RE: Comments on "Is sodium carboxymethyl cellulose (CMC) really completely innocent? It may be triggering obesity" (https://doi.org/10.1016/j.ijbiomac.2020.09.169)

Dear Editor,

First of all, I would like to inform you that the comments in the letter about our article do not reflect the truth and I reject all of them completely. Before moving on to the comments of the author, I would like to start my answer by reminding you of the importance of the subject;

It is a known fact that obesity has become an important public health problem all over the world. Millions of people around the world struggle with this health problem. Obesity and related chronic disorders are increasing at alarming rates and it is estimated that 86 % of Americans will be overweight by 2030 [1]. Unfortunately, the mechanism underlying this health problem has not been fully elucidated. The etiology of obesity is multifactorial and involves a complex interaction between genetics, hormones, and environment [2]. Agents in our food supply have the potential to affect metabolism due to sustained exposure and potential interactions among multiple compounds [1]. "A recently hypothesized factor contributing to the obesity epidemic is our exposure to obesogens, chemicals in our environment that can disrupt metabolism and lead to accumulation of excess fat mass" said Simmons et al in their paper [1]. Therefore, it is important to evaluate every possibility that may be a potential risk factor for obesity.

New products are constantly added to the products containing CMC, which is already widely used. [3, 4]. In our study with the zebrafish model, we obtained findings that this additive, which has no clear limit for its use and is considered to be harmless (innocent), may be effective on obesity, and we shared these findings with the scientific world.

Zebrafish (*Danio rerio*) embryos, whose developmental stages have been described in detail, are a model organism frequently used in developmental toxicology studies due to their transparent properties and short development time [5]. It has also been noticed that there is a remarkable homology (60-80%) between zebrafish and human genes, as well as a high degree of similarity in the catalytic or ligand binding sites of the protein structures [6]. In this respect, zebrafish is an alternative model organism that has been used successfully in the development of various neurophysiological and metabolic conditions and problems [7]. Moreover "As a new and rigorous in vivo model, zebrafish are an increasingly valuable screening model for the ability of compounds to cause obesity" [1]. In ovipor animals, like zebrafish, the embryos use only eggs with reserves of fat droplets in the yolk as a food source, during their embryonic development stage. Because of these features, zebrafish embryos, which are forced to live dependent on yolk, can be defined as "closed systems" in which food intake is not influenced by outside [8]. In this period, the injection of food additives into the yolk sacs (that is their foods) of the embryos makes this organism a good model in which the side effects of food additives can be investigated. Thus, we chose zebrafish animal model and microinjection method in present study to evaluate side effects of CMC as an additive. *So, we did not aim to mimic any realistic human embryo/fetal exposure.* There are many publications using zebrafish to investigate the toxic or side effects of food additives [9-15].

I quote and explain the points of their comments in the following:

In reviewing this publication, IPEC toxicologists concluded that the title of the article was scientifically misleading and alarmist. Further, the data provided in this publication, was scientifically inconsistent with previously reported toxicological safety information for CMC, and provided questionable new data to support revisiting potential safety concerns for CMC. In the interest of publishing accurate scientific data to advance human risk assessment, IPEC-Americas is providing to the Editors a summary of our major concerns:

Here, I would like to remind that the evaluation of substances as "toxic", "side effect" or "potential risk factor" is very different from each other. Because the physiological, metabolic, immunological, behavioral etc. reactions of organisms against these effects are different. In the evaluation of toxic and side effects, test methods with clearer results and approved by the scientific world are used. On the other hand, the "potential risk factor" category is an evaluation criterion used for substances that are likely to be toxic or show side effects. And, it is possible to reveal the effects of these risk factors clearly and to clarify the systemic effect mechanisms in organisms as a result of scientific studies. It should also be noted that innocent substances known to have no toxicity or side effects can preserve their innocence unless scientifically demonstrated otherwise. At this point, I would like to emphasize that the real “scientifically misleading and alarmist” situation is to make a security assessment based on the literature of the 1960s and even 1940s (given in the letter) [16, 17]. In parallel with the developing technology, scientific studies are also developing with an increasing momentum. Technology has given us what we could not have seen before, what we could not have detected before, what we were not aware of before. For example, thanks to high-resolution imaging systems, we can now see and monitor functions even inside the cell. Therefore, it is important to confirm our previous (old) knowledge with new methods and techniques in order to use this blessing of technology for the benefit of humanity. In our study, we tested the toxic effect of CMC using a new method and up-to-date techniques and confirmed that it does not have toxicity in accordance with the information previously reported in the literature. (Contrary to what the author stated in the letter) In addition, we obtained findings that it may trigger obesity, again in accordance with the literature [18, 19]. And our findings were published after scientific review.

