That is why emulsifiers are needed to stabilize them. However, it also means that exactly those infants are exposed to water soluble surfactants who are genetically predisposed to allergy or hypersensitivity.

in vitro studies can be useful prioritizing methods which could also indicate the possible mode of actions (e.g. transcellular, paracellular, modulate p-glycoprotein or BCRP, altering microbiota, making the mucus layer penetrable). However, risk assessors do not take into account the results of *in vitro* tests as it has been stated in re-evaluation of polysorbates regarding the study (discussed in detail earlier in this review) demonstrating increased *E. coli* translocation through Caco2 cells [67]: “as this suggestion was based solely on *in vitro* results and *ex vivo* results, and its relevance to the *in vivo* situation remains unclear in the absence of relevant clinical studies, the Panel could not use the results of these studies for risk assessment.” [11]

In this manner, specific *in vivo* and/or clinical studies would be needed from the academic world for each emulsifier, for each mode of action in order to help risk assessors make adequate evaluations ruling out with certainty that authorized food emulsifiers pose a safety concern to the health of consumers.

With regard to the test methods, the academic world enjoys freedom of choice, as there is no official guidance for scientists to demonstrate the possible risk of a food additive. However, it is reasonable to assume that the Guidance for the industry by EFSA [82] will also guide us on what type of evidence risk assessors will accept. This Guidance describes that only animal studies are required to prove the safety of an additive even in Tier 3 level and generally no human studies are needed. On this basis, *in vivo* studies using experimental animals should be enough to show the potential risk, in other words, risk assessors should accept substantiated evidence from animal studies. Although, the polysorbates re-evaluation mentioned above says that *in the absence of relevant clinical studies, EFSA could not use the in vitro results in their risk assessment* [11], we consider it is highly unlikely that EFSA applies double standard between industry and independent scientists. This is all the more true given that demonstrating a serious health risk of an emulsifier can raise ethical questions. To give an example, look at a situation in which we want to highlight an emulsifier triggering Crohn's disease by human studies. As we have seen earlier, surfactants can increase intestinal permeability and alter microbiota to a greater extent in genetically predisposed people to Crohn's than in healthy ones [74,91]. Therefore, we believe that studies in healthy volunteers would not show colitis triggering potential of an emulsifier but only studies in Crohn's patients (or in genetically predisposed ones). However, such a study would likely constitute a serious risk to these subjects. Accordingly, it would contradict EFSA Guidance [82] namely “*Studies of food additives in humans should only be performed if there are adequate data from animal and other related studies to demonstrate the likely safety in humans.*”

Nevertheless, in some cases human studies might also provide useful information. Obviously, only the cases when they do not pose considerable risk to subjects (e.g. microbiota study in healthy volunteers or short term absorption enhancement study with model p-glycoprotein substrate contaminant with no acute toxicity). However, as described above, these studies are unlikely to demonstrate the most serious health concerns (e.g. triggering autoimmunity or promoting cancer). Therefore it is questionable

that the results of these studies provide sufficient grounds to force risk assessor to change their opinion.

For all these reasons, after the above mentioned prioritization, we propose to study the possible role of each priority food emulsifier in triggering the concerned serious diseases using knock-out animals (predispose to colitis or to type 1 diabetes or to other disease associated with impaired intestinal barrier function) similarly as Chassaing study [9]. As we described in 2.3 section it would be beneficial to clarify the supposed contradictions between body weight gain results of the Chassaing and NTP [68] studies.

Moreover, any other studies, either in animals or in humans, which scientifically prove that a food emulsifier disrupts intestinal barrier function and/or alters microbiota will assist assessors in more proper safety evaluation.

When sufficient evidences will be available demonstrating that a certain emulsifier poses a safety concern to the health of consumers EFSA will have to reassess its safety.

5. Conclusions

A number of scientific studies suggest that consumption of surfactant emulsifiers in foods promote numerous diseases (e.g. autoimmune diseases like type 1 diabetes, celiac disease, IBD) by impairing intestinal barrier function and altering intestinal microbiota. Moreover, they can increase the absorption of several environmental toxins including endocrine disruptors and carcinogens.

