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**Food additive emulsifiers and their impact on gut microbiome, permeability and inflammation: mechanistic insights in inflammatory bowel disease**

**Aaron S Bancel<sup>1</sup>, Alicia M Sandall<sup>1</sup>, Megan Rossi<sup>1</sup>, Benoit Chassaing<sup>2,3,4</sup>, James O Lindsay<sup>5</sup>, Kevin Whelan<sup>1</sup>**

1. King's College London, Department of Nutritional Sciences, London, UK
2. INSERM U1016, team "*Mucosal microbiota in chronic inflammatory diseases*", CNRS UMR 8104, Université de Paris, Paris, France
3. Neuroscience Institute, Georgia State University, Atlanta, GA, USA
4. Institute for Biomedical Sciences, Center for Inflammation, Immunity and Infection, Digestive Disease Research Group, Georgia State University, Atlanta, GA, USA
5. Queen Mary University of London, Blizard Institute, Barts and the London School of Medicine, London, UK

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**Address for correspondence:**

Professor Kevin Whelan, King's College London, Department of Nutritional Sciences, 150 Stamford Street, London, SE1 9NH, United Kingdom

Email [kevin.whelan@kcl.ac.uk](mailto:kevin.whelan@kcl.ac.uk)

Phone +44 (0)207 848 3858

**Abstract**

The global burden of inflammatory bowel disease (IBD) has increased over the 21<sup>st</sup> century. Despite multiple studies investigating the pathogenesis of IBD, the causative mechanisms pertaining to the increased prevalence remain unclear. There is growing evidence that aspects of a ‘Western diet’ increase the risk of developing IBD. More recently, evidence implicating dietary emulsifiers has accumulated, with ecological studies showing a positive correlation with inflammatory bowel disease and emulsifier consumption. Further to these, cell and animal studies have demonstrated plausible mechanisms by which dietary emulsifiers may contribute to IBD pathogenesis through mechanisms including: promotion of pro-inflammatory intestinal microbiota; disruption of mucus architecture; increased intestinal permeability; activation of inflammatory pathways and disruption of the cell cycle. This review critically analyses the current evidence for these mechanisms that may be of pathological relevance to IBD, evaluates recent dietary trials, acknowledges the challenges of dietary intervention studies and gives an overview of ongoing and future clinical trials in this important area.

**Keywords**

Emulsifiers, inflammation, colitis

## **Introduction**

The global increase in inflammatory bowel disease (IBD) over the 21<sup>st</sup> century suggest that environmental exposure and lifestyle factors play an important role in disease pathogenesis.<sup>1</sup> Diet is one such modifiable risk factor implicated in IBD aetiology and disease course.<sup>2</sup> Several epidemiological studies have found associations between aspects of a 'Western diet' and the risk of developing IBD, including higher intakes of processed meats, fast food, high fat intake and conversely lower intakes of fruits, vegetables and fibre.<sup>3-5</sup> A dietary component previously overlooked in the aetiology of IBD are food-additive emulsifiers, which are a major addition to human diet during the 21<sup>st</sup> century. An ecological analysis using data at the country level has shown positive correlations between emulsifier consumption and Crohn's disease incidence in Europe, North America and Japan.<sup>6</sup>

Emulsifiers comprise over 60 different food-additives that form or maintain a uniform emulsion of two or more phases in a food, and are used to stabilise the consistency of food products and prevent an unappetising separation of oil and water.<sup>7</sup> They act as surfactants, with the fat molecules in food adsorbing to the hydrophobic end of the emulsifiers and water adsorbing to the hydrophilic end.<sup>8</sup> Emulsifiers have many applications in processed foods including stabilising emulsions, lengthening shelf life by preventing separation during storage and as gelling agents used as a vegetarian alternative to gelatine.<sup>9,10</sup> Food additive emulsifiers therefore optimise a food's organoleptic properties, including appearance, texture and mouthfeel, explaining why they have become a ubiquitous component of the diet in economically developed countries.<sup>6</sup>

The major food additive emulsifiers include lecithin (E322) which is found in many forms of chocolate, mono- and diglycerides of fatty acids (E471) found in many ice-creams and frozen yoghurts, guar gum (E412) found in some dairy and soy-based products, xanthan gum (E415) found in mayonnaise and sandwich fillers, carrageenan (E407) found in flavoured milks, iced coffee and dairy-based ice cream and frozen desserts, celluloses including carboxymethylcellulose (E460-E469) found in vitamin and dietary supplements and polysorbates (E432-436) found in edible oils, ice cream, cake mixes, icing and chocolate syrup.<sup>8,11</sup>

Several mechanistic studies have examined the potential of emulsifiers to induce intestinal inflammation in models of IBD. There are naturally occurring emulsifiers (e.g. lecithin in eggs) but the main research on the inflammatory effects of emulsifiers relates to food additive emulsifiers. Thus far, the main emulsifiers studied for their deleterious effects on the gut include Polysorbate-60 (P60), Polysorbate-80 (P80), Carrageenan and Carboxymethylcellulose (CMC) (Figure 1). Although the structures of emulsifier molecules vary greatly, they perform the same function due to having both hydrophobic and hydrophilic properties.

Numerous *in vitro* studies (Table 1) and *in vivo* animal models (Table 2), implicate a role for food additive emulsifiers in intestinal inflammation via their impact on intestinal microbiota, permeability and inflammation (summarised in Figure 2). This review synthesizes the existing research linking emulsifiers to intestinal inflammation and discusses the translation of this evidence to human IBD. Limitations of the current evidence are discussed, and priorities for future research are highlighted.

### **Alterations in gut microbiota**

The impact of emulsifiers on the microbiota has been identified as a central mechanism in their ability to drive inflammation. For example, C57BL/6 mice fed glycerol monolaurate, a commonly used food emulsifier, developed a gut dysbiosis and decrease in  $\beta$ -diversity. Glycerol monolaurate ingestion led to significant decreases in the anti-inflammatory genera *Akkermansia* and *Lupinus*, and an increase in the genera *Escherichia*, *Roseburia*, *Bradyrhizobium* and *Turicibacter*. Serum lipopolysaccharide (LPS) levels were 61.1 times higher than the control group, suggesting an accompanying systemic inflammatory response.<sup>33</sup>

Likewise, addition of the emulsifier P80 aggravated indomethacin-induced ileitis and histological injury in C57BL/6 mice when compared to mice fed indomethacin alone. In addition, P80 resulted in altered small intestinal microbiota composition, with an increase in sulphide-producing *Enterobacteriaceae* and enhanced 'swarming' behaviour of *Proteus mirabilis*.<sup>35</sup> Swarming is a cyclical multicellular behaviour in which vegetative bacteria differentiate into hyperflagellated filamentous swarm cells capable of coordinated and rapid population migration, therefore enhancing their motility and ability to colonise the epithelium.<sup>37</sup>

CMC may impact the microbiota through other mechanisms. Interleukin-10 knockout (IL10<sup>-/-</sup>) mice given 100  $\mu$ L of 2% CMC solution developed bacterial overgrowth, with spaces between villi distended, bacteria more adherent to mucosa, as well as migration of bacteria to the crypts of Lieberkühn.<sup>28</sup> In a pivotal study, the commonly used emulsifiers P80 and CMC were fed to wild-type, IL10<sup>-/-</sup> and toll-like receptor-5 knockout (TLR5<sup>-/-</sup>) mice. Both CMC and P80 dramatically altered faecal and mucosa-

adherent microbiota composition in all three mouse models. In IL10<sup>-/-</sup> mice, both CMC and P80 induced a decrease in  $\alpha$ -diversity, reduced microbiota stability, led to a bloom in the Verrucomicrobia phyla *Akkermansia muciniphilia* and mucosa-associated, inflammation-promoting Proteobacteria as well as increased bacterial adherence. Emulsifier exposure increased faecal concentrations of bioactive LPS and flagellin as well as circulating antibodies directed toward these bacterial components, suggesting greater exposure of the immune system to bacterial motifs. In order to confirm these changes were a consequence of emulsifier exposure, rather than simply reflecting a shared cage and environment, multiple litters were split into equal groups fed water, P80 and CMC, an approach confirming that microbiota changes occurred irrespective of cage clustering.<sup>29</sup>

### **Reduced mucus thickness and increased bacterial encroachment**

Mucus-preserving Carnoy fixation and subsequent confocal microscopy demonstrated that when P80 (1.0% v/v) and CMC (1.0% w/v) were administered via drinking water for 12 weeks to wild-type and IL10<sup>-/-</sup> mice, mucus layer thickness was reduced to such an extent that some bacteria were geographically encroaching the epithelium, whilst the distance between bacteria and epithelial cells was more than 50% lower compared to placebo. Emulsifier-induced thinning of mucus did not occur in germ-free mice nor was there a change in mucus penetrability, as assessed by gavage with fluorescent beads that were a similar size to bacteria; therefore, demonstrating that changes in mucus via emulsifiers is driven by changes in microbiota composition and function resulting in bacteria penetrating the normally sterile mucus layer.<sup>29</sup> Interestingly, there was no change in expression of the MUC2 gene encoding mucin, the glycoprotein that is the major component of colonic mucus, suggesting that

emulsifiers have an indirect action on mucus function rather than a direct impact on mucin production.<sup>29,38</sup>

Further studies that report an impact of emulsifier exposure on the mucus include a porcine intestinal mucus model in which CMC exposure resulted in thinning of the mucus layer as assessed by fluorescent nanoparticle tracking and scanning electron microscopy. In the same model, P80 induced clumping of the fibres and slightly smaller pores in the mucus, suggesting increased viscosity. The researchers tracked the speed of movement of *Escherichia coli* through the emulsifier-treated mucus model. CMC slowed *E.coli* speed by 62% when exposed to mucus, possibly due to *E. coli* entanglement within the polymer network of CMC itself. In contrast, P80 accelerated *E.coli* movement by 10% within mucus, demonstrating that both emulsifiers can impact the structural properties of mucus as well modifying interactions with luminal bacteria.<sup>21</sup> Interestingly, in a more recent study, CMC and P80 were found to be able to dramatically alter gene expression by select pathobionts, including genes involved in bacterial motility and induction of chronic intestinal inflammation.<sup>36</sup> Thus, select microbiota members are targeted directly by dietary emulsifiers in a way that promotes chronic intestinal inflammation in the host consuming such additives.