1. The route of exposure was inappropriate for this type of study with CMC. It is well-known that CMC (and other modified celluloses) is not absorbed by the human intestinal tract,1,2,3,4,5 and that this lack of absorption prevents systemic exposure. Since there is no systemic exposure, there cannot be exposure to the human embryo/fetus via either the yolk sac (through first trimester) or the placenta and maternal circulation (second and third trimesters). Directly injecting CMC into the Zebra fish yolk sac does not mimic any realistic human embryo/fetal exposure.

The microinjection method we used in our study is an accepted method in the literature to examine the effects of additives [9, 11]. The information given here with the literature of the 80s or even 60s makes us think that our article was not read correctly and misinterpreted. Because in our article, there is no data/statement that CMC causes obesity as a result of digestion or absorption. Considering that exposure is through digestion, it is obvious that suggesting that the use of zebrafish embryos is a restrictive situation would open up the zebrafish to be a model organism for discussion.

Studies have shown that emulsifiers [18, 20] such as CMC [19] could alter the gut microbiota, promote gout inflammation, promote obesity [18]. In the light of the findings we obtained in our study, we

suggested that CMC may trigger obesity through gut microbiota and gut-brain axis. This situation can be easily understood since we have examined the melanocortin signals. Meanwhile, microbiota colonization in zebrafish begins to form from after hatching [21]. As a result, the statements written here do not reflect the content of our article.

2. There is no evidence from numerous laboratory animal studies that CMC exposure results in increased body weights. CMC (and other modified celluloses) has been shown to decrease body weights in long term dietary studies.[6,7] Shelanski and Clark[8] conducted a 3-generation study feeding study that produced no effects on body weights in any generation. Further, subchronic general toxicity studies have shown no effects on body weight except at high dose levels where body weights were decreased.[1,7]

There is a general concern by the IPEC toxicologists for the misinterpretation of data supporting the contention of CMC's systemic toxicity. Specifically, the authors reference to their data and to the EFSA "Call for technical and toxicological data on sodium carboxymethylcellulose (E466) for uses as a food additive in foods for all population groups including infants below 16 weeks of age" are misinterpreted. The EFSA data call in was not based on increasing safety concerns related to the studies of Viennois et al. and Sökmen et al., but because of EU regulations and the new guidance document on infants under 16 weeks of age:[9]

> **"The reason was that the risk assessment approach followed until now by the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) in the re-evaluation of food additives does not apply to this age group[4]. The ANS Panel has, therefore, specified in its opinion that the re-evaluation of uses for this particular age group will be performed separately.**
>
> **On 31 May 2017, EFSA's Scientific Committee (SC) published a guidance document[5] on the risk assessment of substances present in food intended for infants below 16 weeks of age, enabling the ANS Panel to assess the safe use of sodium carboxymethylcellulose (E466) and of other food additives for the population group below that age"**

There are references that CMC exposure results in increase body weights [19, 22, 23] and may cause obesity [18]. On the other hand, I would like to state that the publication years of the articles (6,7,8) cited as reference in this part of the letter are 1968, 1986 and 1948, respectively. More information and study results could be examined with the current literature review.

The reason why we mentioned the EFSA call in our article is to emphasize the importance of the issue. Sökmen et al. is not related to the information given here. I don't understand why they were talking about it in the letter. This is an article about nanoplastic toxicity published by our team.