However, the toxicity tests of original applications for authorization process are unable to demonstrate these possible effects. The effects on intestinal barriers and microbiota can only be demonstrated by specific toxicity testing. As there is no such requirement on industry, risk assessors are awaiting these data from the academic world.

Declarations of interest

None.

Acknowledgements

We are grateful to Sándor Németh, Anita Maczó, Andrea Zentai and András Csáki for their valuable suggestions.

References

- [1] K.F. Csáki, Synthetic surfactant food additives can cause intestinal barrier dysfunction, *Med. Hypotheses* (2011), <http://dx.doi.org/10.1016/j.mehy.2011.01.030>.
- [2] A. Lerner, T. Matthias, Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease, *Autoimmun. Rev.* (2015), <http://dx.doi.org/10.1016/j.autrev.2015.01.009>.
- [3] A.W. Abu-Qare, E. Elmasry, M.B. Abou-Donia, A role for P-Glycoprotein in environmental toxicology, *J. Toxicol. Environ. Heal. - Part B Crit. Rev.* (2003), <http://dx.doi.org/10.1080/109374003006466>.
- [4] H.T. Gao, R. Xu, W.X. Cao, X. Zhou, Y.H.M. Yan, L. Lu, Q. Xu, Y. Shen, Food emulsifier glycerin monostearate increases internal exposure levels of six priority controlled phthalate esters and exacerbates their male reproductive toxicities in rats, *PLoS One* (2016), <http://dx.doi.org/10.1371/journal.pone.0161253>.
- [5] J. König, J. Wells, P.D. Cani, C.L. García-Ródenas, T. MacDonald, A. Mercenier, J. Whyte, F. Troost, R.J. Brummer, Human intestinal barrier function in health and disease, *Clin. Transl. Gastroenterol.* (2016), <http://dx.doi.org/10.1038/ctg.2016.54>.
- [6] E. Toutou, B.W. Barry (Eds.), *Enhancement in Drug Delivery*, Taylor and Francis Group, Boca Raton, 2007.
- [7] A. Lugea, A. Salas, J. Casalot, F. Guarner, J.R. Malagelada, Surface hydrophobicity of the rat colonic mucosa is a defensive barrier against macromolecules and toxins, *Gut* (2000), <http://dx.doi.org/10.1136/gut.46.4.515>.
- [8] A. Sood, R. Panchagnula, Peroral route: an opportunity for protein and peptide drug delivery, *Chem. Rev.* (2001), <http://dx.doi.org/10.1021/cr000700m>.
- [9] B. Chassaing, O. Koren, J.K. Goodrich, A.C. Poole, S. Srinivasan, R.E. Ley, A.T. Gewirtz, Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome, *Nature* (2015), <http://dx.doi.org/10.1038/nature14232>.
- [10] F. McCartney, J.P. Gleeson, D.J. Brayden, Safety concerns over the use of intestinal permeation enhancers: a mini-review, *Tissue Barriers* (2016), <http://dx.doi.org/10.1080/21688370.2016.1176822>.
- [11] EFSA ANS Panel, Scientific Opinion on the re-evaluation of polyoxyethylene sorbitan monolaurate (E 432), polyoxyethylene sorbitan monooleate (E 433), polyoxyethylene sorbitan monopalmitate (E 434), polyoxyethylene sorbitan monostearate (E 435) and polyoxyethylene sorbita, *EFSA J.* 13 (2015) 4152, <http://dx.doi.org/10.2903/j.efsa.2015.4152>.
- [12] EFSA ANS Panel, Scientific opinion on the re-evaluation of mono- and di-glycerides of fatty acids (E 471) as food additives, *EFSA J.* 15 (2017) 5045, <http://dx.doi.org/10.2903/j.efsa.2017.5045>.
- [13] EFSA ANS Panel, Scientific Opinion on the re-evaluation of sucrose acetate isobutyrate (E 444) as a food additive, *EFSA J.* 14 (2016) 4489, <http://dx.doi.org/10.2903/j.efsa.2016.4489>.
- [14] EFSA ANS Panel, Re-evaluation of polyglycerol polyricinoleate (E 476) as a food additive, *EFSA J.* (2017), <http://dx.doi.org/10.2903/j.efsa.2017.4743>.
- [15] EFSA ANS Panel, Scientific Opinion on the re-evaluation of polyglycerol esters of fatty acids (E 475) as a food additive, *EFSA J.* 15 (2017) 5089, <http://dx.doi.org/10.2903/j.efsa.2017.5089>.
- [16] EFSA ANS Panel, Scientific Opinion on the re-evaluation of sorbitan monostearate (E 491), sorbitan tristearate (E 492), sorbitan monolaurate (E 493), sorbitan monooleate (E 494) and sorbitan monopalmitate (E 495) when used as food additives, *EFSA J.* 15 (2017) 4788, <http://dx.doi.org/10.2903/j.efsa.2017.4788>.