This evidence indicates that emulsifiers impact the microbiota, which then modifies the mucus layer (Figure 2), but that the mechanisms seem to differ between emulsifier sub-classes. Under germ-free conditions, emulsifiers do not affect mucus thickness or penetrability.<sup>29</sup> The changes in mucus seem likely to have an effect on microbiome interactions with the gut epithelium and may lead to changes in intestinal permeability and bacterial translocation.



## **Changes in intestinal permeability and bacterial translocation**

Intestinal epithelial cells maintain an important barrier to potential pathogenic luminal bacteria. A disorder of this barrier may result in increased intestinal permeability, promoting exposure to luminal contents and greater bacterial translocation<sup>39</sup> that subsequently trigger immunological responses.<sup>40</sup> Emulsifiers, such as carrageenan, have been shown to increase intestinal permeability in both rats and guinea-pigs associated with the onset of colitis (Table 2).<sup>24</sup>

Carrageenan has a direct impact on epithelial monolayer permeability in Caco-2 cells with associated alterations in membrane associated Zonula Occludens-1 (ZO-1) protein which recedes from the cell membrane to more central cell locations in a dose-dependent manner, resulting in severely disturbed cell architecture. Actin filaments are also altered by carrageenan leading to disruption of intercellular junctions between adjacent cells and reduced barrier function.<sup>19</sup> Carrageenan triggered a disruption of the epithelial barrier in the colonic adenocarcinoma cell line HCT-8 by decreasing gene expression and subsequent density of ZO-1.<sup>17</sup>

Increased permeability after emulsifier ingestion has been observed in both wild-type and IL10<sup>-/-</sup> mice, and exposure correlates positively with serum antibody levels to flagellin and LPS.<sup>29,41</sup> Increased bacterial translocation across the gastrointestinal mucosa has been implicated as a plausible mechanism for emulsifier-induced gastrointestinal inflammation. The initial studies to explore this relationship used a Caco2-cl1 monoculture model and examined the effect of emulsifiers on the translocation of *E. coli* across M-cells (Table 1).<sup>16</sup> Also known as membranous or microfold cells, M-cells are thought to act as a portal for bacterial translocation within Peyer's patches.<sup>42,43</sup> P80, at 0.1% v/v, led to a 59-fold greater translocation of *E. coli*

across M-cells relative to an untreated control. There was a dose-dependent relationship at higher concentrations of P80. In contrast, P60 did not result in a significant difference in *E. coli* translocation across M-cells at any concentration tested.

The above studies demonstrate that emulsifiers can alter permeability, increase bacterial translocation and therefore activate inflammatory pathways (Figure 2).

### **Inflammatory pathways**

The pro-inflammatory potential of emulsifiers has been examined in numerous *in vitro* (Table 1) and animal models of IBD (Table 2), in particular in relation to the effect of carrageenan.

Thus, exposure of the normal human intestinal epithelial cell line NCM460 to carrageenan activates a distinct inflammatory pathway via the CARD B-cell lymphoma/leukaemia-10 (Bcl-10) and subsequently through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) cascade.<sup>12</sup> NF- $\kappa$ B activation is triggered by the innate immune receptor Toll-like receptor-4 (TLR-4) and blockade of the TLR-4 pathway lowers the carrageenan-induced augmentation of Bcl-10 and interleukin-8 (IL-8).<sup>44</sup> This observation implies that carrageenan may stimulate pro-inflammatory activation (Bcl-10, IL-8) via TLR4, a recognised mediator of intestinal inflammation in IBD.<sup>13</sup> Furthermore, carrageenan can activate LPS-induced inflammation synergistically through the Bcl-10 pathway and therefore magnify pre-existing inflammation.<sup>31</sup>

TLR4, is known to require Bcl-10 to trigger NF- $\kappa$ B pathway activation. Therefore, in a germ-free environment IL10<sup>-/-</sup> mice fed carrageenan experienced significantly greater activation of the NF- $\kappa$ B pathway and subsequent higher faecal calprotectin compared to Bcl10<sup>-/-</sup> mice demonstrating that Bcl10 is required for the development of maximum carrageenan-induced intestinal inflammation.<sup>27</sup>

Carrageenan has also been shown to induce dose-dependent TNF- $\alpha$  production by monocytes *in vitro* with an associated impact on cell aggregation and increased expression of intercellular adhesion molecule-1 (ICAM-1). Degraded carrageenan drives a robust increase in both TNF- $\alpha$  and ICAM-1 mRNA expression, induced by NF- $\kappa$ B activation as confirmed by western blotting of NF- $\kappa$ B in the cell nucleus.<sup>15</sup>

In addition to the above, using an *in vitro* co-culture system consisting of Caco-2 monolayers and human monocytes from the cell-line THP-1, a model used to mimic M-cells, it has been demonstrated that secretion of TNF- $\alpha$ , interleukin 1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6) were significantly higher following the addition of carrageenan. Pre-treatment of the co-culture with anti-TNF $\alpha$  antibodies suppressed the increase in IL-1 $\beta$  and IL-6.<sup>18</sup> Interleukin-8 (IL-8) secretion from human intestinal epithelial cells have also been shown to be greater when treated with carrageenan in an NF- $\kappa$ B-dependent pathway.<sup>12</sup>

Other emulsifiers have also shown a pro-inflammatory effect. For example, it was found that CMC and P80 significantly increased the expression of pro-inflammatory cytokine chemokine (C-X-C motif) ligand 1 (CXCL1).<sup>30</sup> Overall, these experiments suggest that emulsifiers play a role in stimulating various inflammatory pathways that could contribute to intestinal inflammation (Figure 2).

## **Cell proliferation and apoptosis**

High levels of apoptosis have been observed in the intestinal epithelium in IBD as well as with exposure to emulsifiers.<sup>45</sup> Carrageenan exposure to the cell line NCM-460 (derived from normal human colonic mucosa) led to a dose dependant increase in epithelial cell death compared to controls, with a significantly lower percentage in cell cycle survival between G0-G1 at days 6 & 8 and higher p53 activation.<sup>14</sup>

In a mouse model of colorectal cancer, CMC and P80 have been observed to alter epithelial proliferation and apoptosis in a microbiota-dependent manner, with such alterations disappearing under germfree conditions and being transplanted by fecal microbiota transplantation.<sup>30</sup> These experiments further highlight the broad consequences on the host induced by select dietary emulsifier exposure.

## **Emulsifiers and colitis in experimental animal models**

As discussed above, emulsifiers impact the gut microbiome, mucus architecture, intestinal permeability and inflammatory pathways (Figures 1 and 2), therefore it is logical to assess their effect in promoting intestinal inflammation. CMC and P80 induce robust colitis in genetically susceptible IL10<sup>-/-</sup> mice, whilst also inducing low grade inflammation in wild-type mice.<sup>29</sup> Emulsifier-fed IL-10<sup>-/-</sup> mice exhibited reduced colon length, increased histological colitis scores and elevated faecal lipocalin-2 (a sensitive and dynamic marker of intestinal inflammation in murine models). In addition, levels of inflammation inversely correlated with bacterial-epithelial distance in both IL10<sup>-/-</sup> and wild-type mice.

Numerous animal models develop colitis when exposed to carrageenan (Table 2).<sup>46</sup> A study analysing the effect of degraded carrageenan on rabbits demonstrated the rapid

development of caecal ulceration and large discrete rectal ulcers.<sup>23</sup> Similarly, degraded carrageenan induced weight loss, diarrhoea with blood and mucus and caecal ulceration in guinea pigs.<sup>22</sup> In addition, rats fed carrageenan developed inflammation similar to human IBD, with focal lesions, lymphoid hyperplasia and microgranulomas.

