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COMMENT

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Comment on "Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods?" by S. David, C. S. Levi, L. Fahoum, Y. Ungar, E. G. Meyron-Holtz, A. Shpigelman and U. Lesmes, *Food Funct.*, 2018, 9, 1344–1352

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Carrageenan (CGN) is a polysaccharide that is found in various types of sea weed. It is a common food additive used for its gelling and thickening properties and has been used safely throughout the world for decades. CGN is approved as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration and is also considered safe for the general population by the World Health Organizations Joint Expert Committee on Food Additive (JECFA) and the European Food Safety Authority. CGN has been tested for safety in various animal models for many years and more recently in an array of *in vitro* or cell-based models. A recent review published by this journal entitled "Revisiting the Carrageenan controversy: Do we really understand the digestive fate and safety of carrageenan in our foods?" has provided the impetus for this commentary (S. David, *et al.*, *Food Funct.*, 2018, **9**(3), 1344–1352). It is important that our food is safe, and clearly there are examples of food additives that were found to be unsafe after years of use, but the issue is the need for accurate interpretation of previously published studies and the need for designing and conducting experiments that can be used to make decisions on safety. It is our hope that this commentary brings to light some of the important physical and chemical properties of CGN and how information can be easily misinterpreted.

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What's in a name?

If one reads the literature reviewing studies involving CGN, it will become apparent that well-designed dietary safety studies show that food grade CGN of high weight average molecular weight (M_w) (M_w 200 000 to 800 000 Da) is not absorbed from the intestinal tract, nor is it degraded during transit. There are, however, several studies in which poligeenan (PGN and sometimes referred to as degraded CGN, M_w 10 000–20 000 Da) or degraded carrageenan (M_w 20 000–40 000 Da) is used as the test material. It is important to note that PGN and degraded CGN are made in the laboratory under very harsh conditions of low pH (<2.0) and high temperature (>80 °C) and are NOT the same as commercial CGN. Pittman *et al.*[2] demonstrated that degraded CGN could be absorbed after oral administration in animal studies, but no high M_w carrageenan was absorbed under the same conditions. The issue is that the authors of the studies identifying adverse effects refer to degraded CGN as CGN. Degraded CGN and PGN have very low M_ws; are never used as food additives and do not have any regulatory approvals as food additives. By referring to degraded CGN or PGN as CGN, the scientific community tends to consider all forms of CGN as the intact high M_w CGN. This is a significant problem because there is no debate over the harmful effects of degraded CGN and PGN, neither of which is permitted in food.[3]

Let's review route of administration

Another problem in the literature that has negatively impacted both consumer views, as well as the scientific community, is that high M_w CGN when injected into a confined tissue space of a rodent, such as the foot pad or the intraperitoneal cavity, induces a significant inflammatory response. This effect is so pronounced and so well understood that this model of inflammation is often used to evaluate the efficacy of anti-inflammatory drugs. These studies have led many researchers to believe

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that CGN, when eaten with food, induces immune responses leading to inflammation. The problem with this train of thought is that CGN **DOES NOT** enter the body when used as a food additive[4–6] and it is never intentionally injected into humans. So, if food grade CGN is stable in the gut, and is not absorbed into the body, **IT SIMPLY CANNOT INDUCE** these systemic inflammatory responses. Moreover, no human cases of allergic or anaphylactic reactions following carrageenan ingestion have been observed in the published literature.[4] Well-designed safety studies conducted in accordance with Good Laboratory Practices showed NO harmful effects of CGN in diet.[7,8] Long term animal safety studies have also been done to investigate the potential for CGN to cause cancer.[9–13] These results were all NEGATIVE and in recent clinical studies using CGN no adverse effects were observed. These important references[5,6,14–18] were omitted from the David *et al*. review.[1]

The importance of reading the references cited

It should be apparent from the discussion above that although there are many studies in animals evaluating the safety of CGN many of these did not use high M_w CGN. This point is important because in the recently published article,[1] many of the references used to support the idea that CGN causes adverse effects in humans were, in fact, conducted using degraded CGN or poligeenan. Thus, the published work being used to justify current CGN studies did not actually use CGN as the test material and serves only to cloud the regulatory picture regarding the safety of CGN.

Can anyone repeat the work?

In the review by David *et al.*[1] several references are cited to support the notion that CGN causes inflammation in the gastrointestinal tract, as well as other adverse effects. Many of these studies reported that specific signaling processes in the gut (*e.g.*, Toll-Like Receptor 4) were activated by CGN. These studies have also reported that CGN induces oxidative stress and disrupts insulin regulation. Clearly, these findings would be of concern to CGN manufacturers, and in response, the industry supported research by other laboratories to understand these reported effects. Multiple studies were conducted to reproduce the previously published work and many of these were conducted in compliance with Good Laboratory Practices. In every case these studies[16,17] could not reproduce any portion of the published positive *in vitro* findings. There was no binding to key signaling receptors; there was no increase in oxidative stress, and there was no cytotoxicity, even when all three of the major forms of CGN were tested at concentrations much higher than those reported in the studies that produced adverse effects. A fundamental premise of the scientific method is that the reported work should be reproducible by other laboratories. Many of the studies refuting the *in vitro* mechanistic data were not cited by the authors of the review, and when they were mentioned, they were considered insignificant because the test material was not well characterized. In fact, the CGN used in these replication studies underwent extensive chemical characterization. This cannot be said for the *in vitro* work in which CGN was purchased from commercial sources without proper characterization.

Research supported by industry is less credible than academic research, really?

It is true that all laboratories must constantly fight a natural tendency towards bias. The important point is that bias in research can occur in both academic and industrial laboratories. Pressure to prove one's hypothesis correct, or to find the next major issue to improve chances for government funding are just as strong as the desire for industry to have safe products. An important difference is that the contract laboratories performing the industry-funded work do not have only one client. Their reputation as a service laboratory depends on good scientific practices and these laboratories are often under heavy regulatory and scientific scrutiny. So, let's focus on well-designed studies with proper positive and negative controls, demonstrated concentration or dose response, the right number of replicates, correct nomenclature, inclusion of all relevant references, and sound statistical analyses and not the source of funding.

Conclusions

In conclusion, there is no controversy regarding the safety of CGN; there is, however, confusion in the literature. Reviews are important as they are intended to bring all literature and all findings together for discussion. This commentary was prepared to make clear some of the misperceptions surrounding CGN and to emphasize the importance of scientific method and accuracy in the interpretation of other works. To that end, CGN is an approved food additive as determined by well-established regulatory agencies and used worldwide in numerous foods. CGN does not cause inflammatory or gastrointestinal effects when administered to animals in diet under standard protocols to evaluate safety. Confusion exists in the literature due to using the name carrageenan when the actual product tested is another material, degraded carrageenan (Poligeenan). Degraded carrageenan (Poligeenan) may cause adverse effects in animal studies and, therefore, it is NOT permitted to be used as a food additive. Additional confusion exists when considering the effects of injected CGN which are not relevant to the orally administered food additive.

Conflicts of interest

The authors have no conflicts of interest.

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