- [17] EFSA ANS Panel, Re-evaluation of oxidised soya bean oil interacted with mono- and diglycerides of fatty acids (E 479b) as a food additive, *EFSA J.* 16 (2018) 5420, <http://dx.doi.org/10.2903/j.efsa.2018.5420>.
- [18] EFSA ANS Panel, Re-evaluation of sodium, potassium and calcium salts of fatty acids (E 470a) and magnesium salts of fatty acids (E 470b) as food additives, *EFSA J.* 16 (2018) 5180, <http://dx.doi.org/10.2903/j.efsa.2018.5180>.
- [19] EFSA ANS Panel, Re-evaluation of propane-1,2-diol esters of fatty acids (E 477) as a food additive, *EFSA J.* 16 (2018) 5497, <http://dx.doi.org/10.2903/j.efsa.2018.5497>.
- [20] T.W. Wong, D.S. Juras, R.W. Wissler, Effect of concurrent feeding of tween 80 on the carcinogenicity of orally administered 3-methylcholanthrene, *J. Natl. Cancer Inst.* (1959), <http://dx.doi.org/10.1093/jnci/22.2.363>.
- [21] M. Takahashi, S. Fukushima, S.H. Carcinogenic effect of N-methyl-N'-nitro-N-nitrosoguanidine with various kinds of surfactant in the glandular stomach of rats, *Gan* 64 (1973) 211, <http://dx.doi.org/10.20772/cancersci1959.64.3.211>.
- [22] S. Fukushima, M. Tatematsu, M. Takahashi, Combined effect of various surfactants on gastric carcinogenesis in rats treated with N-methyl-N'-nitro-N-nitrosoguanidine, *Gan* 65 (1974) 371, <http://dx.doi.org/10.20772/cancersci1959.65.4.371>.
- [23] S. Miika Takahashi, Fukushima M. Hananouchi, Induction of undifferentiated adenocarcinoma in the stomach of rats by N methyl N' nitro N nitrosoguanidine with various kinds of surfactant, *Gan* 17 (1975) 255.
- [24] C. Tagesson, C. Edling, Influence of surface-active food additives on the integrity and permeability of rat intestinal mucosa, *Food Chem. Toxicol.* (1984), [http://dx.doi.org/10.1016/0278-6915\(84\)90165-0](http://dx.doi.org/10.1016/0278-6915(84)90165-0).
- [25] T. Alama, H. Katayama, S. Hirai, S. Ono, A. Kajiyama, K. Kusamori, H. Katsumi, T. Sakane, A. Yamamoto, Enhanced oral delivery of alendronate by sucrose fatty acids esters in rats and their absorption-enhancing mechanisms, *Int. J. Pharm.* (2016), <http://dx.doi.org/10.1016/j.ijpharm.2016.10.046>.
- [26] A. Yamamoto, H. Katsumi, K. Kusamori, T. Sakane, Improvement of intestinal absorption of poorly absorbable drugs by various sugar esters, *Yakugaku Zasshi* (2014), <http://dx.doi.org/10.1248/yakushi.13-00221-1>.
- [27] H. Onishi, Y. Imura, M. Uchida, Y. Machida, Enhancement potential of sucrose laurate (L-1695) on intestinal absorption of water-soluble high molecular weight compounds, *Curr. Drug Deliv.* (2012), <http://dx.doi.org/10.2174/156720112802650699>.
- [28] S. Maher, R.J. Mrsny, D.J. Brayden, Intestinal permeation enhancers for oral peptide delivery, *Adv. Drug Deliv. Rev.* (2016), <http://dx.doi.org/10.1016/j.addr.2016.06.005>.
- [29] E. Scott Swenson, W.J. Curatolo, (C) means to enhance penetration. (2) Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity, *Adv. Drug Deliv. Rev.* (1992), [http://dx.doi.org/10.1016/0169-409X\(92\)90015-1](http://dx.doi.org/10.1016/0169-409X(92)90015-1).
- [30] E. Toutou, M. Donbrow, Promoted rectal absorption of insulin: formulative parameters involved in the absorption from hydrophilic bases, *Int. J. Pharm.* (1983), [http://dx.doi.org/10.1016/0378-5173\(83\)90063-7](http://dx.doi.org/10.1016/0378-5173(83)90063-7).
- [31] Z. Ujhelyi, F. Fenyvesi, J. Váradi, P. Fehér, T. Kiss, S. Veszélka, M. Deli, M. Vecsernyés, I. Bácskay, Evaluation of cytotoxicity of surfactants used in self-micro emulsifying drug delivery systems and their effects on paracellular transport in Caco-2 cell monolayer, *Eur. J. Pharm. Sci.* (2012), <http://dx.doi.org/10.1016/j.ejps.2012.07.005>.
- [32] T. Yamagata, H. Kusuhara, M. Morishita, K. Takayama, H. Benameur, Y. Sugiyama, Improvement of the oral drug absorption of topotecan through the inhibition of intestinal xenobiotic efflux transporter, breast cancer resistance protein, by excipients, *Drug Metab. Dispos.* (2007), <http://dx.doi.org/10.1124/dmd.106.014217>.