<sup>25 26</sup>

Another emulsifier, methylcellulose (E461), has also been implicated. C57BL/6J mice in a dextran sodium sulphate model of colitis were tested against more than 40 different diets. Those exposed to methylcellulose were found to have a higher severity of colitis (with disease severity measured by percentage weight change from baseline).<sup>34</sup>

### **Emulsifiers and colitis in an experimental model mimicking the human microbiota**

The mucosal-simulated human intestinal microbiota ecosystem (M-SHIME) is a dynamic *in vitro* model that maintains an active human microbiome without a live host. P80-treated M-SHIME microbiota compositions develop a clear cluster which is distinct from the other M-SHIME models tested.<sup>20</sup> The same did not occur during CMC treatment. However, in both P80- and CMC-treated M-SHIME models, an alteration in microbial gene expression was observed, with enrichment of flagella transcript expression, which is hypothesised to lead to low-grade inflammation via activation of TLR-5 and/or the nucleotide binding leucine-rich repeat caspase activating recruitment domain (CARD) (NLRC4) protein.<sup>29</sup> P80 induced increased expression of LPS at higher concentrations.

When transferred to germ free mice, P80 and CMC treated M-SHIME microbiomes led to increased levels of inflammatory markers such as lipocalin and IL-6, and

shortened, thickened colons, which correlated with increased microbiota encroachment onto the epithelium. These results importantly suggest that alterations in the microbiota induced by emulsifiers are sufficient to drive to intestinal inflammation.<sup>20,47</sup>

### **Limitations of experimental models of emulsifiers in IBD**

The cell line and murine studies outlined above are not without limitations that may restrict their applicability to humans. Experimental doses of emulsifiers given in animal models, although administered over a relatively short period, are often much higher than human dietary exposure. Average consumption of P80, CMC and carrageenan in the United Kingdom (UK) are 8.2, 46 and 11-53 mg/kg body weight/day, respectively.<sup>48-50</sup> However, mouse studies report doses as percentage of the drinking water provided, which makes direct comparison to human intake challenging. For example, the doses of P80 and CMC provided in a recent murine study were approximately 610 mg/kg/day. However, two factors may mitigate against this limitation. Firstly, animal studies tend to employ short term emulsifier exposure<sup>29</sup>; thus the chronic human intake over many years is likely to exceed the doses tested in animals. Secondly, although average intakes in humans may be much lower than doses used in experiments, people consuming diets high in processed foods may ingest some emulsifiers in doses more reflective of those used in animal studies. For example, the 95<sup>th</sup> percentile intake of carrageenan in the UK is 142.5 mg/kg/day which exceeds the European Union Acceptable Daily Intake of 75 mg/kg/day.<sup>50</sup> Lastly, in-depth mechanistic investigation of CMC- and P80-driven alteration in microbiota composition and function revealed that they are acting through different mechanisms, with CMC promoting the expression of genes involved in the promotion of inflammation without impact on microbiota composition, while P80 alters microbiota

composition by favouring the expansion of pro-inflammatory bacteria.<sup>20,29</sup> Hence, while these two compounds were tested individually in mice, it remains necessary to investigate their synergistic effects even at lower concentrations.

Caution should be taken when applying animal models to human disease. For instance, there are anatomical, physiological and histological differences between mice and humans, as well as disparity between microbial composition, all of which can affect the outcomes measured.<sup>51,52</sup> Experiments using animal models include a homogenous population which obviously is not the case with the heterogeneity of human disease and cannot fully replicate the complexity of a multifactorial disease such as IBD.<sup>53</sup> This could explain the lack of confirmatory translation between pre-clinical research and some subsequent human clinical trials.

A further challenge of extrapolating the mechanistic study findings to humans pertains to whether the emulsifiers used in these models were food-grade, a detail that is not reported in the majority of *in vitro* and animal studies.<sup>12,13,54</sup> Non-food grade carrageenan, used in applications such as cosmetics<sup>55</sup> is predominantly 'degraded carrageenan' which has a lower molecular weight than food-additive carrageenan.<sup>54</sup> Degraded carrageenan is created when it is subjected to high temperatures >80°C and acidic environments (pH 0.9-1.3) for several hours.<sup>50</sup> Under conditions comparable to gastric digestion (pH 1.9 for 120 minutes at 37°C), only 10% of food-grade carrageenan is actually degraded.<sup>56</sup> Therefore, many *in vitro* and animal studies examine the effects of a degraded carrageenan that is neither permitted in foods and is only produced in small amounts under physiological conditions in the gut.<sup>50</sup>

There are yet to be any human studies establishing the effect of emulsifiers on the pathways identified from *in vivo* and *in vitro* models. Given these observations, it is important to assess the effects of chronic daily exposure to emulsifiers on the microbiome, permeability and intestinal inflammatory pathways in well-designed human studies. Three study designs could be considered in such human studies. Firstly, epidemiological studies that report emulsifier exposure against the risk of inflammatory and metabolic disorders such as IBD. The European Prospective Investigation into Cancer (EPIC) study, a nested matched case-control study, reported 256 cases of UC and 117 of CD that occurred at least 2 years after baseline and therefore excluded prevalent cases. Analysis of baseline, validated food frequency questionnaires demonstrated an association between high sugar and soft drink consumption and UC risk. However, although it employed prospective dietary assessment using a validated tool, the study has several limitations including a sample size that is likely underpowered to detect risk of IBD, median participant age of 50 years which will only detect later onset IBD, the lack of adjustment for many possible confounders and most importantly no ability to measure actual emulsifier exposure, only intakes of some examples of processed foods.<sup>57</sup> A further study investigated the available data for food and beverage emulsifier consumption by country. When compared to known CD incidence on a matched year basis, a strong positive correlation with emulsifier consumption was found in Europe, the United States of America, Canada and Japan.<sup>6</sup> However, this analysis is based upon country-level data for both emulsifier intake and IBD prevalence rather than individual-level data for intake and IBD risk. Ecological studies and statistically significant correlations cannot provide evidence of causality<sup>58</sup> and thus human studies are required to confirm test these hypotheses. It should also be acknowledged that there is a lack of comprehensive



information on emulsifier content in food composition databases or elsewhere, which therefore makes it challenging to accurately quantify emulsifier exposure.

Secondly, feeding studies in which emulsifiers are provided to healthy humans or patients with IBD would identify any deleterious impact on the microbiome, permeability and inflammation (similar to animal experiments described, Table 2). However, there are no such feeding studies in humans thus far. Thirdly, studies that restrict emulsifier intake with subsequent investigation of any beneficial impacts on these outcomes of emulsifier restriction, may therefore allude to emulsifiers' harmful effects *in vivo*.

## **Human trials of dietary emulsifier restriction**

### ***(i) Emulsifier-restricted diets***

Two studies have investigated the effect of dietary emulsifier restriction in IBD (Table 3). The first was a double-blind, randomised controlled trial in 15 patients with UC in remission investigating whether a 'no-carrageenan diet' prevented relapse.<sup>59</sup> All participants followed a 'no-carrageenan diet' and in conjunction were randomised to receive either 200 mg/day carrageenan capsules (carrageenan-containing diet, control group) or placebo dextrose tablet ('no-carrageenan diet', intervention group). At the one-year endpoint, 3 out of 5 patients in the carrageenan-containing diet group relapsed ( $\geq 2$  point increase in Simple Clinical Colitis Activity Index plus escalation of treatment) whereas only 1 out of 7 relapsed in the 'no-carrageenan diet' ( $p=0.046$ ). The carrageenan-containing diet group experienced an increase in faecal calprotectin between baseline and end of study, although this did not reach statistical significance, whereas faecal calprotectin was stable in the 'no-carrageenan diet'.

The use of a re-supplementation design allowed an appropriate control and enabled double-blinding of the intervention, which can be a particular problem in trials of whole diet interventions.<sup>64</sup> However, with only 12 patients completing the study (three withdrawals), this study was underpowered as the *a priori* sample size required was 36, and therefore findings are at risk of both type 1 and type 2 error. Additionally, time-to-relapse studies in IBD usually have a minimum time period of 2-5 years follow-up.<sup>65</sup> Another limitation was the re-supplementation of carrageenan in capsules that does not reflect real world exposure. Emulsifiers are normally consumed in a food matrix that may alter the impact of gastric conditions on degradation of carrageenan (as discussed earlier) as well as on their activity in the gut. This limitation is also shared by the murine studies in which emulsifiers were added to drinking water. The dose of carrageenan re-supplemented in this study (200 mg/d) is much lower than estimated UK intakes of 11–53 mg/kg/day<sup>50</sup>. Finally, the study did not analyse microbiome, intestinal permeability or immune function to explore the mechanism of action.

The second trial to restrict emulsifier intake was an uncontrolled, feasibility study of a low emulsifier diet in adults with Crohn's disease (n=20).<sup>60</sup> During a baseline 7-day food diary, the majority of participants (15/20, 75%) consumed emulsifiers every day, with the remainder (5/20, 25%) consuming emulsifiers on 6 of the 7 days. The intervention involved a 14-day low emulsifier diet designed to exclude all food-additives classified as emulsifiers. The diet was delivered using counselling from a dietitian, an educational booklet and a novel smartphone application. Adherence (defined *a priori* as at least a 75% reduction in frequency of emulsifier intake between baseline and intervention) was achieved by 19/20 participants (95%). Food-related quality of life (FR-QOL) improved significantly on the low emulsifier diet. Interestingly, Crohn's disease related symptoms evaluated by the patient related

outcomes questionnaire (PRO-2) and perceived symptom control assessed by the IBD-Control-8 questionnaire improved significantly following the low emulsifier diet, however there was no other measure of disease activity such as CDAI or faecal calprotectin. This was indeed a feasibility study only, designed to demonstrate that a low emulsifier diet is deliverable and acceptable to patients. Appropriately designed and adequately powered studies are now required to evaluate its effect on inducing and maintaining disease remission.

***(ii) Emulsifier-restriction as part of other complex diets***

Despite the paucity of human evidence implicating emulsifiers in IBD specifically, recently developed complex dietary interventions that exclude many dietary components of the Western diet including emulsifiers, have been shown to reduce inflammation in Crohn's disease (Table 3). Whilst these novel dietary interventions cannot implicate the effect of emulsifiers specifically on gut inflammation (as they exclude many other dietary components in addition), they lend weight to the potential of emulsifier restriction in IBD.

Firstly, in a randomised controlled trial of the Crohn's Disease Exclusion Diet (CDED)<sup>62</sup>, children with mild to moderately active Crohn's disease (PCDAI 10-40) received either exclusive enteral nutrition (EEN) or partial enteral nutrition (PEN) (50% of energy requirements) and CDED (50% of energy requirements) for 6 weeks. The CDED group experienced significantly higher tolerability by week 6 (39/40, 97.5%) than the EEN group (28/38, 73.6%,  $p=0.002$ ). Although not powered to detect clinical outcomes, there were no significant differences between groups in the proportion achieving corticosteroid-free remission at week 6. Both groups had a significant decrease in faecal calprotectin, however, there was no statistically significant

difference in absolute or delta calprotectin between the two groups at week 6. In line with clinical practice, both groups then reduced enteral nutrition to 25% of energy requirements from week six to week 12, with the remaining 75% of energy requirements provided by an unrestricted diet to those previously following EEN group and the CDED in those previously following PEN plus CDED. At week 12, significantly more patients in the CDED group maintained corticosteroid-free remission (28/37, 75.6%) compared to those following unrestricted diet (14/31, 45.1%,  $p=0.01$ ).

This trial demonstrates that reintroduction onto a liberalised diet after a period of EEN is associated with higher relapse rates compared to patients who reintroduce onto a controlled diet that excludes components of a Western diet. Additionally, the reintroduction of a restriction-free diet led to a major rebound in microbiota towards pre-treatment baseline profiles, most notably an increase in Proteobacteria, whereas the CDED group sustained the decrease in Proteobacteria. This study demonstrates the potential of a diet restricting many food components, including some but not all emulsifiers, animal and dairy fat, red meat, artificial sweeteners, sulphites, protein sources rich in taurine and wheat, on inducing and sustaining remission in active Crohn's disease. Interestingly, participants were recruited from centres in Israel and Canada, countries with differing dietary patterns, and yet the effect of reintroduction of free-food was comparable, incriminating dietary components of an unrestricted free-diet in the exacerbation of Crohn's disease. It is important to note that the PEN given in conjunction with the CDED was a formula containing added emulsifiers (soy lecithin), and so overall neither group was emulsifier-free. Future trials should compare CDED to a control diet to investigate whether it is the diet alone or its combination with PEN that accounts for any clinical benefit.

Secondly, the Specific Carbohydrate (SCD) diet, initially used for the treatment of coeliac disease<sup>66</sup>, is another popular diet used in patients with IBD that restricts carbohydrates containing polysaccharides and disaccharides, processed foods, all grains, milk, some fruit and vegetables (corn, potatoes, yams), some legumes (chickpeas and soy beans), canned fruits and vegetables or meats that have been smoked or canned. It therefore restricts many emulsifiers.<sup>67</sup> To date, there have been no RCTs of the SCD and no prospective and robust evidence of improvements in inflammation or mucosal healing in IBD.<sup>68</sup> However, observational studies have shown improvements in gastrointestinal symptoms with the SCD.<sup>69</sup>

Finally, the CD-TREAT diet mimics EEN using a food based approach that restricts components such as gluten, lactose, emulsifiers, fibre, carbohydrates and alcohol.<sup>63</sup> Studies in mice and healthy humans demonstrated the CD-TREAT diet induced similar changes in the microbiome to EEN. Following which, a case series of five children with active Crohn's disease (weighted PCDAI  $\geq 12.5$ ) received CD-TREAT diet for 8 weeks, with four (80%) experiencing a clinical response and three (60%) entering remission, with significant concurrent decreases in faecal calprotectin (mean change -  $918 \pm 555$  mg/kg;  $P=0.002$ ). The data above are promising and a randomised controlled trial is now underway to fully evaluate CD-TREAT (ClinicalTrials.gov Identifier: NCT04225689).

Whilst diets such as the CDED, SCD and CD-TREAT demonstrate that a reduction in food components including many emulsifiers, may reduce intestinal inflammation in Crohn's disease, these diets also restrict other food groups and aspects of the Western diet. Compositional analysis of 61 available enteral nutrition formulas indicates that many contain food additives putatively linked to Crohn's disease aetiology, including

emulsifiers implicated in colitis from cell line and animal models.<sup>70</sup> However, the relationship between food additive concentration within these formulas and their subsequent clinical effectiveness was not analysed. It is also not known whether emulsifiers play a role in triggering IBD, or whether emulsifiers are only a crucial factor in a specific subset of patients. Still, such observations challenge the theory that these food additives are harmful and reinforces the fact that preclinical data do not always translate to real-life findings. Therefore, further studies are required to disentangle which dietary components, if any, are implicated in the induction of intestinal inflammation in IBD.

Furthermore, following a restrictive prescribed diet is challenging; the diet must be feasible to allow adequate compliance and not result in nutritional deficiency or an overly restrictive lifestyle that might impact on psychosocial function and food-related quality of life that are already impaired in IBD.<sup>71</sup> With further understanding of the roles of specific components in IBD, tailored and minimally restrictive advice can be developed, as well as therapeutic dietary interventions that improve clinical disease without these deleterious impairments.

Although there is compelling evidence from *in vitro* and animal studies of the impact of emulsifiers on gastrointestinal inflammation (Figure 2), when examining applicability of these findings to humans, we must also consider whether excluding emulsifiers from the diet is a viable long-term therapeutic option. Of the emulsifiers implicated in IBD, carrageenan (labelled also as E407) is used commonly in bakery foods, meat products, pre-made soups and flavoured drinks <sup>50</sup>; P80 (E433) is used in breads, flavoured drinks, confectionary, ice-creams and sorbets, bakery foods and desserts <sup>48</sup>; and CMC (E466) is used in flavoured drinks, meat substitutes, ice lollies,

sorbets and artificially sweetened products.<sup>49</sup> Therefore, exclusion of these emulsifiers presents a challenge for people following a “Western diet”, and a further degree of difficulty will arise if additional emulsifiers are found to induce gastrointestinal inflammation. Hence, further investigation of the effect of numerous dietary emulsifiers on the human microbiota are needed to identify emulsifiers without deleterious impact on the microbiota or the host. Nonetheless, any therapeutic intervention restricting or excluding emulsifiers is likely to involve a major change to daily dietary patterns.

### **Future research on emulsifiers**

A range of *in vitro* and *in vivo* studies have implicated emulsifiers in intestinal inflammation and IBD, which have been recognised by recent consensus recommendations from the International Organisation for the study of IBD (IOIBD) that suggest it is prudent to limit the intake of emulsifiers and thickeners in IBD.<sup>72</sup> However, high-quality human studies required to corroborate the *in vitro* and *in vivo* evidence to patients with IBD and to support these recommendations are lacking. For example, emulsifiers appear to disrupt epithelial tight junctions in Caco-2 monolayers<sup>19</sup>, but whether this translates to increased gut permeability in humans is yet to be investigated. Questions also remain regarding whether the mechanisms of emulsifier-induced inflammation differ between the small and large intestine which may impact applicability to different IBD disease phenotypes. Additionally, whether the activation of the NF- $\kappa$ B inflammatory pathway observed in emulsifier-exposed cell lines<sup>17</sup> translates to a robust colitis in humans requires an adequately powered randomised controlled trial. Unlike germ-free cell lines, human studies inevitably involve emulsifiers interacting with the gut microbiome; this is an essential consideration as

mouse studies indicate that emulsifiers modulate the gut microbiota that in turn mediates the inflammatory response.<sup>20</sup>

The impact of dietary emulsifiers on disease severity, gut microbiome, barrier function and intestinal inflammation in IBD patients is therefore yet to be fully elucidated. Whilst recent research into whole-food exclusion diets show promise<sup>62,63</sup>, the European Crohn's and Colitis Organisation's current report on gaps in the evidence-base for diet and IBD has identified emulsifier research as an important priority to progress our understanding of the dietary management of IBD.<sup>73</sup> There is also a wider European food safety concern and the European Food Safety Authority's Emerging Risks report identified "food emulsifiers, the gut microbiome and long-term health effects" as requiring urgent research.<sup>74</sup> Therefore, a human RCT to investigate the role of dietary emulsifier exclusion on the induction and maintenance of remission in IBD is warranted. Such a dietary trial has been designed and is underway (ClinicalTrials.gov Identifier: NCT04046913). A further trial investigating the effect of the commonly consumed emulsifier soy lecithin on healthy volunteers using a controlled dietary intervention is also proceeding and will give further information on the role of emulsifiers in gut health (ClinicalTrials.gov Identifier: NCT03842514).<sup>11</sup>

Future emulsifier research could add to the emerging evidence-base in IBD that microbiota profiles can be used to predict treatment response. Personalised medicine using baseline faecal microbiota as predictive biomarkers show promising findings in drug interventions.<sup>75</sup> However, there are inconsistent findings in the nutrition field to demonstrate that microbiota, or its metabolites, predict response to dietary interventions.<sup>76,77</sup> Future human studies measuring the gut microbiota before and



after a low emulsifier diet will inform this field of predicting diet response and personalised IBD dietary management translated into clinical practice.