- [33] A.A.A. Al-Ali, J.R.C. Quach, C. Bundgaard, B. Steffansen, R. Holm, C.U. Nielsen, Polysorbate 20 alters the oral bioavailability of etoposide in wild type and mdr1a deficient Sprague-Dawley rats, *Int. J. Pharm.* (2018), <http://dx.doi.org/10.1016/j.ijpharm.2018.04.006>.
- [34] N. Takaiishi, H. Satsu, M. Shimizu, Enhanced daunomycin accumulation in human intestinal Caco-2 cells from non-ionic food emulsifiers unrelated to the p-glycoprotein inhibitory mechanism, *Biosci. Biotechnol. Biochem.* (2006), <http://dx.doi.org/10.1271/bbb.60306>.
- [35] R.L. Oberle, T.J. Moore, D.A.P. Krummel, Evaluation of mucosal damage of surfactants in rat jejunum and colon, *J. Pharmacol. Toxicol. Methods* (1995), [http://dx.doi.org/10.1016/1056-8719\(94\)00060-H](http://dx.doi.org/10.1016/1056-8719(94)00060-H).
- [36] A. Bernkop-Schnürch, C. Valenta, S.M. Daege, Peroral polypeptide delivery. A comparative in vitro study of mucolytic agents, *Arzneimittel-Forschung/Drug Res.* 49 (1999) 799–803, <http://dx.doi.org/10.1055/s-0031-1300504>.
- [37] S.C. Bischoff, G. Barbara, W. Buurman, T. Ockhuizen, J.D. Schulzke, M. Serino, H. Tilg, A. Watson, J.M. Wells, Intestinal permeability - a new target for disease prevention and therapy, *BMC Gastroenterol.* (2014), <http://dx.doi.org/10.1186/s12876-014-0189-7>.
- [38] C.A. Barta, K. Sachs-Barrable, F. Feng, K.M. Wasan, Effects of monoglycerides on P-glycoprotein: modulation of the activity and expression in Caco-2 cell monolayers, *Mol. Pharm.* (2008), <http://dx.doi.org/10.1021/mp800050q>.
- [39] H. Thakkar, J. Desai, Influence of excipients on drug absorption via modulation of intestinal transporters activity, *Asian J. Pharm.* (2015), <http://dx.doi.org/10.4103/0973-8398.154688>.
- [40] S. Zhu, R. Huang, M. Hong, Y. Jiang, Z. Hu, C. Liu, Y. Pei, Effects of polyoxyethylene (40) stearate on the activity of P-glycoprotein and cytochrome P450, *Eur. J. Pharm. Sci.* (2009), <http://dx.doi.org/10.1016/j.ejps.2009.05.001>.
- [41] T. Yamagata, H. Kusuha, M. Morishita, K. Takayama, H. Benamer, Y. Sugiyama, Effect of excipients on breast cancer resistance protein substrate uptake activity, *J. Control. Release* (2007), <http://dx.doi.org/10.1016/j.jconrel.2007.08.021>.
- [42] A.E. van Herwaarden, E. Wagenaar, B. Karnekamp, G. Merino, J.W. Jonker, A.H. Schinkel, Breast cancer resistance protein (Bcrp1/Abcg2) reduces systemic exposure of the dietary carcinogens aflatoxin B1, IQ and Trp-P-1 but also mediates their secretion into breast milk, *Carcinogenesis* 27 (2005) 123–130, <http://dx.doi.org/10.1093/carcin/bgi176>.
- [43] C.L. Roberts, S.L. Rushworth, E. Richman, J.M. Rhodes, Hypothesis: increased consumption of emulsifiers as an explanation for the rising incidence of Crohn's disease, *J. Crohns Colitis* (2013), <http://dx.doi.org/10.1016/j.crohns.2013.01.004>.