Although our armamentarium of drug therapy in IBD has and will continue to increase, it is clear that the role played by emulsifiers in IBD requires clarification with ambitious human studies to unravel the potential to augment, complement or replace current therapeutic strategies.

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### **Conflicts of Interest**

ASB, AMS, BC and JOL declare no conflicts of interest relevant to this work. KW is the co-inventor of a mobile phone application to assist patients to follow dietary restrictions (FoodMaestro FODMAP app). MR is the co-founder of a plant-based food brand (Bio&Me).

### **Author Contributions**

ASB, AMS and KW conceived the idea of the manuscript and were responsible for the planning, content and structure of the article. ASB wrote the initial draft, with critical review and oversight by KW. AMS, MR, BC and JOL provided critical review of the manuscript and contributed to subsequent revisions. All authors approved the final version of this manuscript.

## Data availability statement

No new data were generated or analysed in support of this manuscript.

## References

1. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet* 2018; **390**(10114): 2769-78.
2. Levine A, Sigall Boneh R, Wine E. Evolving role of diet in the pathogenesis and treatment of inflammatory bowel diseases. *Gut* 2018; **67**(9): 1726-38.
3. D'Souza S, Levy E, Mack D, et al. Dietary patterns and risk for Crohn's disease in children. *Inflamm Bowel Dis* 2007; **14**(3): 367-73.
4. Niewiadomski O, Studd C, Wilson J, et al. Influence of food and lifestyle on the risk of developing inflammatory bowel disease. 2016; **46**(6): 669-76.
5. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. A Prospective Study of Long-term Intake of Dietary Fiber and Risk of Crohn's Disease and Ulcerative Colitis. *Gastroenterology* 2013; **145**(5): 970-7.
6. Roberts CL, Rushworth SL, Richman E, Rhodes JM. Hypothesis: Increased consumption of emulsifiers as an explanation for the rising incidence of Crohn's disease. *J Crohns Colitis* 2013; **7**(4): 338-41.
7. Nations FaAOotU. Codex Alimentarius. Class names and the international numbering system for food additives 1989.
8. Cox S, Sandall A, Smith L, Rossi M, Whelan K. Food additive emulsifiers: a review of their role in foods, legislation and classifications, presence in food supply, dietary exposure, and safety assessment. *Nutrition Reviews* 2020.
9. McClements D. Food Emulsions Principles, Practices and Techniques. . 3rd ed ed. Boca Ranton, FL: Taylor & Francis Group; 2015.
10. Garzon R, Hernando I, Llorca E, Rosell CM. Understanding the effect of emulsifiers on bread aeration during breadmaking. *J Sci Food Agric* 2018; **98**(14): 5494-502.
11. Partridge D, Lloyd KA, Rhodes JM, Walker AW, Johnstone AM, Campbell BJ. Food additives: Assessing the impact of exposure to permitted emulsifiers on bowel and metabolic health - introducing the FADiets study. *Nutr Bull* 2019; **44**(4): 329-49.
12. Borthakur A, Bhattacharyya S, Dudeja PK, Tobacman JK. Carrageenan induces interleukin-8 production through distinct Bcl10 pathway in normal human colonic epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**(3): G829-38.
13. Bhattacharyya S, Gill R, Chen ML, et al. Toll-like receptor 4 mediates induction of the Bcl10-NFkappaB-interleukin-8 inflammatory pathway by carrageenan in human intestinal epithelial cells. *J Biol Chem* 2008; **283**(16): 10550-8.
14. Bhattacharyya S, Borthakur A, Dudeja PK, Tobacman JK. Carrageenan induces cell cycle arrest in human intestinal epithelial cells in vitro. *J Nutr* 2008; **138**(3): 469-75.
15. Benard C, Cultrone A, Michel C, et al. Degraded carrageenan causing colitis in rats induces TNF secretion and ICAM-1 upregulation in monocytes through NF-kappaB activation. *PLoS One* 2010; **5**(1): e8666.
16. Roberts CL, Keita AV, Duncan SH, et al. Translocation of Crohn's disease Escherichia coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers. *Gut* 2010; **59**(10): 1331-9.

17. Choi HJ, Kim J, Park SH, Do KH, Yang H, Moon Y. Pro-inflammatory NF-kappaB and early growth response gene 1 regulate epithelial barrier disruption by food additive carrageenan in human intestinal epithelial cells. *Toxicol Lett* 2012; **211**(3): 289-95.
18. Jiang HY, Wang F, Chen HM, Yan XJ. kappa-carrageenan induces the disruption of intestinal epithelial Caco-2 monolayers by promoting the interaction between intestinal epithelial cells and immune cells. *Mol Med Rep* 2013; **8**(6): 1635-42.
19. Fahoum L, Moscovici A, David S, et al. Digestive fate of dietary carrageenan: Evidence of interference with digestive proteolysis and disruption of gut epithelial function. *Mol Nutr Food Res* 2017; **61**(3).
20. Chassaing B, Van de Wiele T, De Bodt J, Marzorati M, Gewirtz AT. Dietary emulsifiers directly alter human microbiota composition and gene expression ex vivo potentiating intestinal inflammation. *Gut* 2017; **66**(8): 1414-27.
21. Lock JY, Carlson TL, Wang CM, Chen A, Carrier RL. Acute Exposure to Commonly Ingested Emulsifiers Alters Intestinal Mucus Structure and Transport Properties. *Sci Rep* 2018; **8**(1): 10008.
22. Onderdonk AB, Franklin ML, Cisneros RL. Production of experimental ulcerative colitis in gnotobiotic guinea pigs with simplified microflora. *Infect Immun* 1981; **32**(1): 225-31.
23. Al-Suhail AA, Reid PE, Culling CF, Dunn WL, Clay MG. Studies of the degraded carrageenan-induced colitis of rabbits. I. Changes in the epithelial glycoprotein O-acetylated sialic acids associated with ulceration. *Histochem J* 1984; **16**(5): 543-53.
24. Delahunty T, Recher L, Hollander D. Intestinal permeability changes in rodents: a possible mechanism for degraded carrageenan-induced colitis. *Food Chem Toxicol* 1987; **25**(2): 113-8.
25. Moyana TN, Lalonde JM. Carrageenan-induced intestinal injury in the rat--a model for inflammatory bowel disease. *Ann Clin Lab Sci* 1990; **20**(6): 420-6.
26. Pricolo VE, Madhere SM, Finkelstein SD, Reichner JS. Effects of lambda-carrageenan induced experimental enterocolitis on splenocyte function and nitric oxide production. *J Surg Res* 1996; **66**(1): 6-11.
27. Bhattacharyya S, Xue L, Devkota S, Chang E, Morris S, Tobacman JK. Carrageenan-induced colonic inflammation is reduced in Bcl10 null mice and increased in IL-10-deficient mice. *Mediators Inflamm* 2013; **2013**: 397642.
28. Swidsinski A, Ung V, Sydora BC, et al. Bacterial overgrowth and inflammation of small intestine after carboxymethylcellulose ingestion in genetically susceptible mice. *Inflamm Bowel Dis* 2009; **15**(3): 359-64.
29. Chassaing B, Koren O, Goodrich JK, et al. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 2015; **519**(7541): 92-6.
30. Viennois E, Merlin D, Gewirtz AT, Chassaing B. Dietary Emulsifier-Induced Low-Grade Inflammation Promotes Colon Carcinogenesis. *Cancer Res* 2017; **77**(1): 27-40.
31. Wu W, Zhen Z, Niu T, et al. kappa-Carrageenan Enhances Lipopolysaccharide-Induced Interleukin-8 Secretion by Stimulating the Bcl10-NF-kappaB Pathway in HT-29 Cells and Aggravates *C. freundii*-Induced Inflammation in Mice. *Mediators Inflamm* 2017; **2017**: 8634865.
32. Shang Q, Sun W, Shan X, et al. Carrageenan-induced colitis is associated with decreased population of anti-inflammatory bacterium, *Akkermansia muciniphila*, in the gut microbiota of C57BL/6J mice. *Toxicol Lett* 2017; **279**: 87-95.
33. Jiang Z, Zhao M, Zhang H, Li Y, Liu M, Feng F. 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; **62**(3).
34. Llewellyn SR, Britton GJ, Contijoch EJ, et al. Interactions Between Diet and the Intestinal Microbiota Alter Intestinal Permeability and Colitis Severity in Mice. *Gastroenterology* 2018; **154**(4): 1037-46 e2.
35. Furuhashi H, Higashiyama M, Okada Y, et al. Dietary emulsifier polysorbate-80-induced small-intestinal vulnerability to indomethacin-induced lesions via dysbiosis. *J Gastroenterol Hepatol* 2020; **35**(1): 110-7.

36. Viennois E, Bretin A, Dube PE, et al. Dietary Emulsifiers Directly Impact Adherent-Invasive E. coli Gene Expression to Drive Chronic Intestinal Inflammation. *Cell Rep* 2020; **33**(1): 108229.
37. Allison C, Coleman N, Jones PL, Hughes C. Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Infect Immun* 1992; **60**(11): 4740-6.
38. Johansson ME, Ambort D, Pelaseyed T, et al. Composition and functional role of the mucus layers in the intestine. *Cell Mol Life Sci* 2011; **68**(22): 3635-41.
39. Galipeau HJ, Verdu EF. The complex task of measuring intestinal permeability in basic and clinical science. *Neurogastroenterol Motil* 2016; **28**(7): 957-65.
40. Michielan A, D'Inca R. Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. *Mediators Inflamm* 2015; **2015**: 628157.
41. Ziegler TR, Luo M, Estivariz CF, et al. Detectable serum flagellin and lipopolysaccharide and upregulated anti-flagellin and lipopolysaccharide immunoglobulins in human short bowel syndrome. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**(2): R402-10.
42. Owen RL, Jones AL. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology* 1974; **66**(2): 189-203.
43. Neutra MR. M cells in antigen sampling in mucosal tissues. *Curr Top Microbiol Immunol* 1999; **236**: 17-32.
44. Tsuji RF, Hoshino K, Noro Y, et al. Suppression of allergic reaction by lambda-carrageenan: toll-like receptor 4/MyD88-dependent and -independent modulation of immunity. *Clin Exp Allergy* 2003; **33**(2): 249-58.
45. Nunes T, Bernardazzi C, de Souza HS. Cell death and inflammatory bowel diseases: apoptosis, necrosis, and autophagy in the intestinal epithelium. *Biomed Res Int* 2014; **2014**: 218493.
46. Watt J, Marcus R. Experimental ulcerative disease of the colon in animals. *Gut* 1973; **14**(6): 506-10.
47. Viennois E, Chassaing B. First victim, later aggressor: How the intestinal microbiota drives the pro-inflammatory effects of dietary emulsifiers? *Gut Microbes* 2018: 1-4.
48. European Food Safety Authority. 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 sorbitan tristearate (E 436) as food additives. *EFSA Journal* 2015; **13**(7): 1 - 74.
49. European Food Safety Authority. Re-evaluation of celluloses E 460(i), E 460(ii), E 461, E 462, E 463, E 464, E 465, E 466, E 468 and E 469 as food additives. *EFSA Journal* 2017; **16**(1): 1 - 104.
50. European Food Safety Authority. Re-evaluation of carrageenan (E 407) and processed *Eucaema* seaweed (E 407a) as food additives. *EFSA Journal* 2018; **16**(4): 1 - 112.
51. Hugenholtz F, de Vos WM. Mouse models for human intestinal microbiota research: a critical evaluation. *Cell Mol Life Sci* 2018; **75**(1): 149-60.
52. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A* 2013; **110**(9): 3507-12.
53. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech* 2015; **8**(1): 1-16.
54. Weiner ML. Parameters and pitfalls to consider in the conduct of food additive research, Carrageenan as a case study. *Food Chem Toxicol* 2016; **87**: 31-44.
55. Prajapati VD, Maheriya PM, Jani GK, Solanki HK. Carrageenan: a natural seaweed polysaccharide and its applications. *Carbohydr Polym* 2014; **105**: 97-112.
56. Tobacman JK. Review of harmful gastrointestinal effects of carrageenan in animal experiments. *Environ Health Perspect* 2001; **109**(10): 983-94.
57. Racine A, Carbonnel F, Chan SS, et al. Dietary Patterns and Risk of Inflammatory Bowel Disease in Europe: Results from the EPIC Study. *Inflamm Bowel Dis* 2016; **22**(2): 345-54.
58. Georges M. The long and winding road from correlation to causation. *Nat Genet* 2011; **43**(3): 180-1.

59. Bhattacharyya S, Shumard T, Xie H, et al. A randomized trial of the effects of the no-carrageenan diet on ulcerative colitis disease activity. *Nutr Healthy Aging* 2017; **4**(2): 181-92.
60. Sandall AM, Cox, S.R.; Lindsay, J.O.; Gewirtz, A.T.; Chassaing, B.; Rossi, M.; Whelan, K. Emulsifiers Impact Colonic Length in Mice and Emulsifier Restriction is Feasible in People with Crohn's Disease. *Nutrients* 2020; **12**, 2827.
61. Sigall-Boneh R, Pfeffer-Gik T, Segal I, Zangen T, Boaz M, Levine A. Partial enteral nutrition with a Crohn's disease exclusion diet is effective for induction of remission in children and young adults with Crohn's disease. *Inflamm Bowel Dis* 2014; **20**(8): 1353-60.
62. Levine A, Wine E, Assa A, et al. Crohn's Disease Exclusion Diet Plus Partial Enteral Nutrition Induces Sustained Remission in a Randomized Controlled Trial. *Gastroenterology* 2019; **157**(2): 440-50.e8.
63. Svolos V, Hansen R, Nichols B, et al. Treatment of Active Crohn's Disease With an Ordinary Food-based Diet That Replicates Exclusive Enteral Nutrition. *Gastroenterology* 2019; **156**(5): 1354-67.e6.
64. Staudacher HM, Irving PM, Lomer MCE, Whelan K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. *Proc Nutr Soc* 2017; **76**(3): 203-12.
65. Liverani E, Scaiola E, Digby RJ, Bellanova M, Belluzzi A. How to predict clinical relapse in inflammatory bowel disease patients. *World J Gastroenterol* 2016; **22**(3): 1017-33.
66. Haas SV, Haas MP. The treatment of celiac disease with the specific carbohydrate diet; report on 191 additional cases. *Am J Gastroenterol* 1955; **23**(4): 344-60.
67. Lewis JD, Abreu MT. Diet as a Trigger or Therapy for Inflammatory Bowel Diseases. *Gastroenterology* 2017; **152**(2): 398-414.e6.
68. Wahbeh GT, Ward BT, Lee DY, Giefer MJ, Suskind DL. Lack of Mucosal Healing From Modified Specific Carbohydrate Diet in Pediatric Patients With Crohn Disease. *Journal of pediatric gastroenterology and nutrition* 2017; **65**(3): 289-92.
69. McCormick NM LJ. The Specific Carbohydrate Diet in the Treatment of Crohn's Disease: A Systematic Review. *Journal of Gastroenterology and Hepatology Research* 2017; **6**(4): 2392-9.
70. Logan M, Gkikas K, Svolos V, et al. Analysis of 61 exclusive enteral nutrition formulas used in the management of active Crohn's disease-new insights into dietary disease triggers. *Aliment Pharmacol Ther* 2020; **51**(10): 935-47.
71. Czuber-Dochan W, Morgan M, Hughes LD, Lomer MCE, Lindsay JO, Whelan K. Perceptions and psychosocial impact of food, nutrition, eating and drinking in people with inflammatory bowel disease: a qualitative investigation of food-related quality of life. *J Hum Nutr Diet* 2020; **33**(1): 115-27.
72. Levine A, Rhodes JM, Lindsay JO, et al. Dietary Guidance From the International Organization for the Study of Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2020; **18**(6): 1381-92.
73. Sigall-Boneh R, Levine A, Lomer M, et al. Research Gaps in Diet and Nutrition in Inflammatory Bowel Disease. A Topical Review by D-ECCO Working Group [Dietitians of ECCO]. *Journal of Crohn's and Colitis* 2017; **11**(12): 1407-19.
74. European Food Safety Authority. EFSA's activities on emerging risks in 2015. EFSA Supporting publication 2016:EN-1100. 2015.
75. Sprockett D, Fischer N, Boneh RS, et al. Treatment-Specific Composition of the Gut Microbiota Is Associated With Disease Remission in a Pediatric Crohn's Disease Cohort. *Inflamm Bowel Dis* 2019; **25**(12): 1927-38.
76. De Filippis F, Vitaglione P, Cuomo R, Berni Canani R, Ercolini D. Dietary Interventions to Modulate the Gut Microbiome—How Far Away Are We From Precision Medicine. *Inflamm Bowel Dis* 2018; **24**(10): 2142-54.
77. Rossi M, Aggio R, Staudacher HM, et al. Volatile Organic Compounds in Feces Associate With Response to Dietary Intervention in Patients With Irritable Bowel Syndrome. *Clin Gastroenterol Hepatol* 2018; **16**(3): 385-91 e1.