- [44] D. Hollander, Crohn's disease - a permeability disorder of the tight junction? *Gut* 29 (1988) 1621 <https://gut.bmj.com/content/29/12/1621.long>.
- [45] A. Michielan, R. D'Inca, Intestinal permeability in inflammatory bowel disease: pathogenesis, clinical evaluation, and therapy of leaky gut, *Mediators Inflamm.* (2015), <http://dx.doi.org/10.1155/2015/628157>, Article ID 628157.
- [46] Q. Mu, J. Kirby, C. Reilly, X. Luo, Leaky Gut as a danger signal for autoimmune diseases, *Front. Immunol.* 8 (2017) 598, <http://dx.doi.org/10.3389/fimmu.2017.00598>.
- [47] M.C. Arrieta, L. Bistritz, J.B. Meddings, Alterations in intestinal permeability, *Gut* 55 (2006) 1512, <http://dx.doi.org/10.1136/gut.2005.085373>.
- [48] A. Fasano, T. Shea-Donohue, Mechanisms of Disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases, *Nat. Clin. Pract. Gastroenterol. Hepatol.* 2 (2005) 416, <http://dx.doi.org/10.1038/ncpgasthep0259>.
- [49] T. Watts, I. Berti, A. Sapone, T. Gerarduzzi, T. Not, R. Zielke, A. Fasano, Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats, *PNAS.* 102 (2005) 2916, <http://dx.doi.org/10.1073/pnas.0500178102>.
- [50] A. Fasano, R. Troncone, D. Branski (Eds.), *Frontiers in Celiac Disease*, Pediatric, KARGER, 2008, <http://dx.doi.org/10.1159/isbn.978-3-8055-8527-9>.
- [51] R. D'Inca, V. Di Leo, G. Corrao, D. Martines, A. D'Odorico, C. Mestriner, C. Venturi, G. Longo, G. Sturmiolo, Intestinal permeability test As a predictor of clinical course in Crohn's disease, *Am. J. Gastroenterol.* 94 (1999) 2956–2960, <http://dx.doi.org/10.1111/j.1572-0241.1999.01444.x>.
- [52] J. Wyatt, H. Vogelsang, W. Hübl, T. Waldhöer, H. Lochs, Intestinal permeability and the prediction of relapse in Crohn's disease, *Lancet* 341 (1993) 1437–1439, [http://dx.doi.org/10.1016/0140-6736\(93\)90882-H](http://dx.doi.org/10.1016/0140-6736(93)90882-H).
- [53] J. Tibble, G. Sighthorsson, S. Bridger, M. Fagerhol, I. Bjarnason, Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease, *Gastroenterology* 119 (2000) 15–22, <http://dx.doi.org/10.1053/gast.2000.8523>.
- [54] I. Pierantonelli, G. Svegliati-Baroni, Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH, *Transplantation* 103 (2019) 1–13, <http://dx.doi.org/10.1097/TP.0000000000002480>.
- [55] P. Kwang-Il, K. Kwang-Youn, W.O. Tae, K. Du-Seock, K. Eung-Kyun, R.Y. Yong, S. Young-Kyo, M. Jin-Yeul, S. Pann-Ghill, Phospholipase C γ 1 links inflammation and tumorigenesis in colitis-associated cancer, *Oncotarget* 9 (2019) 5752–5763, <http://dx.doi.org/10.18632/oncotarget.23430>.
- [56] V. Stadlbauer, B. Leber, S. Lemesch, S. Trajanoski, M. Bashir, A. Horvath, M. Tawdrous, T. Stojakovic, G. Fauler, P. Fickert, C. Högenauer, I. Klymiuk, P. Stiegler, M. Lamprecht, T. Pieber, N. Tripolt, H. Sourij, Lactobacillus casei shirota supplementation does not restore gut microbiota composition and gut barrier in metabolic syndrome: a randomized pilot study, *PLoS One* 10 (2015), e0141399, <http://dx.doi.org/10.1371/journal.pone.0141399>.