## Table titles

**Table 1: Summary of key *in vitro* studies of the effect of emulsifiers on microbiota, mucus and bacterial encroachment, intestinal permeability and bacterial translocation; and inflammation & colitis**

**Table 2: Summary of key animal model studies of the effect of emulsifiers on microbiota, mucus and bacterial encroachment, intestinal permeability and bacterial translocation; and inflammation & colitis**

**Table 3: Summary of key human trials of emulsifier restriction and emulsifier restriction as part of other diets**

## Figure titles and legends

**Figure 1: The chemical formulae and molecular structures of three commonly used food additive emulsifiers.**

Image courtesy of the National Center for Biotechnology Information. PubChem Database. CMC sodium, CID=6328154,

<https://pubchem.ncbi.nlm.nih.gov/compound/Carboxymethylcellulose-sodium>;

Carrageenan, CID=71597331,

<https://pubchem.ncbi.nlm.nih.gov/compound/71597331>; Polysorbate 80,

CID=5284448, <https://pubchem.ncbi.nlm.nih.gov/compound/5284448>

**Figure 2: Mechanisms of emulsifier driven intestinal inflammation**

(1) The microbiome and mucus are exposed to food additive emulsifiers in the intestinal lumen. (2) This leads to an alteration in microbiome with decreased diversity

and increased pro-inflammatory potential. (3) Some emulsifiers increase bacterial expression of flagellin & lipopolysaccharide, which enhances motility and the ability of bacteria to translocate through the mucus layer to the epithelial cell. (4) Thinning of the mucus, which is also driven by emulsifier interactions with the microbiome, leads to a decreased gut barrier function and increases penetrability. (5) The combination of these effects results in bacteria penetrating the mucus and encroaching upon the epithelial cell. (6) Increased permeability through alterations in membrane associated proteins such as Zonula Occludens-1 also allows for higher levels of bacterial translocation. (7) Inflammatory pathways are activated through the B-cell lymphoma/leukaemia-10 (Bcl-10) and toll like receptor-4 (TLR-4), which activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) cascade. (8) The increase in NF $\kappa$ B activation leads to the secretion of pro-inflammatory cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and the subsequent development of colitis.

**Table 1 - The effects of emulsifiers in *in vitro* models**

Study	Design Methods	Emulsifier(s) analysed	Effects
Borthakur et al. (2007) <sup>12</sup>	Human intestinal epithelial cells	Carrageenan	<b>Inflammation and colitis</b> - Carrageenan increased Bcl-10, nuclear and cytoplasmic NF-kappaB, IL-8 promoter activation, and IL-8 secretion.
Bhattacharyya et al. (2008) <sup>13</sup>	Human intestinal epithelial cells	Carrageenan	<b>Inflammation and colitis</b> - Carrageenan triggers TLR-4 which mediates intestinal inflammation via the Bcl10-NFkappaB-interleukin-8 inflammatory pathway
Bhattacharyya et al. (2008) <sup>14</sup>	Human intestinal epithelial cells	Carrageenan	<b>Inflammation and colitis</b> - Carrageenan may effect cell survival, demonstrated by fewer cells re-entering Go-G1 of the cell phase.
Benard et al., (2010) <sup>15</sup>	Rats & peripheral blood monocytes	Carrageenan	<b>Inflammation and colitis</b> - Degraded carrageenan stimulated monocyte aggregation and TNF- $\alpha$ expression and secretion.
Roberts et al. (2010) <sup>16</sup>	Caco-2 cell model	P80 and P80	<b>Intestinal permeability and bacterial translocation</b> - P80 increases bacterial translocation of E.coli across M-cells
Choi et al. (2012) <sup>17</sup>	Human intestinal epithelial cells	Carrageenan	<b>Intestinal permeability and bacterial translocation</b> - Carrageenan triggered disruption of the epithelial barrier by decreasing density of tight junction component zonula occludens-1, and also decreasing its gene expression.
Jiang et al. (2013) <sup>18</sup>	Caco-2 cell model	Carrageenan	<b>Inflammation and colitis</b> - $\kappa$ -Carrageenan-induced TNF- $\alpha$ secretion is the main contributor to cellular damage in Caco-2 monolayers exposed to $\kappa$ -CGN
Fahoum et al. (2017) <sup>19</sup>	Caco-2 cell model	Carrageenan	<b>Intestinal permeability and bacterial translocation</b> - Carrageenan increased intestinal permeability, and redistributed cellular proteins such as Zonula-Occludens-1 and actin, to disrupt normal epithelial function.
Chassaing et al. (2017) <sup>20</sup>	Murine & M-SHIME model	P80 & CMC	<b>Microbiota</b> - In a mucosal-simulated human intestinal microbiota ecosystem (M-SHIME), P80 altered microbiota composition, while CMC have potent effect on microbiota gene expression. When P80- or CMC-treated M-SHIME microbiome are transferred to germ-free mice, they both led to some intestinal inflammation.
Lock et al. (2018) <sup>21</sup>	Porcine mucus model	P80 & CMC	<b>Mucus and bacterial encroachment</b> - P80 and CMC altered mucus structure and E.coli speed of movement.



Carboxymethylcellulose (CMC), Polysorbate-80 (P80), interleukin-8 (IL-8), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), B-cell lymphoma/leukaemia-10 (Bcl-10) and toll like receptor-4 (TLR-4), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B).

**Table 2 – The effects of emulsifiers in animal models**

Study	Design Methods	Emulsifer(s) analysed	Effects
Onderdonk et al. (1981) <sup>22</sup>	Guinea Pigs	Carrageenan	<b>Inflammation and colitis</b> - Degraded carrageenan induced bloody diarrhoea, mucus and caecal ulceration after 3 weeks
Al-Suhail et al. (1984) <sup>23</sup>	Rabbit model	Carrageenan	<b>Inflammation and colitis</b> - Degraded carrageenan induced caecal ulceration and large discrete rectal ulcers in rabbits
Delahunty et al (1987) <sup>24</sup>	Rat & guinea pig models	Carrageenan	<b>Intestinal permeability and bacterial translocation</b> - Degraded carrageenan increased intestinal permeability in rats. Inflammation and colitis - Degraded carrageenan induced distinct colonic ulceration in guinea pigs.
Moyana & Lalonde, (1990) <sup>25</sup>	Rat model	Carrageenan	<b>Inflammation and colitis</b> - Oral carrageenan induced intestinal injury similar to human IBD.
Pricolo et al., (1996) <sup>26</sup>	Rat model	Carrageenan	<b>Inflammation and colitis</b> - Carrageenan induced lesions with proximal to mid small bowel involvement at 2-6 weeks, developing colonic lesions after 8 weeks.
Bhattacharyya et al. (2008) <sup>27</sup>	Murine model	Carrageenan	<b>Inflammation and colitis</b> - Carrageenan-induced colonic inflammation is reduced in Bcl10 null mice and increased in IL-10-deficient mice, demonstrating a Bcl10 requirement for development of carrageenan-induced inflammation.
Swidsinski, et al., (2009) <sup>28</sup>	Murine model	CMC	<b>Microbiota</b> - CMC increased bacterial adherence and caused a massive bacterial overgrowth
Benard et al., (2010) <sup>15</sup>	Rats & peripheral blood monocytes	Carrageenan	<b>Inflammation and colitis</b> -Degraded carrageenan caused a shortening of the rat large colon and an infiltration of macrophages similar to that seen in DSS-induced colitis.
Chassaing et al. (2015) <sup>29</sup>	Murine model	P80 & CMC	<b>Microbiota</b> - Bacterial genes for flagellin and LPS were upregulated in the emulsifier exposed group. Emulsifier dramatically altered faecal and intestinal adherent microbiome in wildtype, IL10 -/- and TLR 5 -/- mice. IL10 -/- mice had a reduction in $\alpha$ diversity and stability. <b>Mucus and bacterial encroachment</b> – Emulsifier exposure decreased the distance between bacteria and the epithelial cells by an average of more than 50%.

			<b>Intestinal permeability and bacterial translocation</b> -Exposure to emulsifiers in wildtype and IL10 -/- mice correlated positively with increased antibody levels to flagellin and LPS (thought to reflect gut permeability). <b>Inflammation and colitis</b> - Emulsifiers P80 and CMC cause low grade inflammation in WT mice, and robust colitis in IL10 -/- mice.
Viennois et al (2017) <sup>30</sup>	Murine model	P80 & CMC	<b>Microbiota</b> - P80 and CMC led to a significantly reduced microbiota diversity characterised by an increase in Bacteroidales and a decrease in Clostridiales. <b>Colitis-associated cancer</b> -In a colitis associated cancer model, mice fed P80 and CMC significantly increased the expression of pro-inflammatory cytokine chemokine (C-X-C motif) ligand 1 (CXCL1). Moreover, these animals developed significantly more colonic tumors.
Wu et al (2017) <sup>31</sup>	Murine model	Carrageenan	<b>Microbiota</b> - $\kappa$ -Carrageenan can synergistically increase LPS-induced inflammation through the Bcl10-NF- $\kappa$ B pathway.
Shang et al (2017) <sup>32</sup>	Murine model	Carrageenan	<b>Microbiota</b> – Carrageenan reduced the abundance of the anti-inflammatory bacterium <i>Akkermansia muciniphila</i> in C57BL/6 mice.
Jiang et al (2018) <sup>33</sup>	Murine model	Glyceryl Monolaurate	<b>Microbiota</b> - Glyceryl Monolaurate induced dysbiosis in C57BL/6 mice. Inflammation and colitis -Mice fed glyceryl monolaurate developed increased serum LPS concentrations and pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$ .
Llewellyn et al (2018) <sup>34</sup>	Murine model	Methylcellulose	<b>Inflammation and colitis</b> - Methylcellulose increased severity of colitis in a C57BL/6 DSS-induced colitis model.
Furuhashi et al (2020) <sup>35</sup>	Murine model	P80	<b>Microbiota</b> - P80 exacerbated indomethacin-induced small bowel intestinal lesions by inducing ileal dysbiosis .
Viennois et al (2020) <sup>36</sup>	Murine model	P80 & CMC	<b>Microbiota</b> - P80 and CMC are sufficient to directly alter gene expression in the pathobiont AIEC (adherent and invasive <i>Escherichia coli</i> ) associated with Crohn's disease. Such alterations are characterized by an increased ability to adhere to and invade intestinal epithelial cells, as well as an increased expression of various virulence factor. <b>Inflammation and colitis</b> – While germfree mice, or mice colonized with a microbiota of low complexity (Altered Schaedler Flora, containing 8 bacterial species only) are completely protected from the detrimental effect of P80 and CMC, colonization with AIEC bacteria is sufficient to restore dietary emulsifier-induced intestinal inflammation and downstream consequences.