- [57] M. Vazquez-Roque, M. Camilleri, T. Smyrk, J. Murray, J. O'Neill, P. Carlson, J. Lamsam, D. Eckert, D. Janzow, D. Burton, M. Ryks, D. Rhoten, A. Zinsmeister, Association of HLA-DQ gene with bowel transit, barrier function, and inflammation in irritable bowel syndrome with diarrhea, *Am. J. Physiol. Gastrointest. Liver Physiol.* 303 (2012) 1262–1269, <http://dx.doi.org/10.1152/ajpgi.00294.2012>.
- [58] C. Schmidt, *Thinking from the gut*, *Nature* 518 (2015) S12–S15.
- [59] B. Wang, M. Yao, L. Lv, Z. Ling, L. Lanjuan, The human microbiota in health and disease, *Engineering* 3 (2017) 71–82, <http://dx.doi.org/10.1016/j.ENG.2017.01.008>.
- [60] V. Norn, *Emulsifiers in Food Technology*, second edition, 2015, <http://dx.doi.org/10.1002/9781118921265>.
- [61] N.S. Neta, J.A. Teixeira, L.R. Rodrigues, Sugar ester surfactants: enzymatic synthesis and applications in food industry, *Crit. Rev. Food Sci. Nutr.* (2015), <http://dx.doi.org/10.1080/10408398.2012.667461>.
- [62] EFSA ANS Panel, Scientific Opinion on the refined exposure assessment of sucrose esters of fatty acids (E 473) from its use as a food additive, *EFSA J.* 16 (2018) 5087, <http://dx.doi.org/10.2903/j.efsa.2018.5087>.
- [63] M.E. Meezan E, D.J. Pillion, *Absorption Enhancers for Drug Administration*, 2006, doi:US 2006/0045869 A1.
- [64] Y. Mine, J.W. Zhang, Surfactants enhance the tight-junction permeability of food allergens in human intestinal epithelial Caco-2 cells, *Int. Arch. Allergy Immunol.* (2003), <http://dx.doi.org/10.1159/000069009>.
- [65] A. Glynn, A.M. Igra, S. Sand, N.G. Ibbäck, K.E. Hellenäs, J. Rosén, B. Aspenström-Fagerlund, Are additive effects of dietary surfactants on intestinal tight junction integrity an overlooked human health risk? – a mixture study on Caco-2 monolayers, *Food Chem. Toxicol.* (2017), <http://dx.doi.org/10.1016/j.fct.2017.05.068>.
- [66] T. Weangsripanaval, T. Moriyama, T. Kageura, T. Ogawa, T. Kawada, Dietary fat and an exogenous emulsifier increase the gastrointestinal absorption of a major soybean allergen, gly m bd 30K, in mice, *J. Nutr.* (2005).
- [67] C.L. Roberts, A.V. Keita, S.H. Duncan, N. O'Kennedy, J.D. Söderholm, J.M. Rhodes, B.J. Campbell, Translocation of Crohn's disease *Escherichia coli* across M-cells: contrasting effects of soluble plant fibres and emulsifiers, *Gut* (2010), <http://dx.doi.org/10.1136/gut.2009.195370>.
- [68] National Toxicology Program, Toxicology and carcinogenesis studies of polysorbate 80 in F344/N rats and B6C3F1 mice, *J. Chem. Inf. Model.* (1992), <http://dx.doi.org/10.1017/CBO9781107415324.004>.