Carboxymethylcellulose (CMC), Polysorbate-80 (P80), interleukin-8 (IL-8), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), B-cell lymphoma/leukaemia-10 (Bcl-10), toll like receptor-4 (TLR-4), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), interleukin 1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), toll-like receptor-5 (TLR-5), dextran sodium sulphate (DSS), lipopolysaccharide (LPS).

**Table 3 – Human dietary trials restricting emulsifiers**

<b>Study</b>	<b>Study design</b>	<b>Participants</b>	<b>Intervention</b>	<b>Outcomes</b>
<b>Emulsifier restriction</b>				
Bhattacharyya et al, 2017 <sup>59</sup>	Randomised, double-blind, placebo-controlled trial	12 patients with ulcerative colitis	Carrageenan free diet for 12 months vs carrageenan-containing diet (re-supplementation)	<p><b>Tolerance</b> – 3 patients declined further participation due to reluctance to comply with the diet.</p> <p><b>Disease activity</b> - At the one-year endpoint, 3/5 patients in the carrageenan-containing diet group relapsed; Only 1 out of 7 relapsed in the ‘no-carrageenan diet’ (p=0.046).</p> <p><b>Microbiome</b> – not measured</p> <p><b>Inflammatory markers</b> – Between the beginning and the end of the study, increases in interleukin-6 and faecal calprotectin were demonstrated in the carrageenan-containing diet group, but not in the carrageenan free diet.</p>
Sandall et al, 2020 <sup>60</sup>	Uncontrolled, feasibility study	20 patients with Crohn’s disease in remission	Low emulsifier diet for 2 weeks	<p><b>Tolerance</b> - At least a 75% reduction in frequency of emulsifier intake between baseline and intervention was achieved by 19/20 participants. Food-related quality of life (FR-QOL) improved significantly on the low emulsifier diet.</p> <p><b>Disease activity</b> – Although not measures of disease activity, Crohn’s disease-related symptoms (measured using the PRO-2 questionnaire) as well as perceived disease control (assessed by the IBD Control-8 questionnaire) improved significantly between baseline and the low emulsifier diet.</p> <p><b>Microbiome</b> – not measured</p> <p><b>Inflammatory markers</b> – not measured</p>
<b>Diets restricting emulsifiers as part of other diets</b>				
Sigall-Boneh et al, 2014 <sup>61</sup>	Uncontrolled, retrospective study	47 children and young adults with active Crohn’s disease (PCDAI >7.5 or HBI ≥4)	Crohn’s Disease Exclusion diet (CDED) plus 50% PEN or CDED alone for 6 weeks then an additional 6-week stepdown diet.	<p><b>Tolerance</b> – Five patients were not compliant (2 did not comply at all; 3 complied most of the time, with 2/3 achieving full remission).</p> <p><b>Disease activity</b> - Remission occurred in 70% of children and 69% of adults. Previously elevated C-reactive protein reached normal levels in 21 of 30 (70%) of patients in remission. Seven patients used the diet without PEN with 6 of 7 entering remission.</p> <p><b>Microbiome</b> – not measured</p> <p><b>Inflammatory markers</b> – Normalisation of previously elevated C-reactive protein was demonstrated in 70% of patients in remission.</p>

Levine et al, 2017 <sup>62</sup>	Randomised controlled trial	78 children with mild to moderate CD (PCDAI) $\geq 10$ and $\leq 40$ .	Children were randomly assigned to CDED plus 50% of energy from PEN for 6 weeks (stage 1) followed by CDED with 25% PEN from weeks 7 to 12 (stage 2) (n = 40, group 1) or a group that received EEN for 6 weeks followed by a free diet with 25% PEN from weeks 7 to 12 (n = 38, group 2).	<p><b>Tolerance</b> - The combination of CDED and PEN was tolerated in 39 children (97.5%), whereas EEN was tolerated by 28 children (73.6%) (<math>P = 0.002</math>).</p> <p><b>Disease activity</b> - At week 12, 28 (75.6%) of 37 children given CDED plus PEN were in corticosteroid-free remission compared with 14 (45.1%) of 31 children given EEN and then PEN (<math>P = 0.01</math>).</p> <p><b>Microbiome</b> – CDED plus PEN was associated with a reduction of faecal Proteobacteria</p> <p><b>Inflammatory markers</b> - In children given CDED plus PEN, corticosteroid-free remission was associated with sustained reductions in C-reactive protein, faecal calprotectin.</p>
Svolos et al, 2019 <sup>63</sup>	Open-label pilot study in Crohn's disease. Randomised open-label trial in healthy adults	5 children with active Crohn's disease (PCDAI $\geq 12.5$ ) and 28 healthy adults received CD-TREAT diet for 8 weeks.	Crohn's disease Treatment with EATING (CD-TREAT) for 8 weeks	<p><b>Tolerance</b> – In healthy adults, CD-TREAT was easier to comply with than EEN. Four children completed the 8-week trial, with 1 child withdrawn due to symptom exacerbation.</p> <p><b>Disease activity</b> - Four children (80%) experienced a clinical response and three (60%) entered remission.</p> <p><b>Microbiome</b> - The concentration of total faecal bacteria decreased with both diets as well as inducing similar changes to <math>\beta</math>-diversity and faecal bacterial metabolites.</p> <p><b>Inflammatory markers</b> – Children receiving CD-TREAT had a significant decrease in faecal calprotectin (mean change <math>-918 \pm 555</math> mg/kg; <math>P=0.002</math>).</p>

Partial enteral nutrition (PEN), exclusive enteral nutrition (EEN), paediatric Crohn's disease activity index (pCDAI).

Figure 1

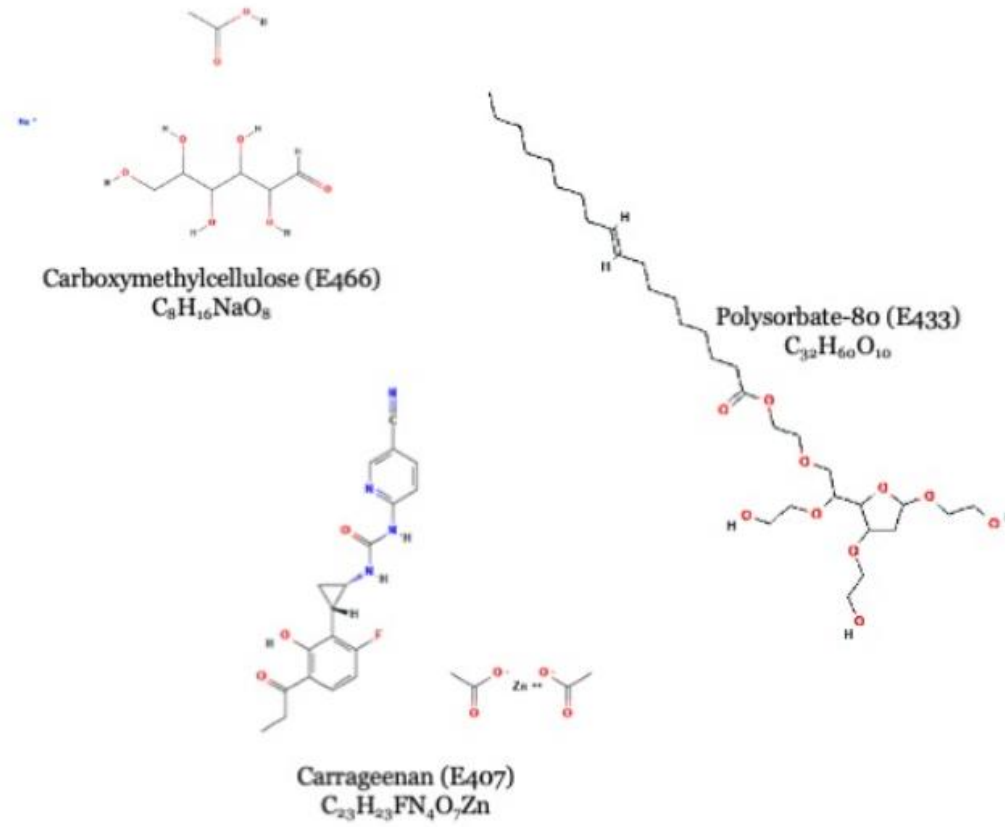


Figure 2