- [69] A.T.G.B. Chassaing, O. Koren, J.K. Goodrich, A.C. Poole, S. Srinivasan, R.E. Ley, Corrigendum: dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome, *Nature* 536 (2016) 238, <http://dx.doi.org/10.1038/nature18000>.
- [70] V. Delorme, R. Dhoub, S. Canaan, F. Fotiadu, F. Carrière, J.F. Cavalier, Effects of surfactants on lipase structure, activity, and inhibition, *Pharm. Res.* (2011), <http://dx.doi.org/10.1007/s11095-010-0362-9>.
- [71] C.A. Merian Nassra, Christine Bourgeois, Muriel Subirade, Patrick Sauvart, Oral administration of lipid oil-in-water emulsions performed with synthetic or protein-type emulsifiers differentially affects post-prandial triacylglycerolemia in rats, *J. Physiol. Biochem.* (2018), <http://dx.doi.org/10.1007/s13105-018-0634-0>.
- [72] Z. Jiang, M. Zhao, H. Zhang, Y. Li, M. Liu, F. Feng, Antimicrobial emulsifier-Glycerol monolaurate induces metabolic syndrome, gut microbiota dysbiosis, and systemic low-grade inflammation in low-fat diet fed mice, *Mol. Nutr. Food Res.* (2018), <http://dx.doi.org/10.1002/mnfr.201700547>.
- [73] 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, 2019 (n.d.) <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02012R0231-20180812&qid=1539973633300&from=HU>.
- [74] B. Chassaing, T. Van De Wiele, J. De Bodt, M. Marzorati, A.T. Gewirtz, Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation, *Gut* (2017), <http://dx.doi.org/10.1136/gutjnl-2016-313099>.
- [75] E. Viennois, B. Chassaing, First victim, later aggressor: How the intestinal microbiota drives the pro-inflammatory effects of dietary emulsifiers? *Gut Microbes* (2018), <http://dx.doi.org/10.1080/19490976.2017.1421885>.
- [76] E. Viennois, D. Merlin, A.T. Gewirtz, B. Chassaing, Dietary emulsifier-induced low-grade inflammation promotes colon carcinogenesis, *Cancer Res.* (2017), <http://dx.doi.org/10.1158/0008-5472.CAN-16-1359>.
- [77] WHO IARC, *Cancer Fact Sheets* (n.d.), 2019 <http://gco.iarc.fr/today/fact-sheets-cancers>.
- [78] J.Y. Lock, T.L. Carlson, C.M. Wang, A. Chen, R.L. Carrier, Acute exposure to commonly ingested emulsifiers alters intestinal mucus structure and transport properties, *Sci. Rep.* (2018), <http://dx.doi.org/10.1038/s41598-018-27957-2>.
- [79] Y. Lu, Y.Y. Wang, N. Yang, D. Zhang, F.Y. Zhang, H.T. Gao, W.T. Rong, S.Q. Yu, Q. Xu, Food emulsifier polysorbate 80 increases intestinal absorption of Di-(2-Ethylhexyl) phthalate in rats, *Toxicol. Sci.* (2014), <http://dx.doi.org/10.1093/toxsci/kfu055>.
- [80] R. Xu, H.T. Gao, F. Zhu, W.X. Cao, Y.H.M. Yan, X. Zhou, Q. Xu, W.L. Ji, SPE-UPLC-MS/MS for the determination of phthalate monoesters in rats urine and its application to study the effects of food emulsifier on the bioavailability of priority controlling PAEs, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* (2016), <http://dx.doi.org/10.1016/j.jchromb.2016.01.007>.

- [81] H.T. Gao, R. Xu, W.X. Cao, Q.N. Di, R.X. Li, L. Lu, Q. Xu, S.Q. Yu, Combined effects of simultaneous exposure to six phthalates and emulsifier glycerol monostearate on male reproductive system in rats, *Toxicol. Appl. Pharmacol.* (2018), <http://dx.doi.org/10.1016/j.taap.2018.01.013>.
- [82] EFSA ANS Panel, Guidance for submission for food additive evaluations, *EFSA J.* 10 (2012) 2760, <http://dx.doi.org/10.2903/j.efsa.2012.2760>.
- [83] National Food Chain Safety Office, Safety Concerns of Citric Acid Esters on Mono- and Diglycerides Usage in Infant Formula and Formulas for Special Medical Purposes Intended for Infants, 2014, Submitted to WHO, https://apps.who.int/iris/bitstream/handle/10665/171781/9789240693982_eng.pdf;jsessionid=75D91B0CB23B6252820AA69F85BF1FE9?sequence=3.
- [84] JECFA (Joint FAO/WHO Expert Committee on Food Additives), *Safety Evaluation of Certain Food Additives, 2015, WHO Food Addit. Ser. 70*.
- [85] EFSA, Annual Report of the Emerging Risks Exchange Network 2014, 2015, <http://dx.doi.org/10.2903/sp.efsa.2015.EN-839>.
- [86] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives, 2008 <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02008R1333-20180812&qid=1539977860253&from=HU>.
- [87] A. Lerner, P. Jeremias, T. Matthias, The world incidence and prevalence of autoimmune diseases is increasing, *Int. J. Celiac Dis.* (2016), <http://dx.doi.org/10.12691/ijcd-3-4-8>.
- [88] R. Sigall-Boneh, A. Levine, M. Lomer, N. Wierdsma, P. Allan, G. Fiorino, S. Gatti, D. Jonkers, J. Kierkus, K.H. Katsanos, S. Melgar, E.S. Yuksel, K. Whelan, E. Wine, K. Gerasimidis, Research gaps in diet and nutrition in inflammatory bowel disease. A topical review by D-ECCO working group [Dietitians of ECCO], *J. Crohns Colitis* (2017), <http://dx.doi.org/10.1093/ecco-jcc/jjx109>.
- [89] S. Maher, J. Heade, F. McCartney, S. Waters, S.B. Bleiel, D.J. Brayden, Effects of surfactant-based permeation enhancers on mannitol permeability, histology, and electrogenic ion transport responses in excised rat colonic mucosae, *Int. J. Pharm.* (2018), <http://dx.doi.org/10.1016/j.ijpharm.2018.01.008>.
- [90] R.C. Anderson, J.E. Dalziel, P.K. Gopal, The role of intestinal barrier function in early life in the development of colitis, *Intechopen*. (2009), <http://dx.doi.org/10.5772/25753>.
- [91] J. Söderholm, G. Olaison, K. Peterson, L. Franzén, T. Lindmark, M. Wirén, C. Tagesson, R. Sjö Dahl, Augmented increase in tight junction permeability by luminal stimuli in the non-inflamed ileum of Crohn's, *Gut* 50 (2002) 307–313.
- [92] Jim Smith, *Food Additive User's Handbook*, Springer, 1991.