On the other hand, the information about the EFSA call in the letter does not reflect the entire text of the call. In fact, the text of this call also includes the following statements;

"For the sake of efficiency, the European Commission asked EFSA to address the above lack of data (data gaps) during its risk assessment of food additives for uses in food for young infants. Therefore specific data requirements for all uses of sodium carboxymethylcellulose (E466) are included in this call for data."

"A repeated dose study with direct oral administration of sodium carboxymethylcellulose (E466) to neonatal animals, which includes gross and histopathological examination of gastrointestinal tract, influence on the microbiota and a possible modification in the bioavailability of nutrients, that are normally contained in food for infants."

Viennois et al, criticized in the letter, showed in their study that CMC alters the microbiota composition.

3. Additionally, Baran et al. claim both that "the side effects and toxic properties have not been fully elucidated" and "…it has been reported in most studies that CMC is not toxic." In fact, the potential toxic properties of CMC have been fully investigated by ADME, acute, subchronic, chronic, genetic, development and reproductive toxicology studies, which have been thoroughly reviewed by JECFA,[1] the Cosmetic Ingredient Review Panel,[7] and EFSA.[10,11]

References 1 and 7 cited here were published in 1989 and 1986, respectively. I mentioned above that these data should be confirmed with current methods. It has already been stated in EFSA reports that the data on this subject are insufficient and most of them are old (see below). The EFSA reports given as references 10 (EFSA 2017) and 11 (EFSA 2020) in the letter include the following statements about CMC;

> *"The Panel noted that carboxy methyl cellulose was one of the food additives reported to alter the gut microbiota, promote gut inflammation, promote obesity and to impair glycaemic control in mice. (EFSA 2017)"*
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> *"No adequate specific studies addressing the safety of use of sodium carboxy methyl cellulose (E 466) in this population under certain medical conditions were available; (EFSA 2017)"*
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> *"The Panel concluded, that the available data did not allow for an adequate assessment of the safety of use of sodium carboxy methyl cellulose (E 466) in infants and young children consuming foods belonging to the categories 13.1.5.1 and 13.1.5.2. (EFSA 2017)"*
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> *"Although the data set available for the different celluloses is not complete and most of the studies were old and do not meet the current requirements of toxicological testing, the ANS Panel considered that the physico-chemical, structural, biological and kinetic similarities between the modified celluloses make it possible to apply a read-across approach among the different celluloses (EFSA 2020)."*

Although CMC has been reported to be non-toxic in most studies in the literature, there are also studies suggesting that it has side effects [18, 19, 22, 23]. Therefore, according to all this information, we can easily say that the side effects and toxic properties of this additive are not fully elucidated due to insufficient and old data. Finally, I would like to state that EFSA 2020 was published after the date we submitted the article.

4. Baran et al. also used intraperitoneal CMC exposure to justify oral exposure as a potential hazard (Klugman et al.12). In applying route-to-route extrapolation, such as going from yolk sac to oral administration, there are important pharmacokinetic and pharmacodynamic considerations that

must be considered for bridging to potential systemic toxicity, such as that intraperitoneal exposure is not interchangeable with oral exposure for a non-absorbable polymer.

We did not use intraperitoneal CMC exposure. We directly microinjected CMC to the yolk sac, not intraperitoneally. These two are completely different from each other. In addition, as I mentioned above, the microinjection method we use is an accepted method in the literature to examine the effects of additives [9, 11].

On the other hand, the author stated that the pharmacodynamic aspects of CMC should also be addressed. But in item 1 in the letter the author referred to pharmacodynamics by saying that it was not absorbed in any way. In other words, it should be questioned whether there is a difference between taking a substance that comes out without being affected by digestion or giving it in the yolk sac. If it is claimed that this polymer comes out without a significant change in the digestive system, what difference can there be when the fish are exposed to the same substance from the yolk sac? In addition, obesity mechanism does not only arise from problems at the digestive level. Ultimately, it has been declared that the gut-brain axis is an important factor in obesity. In the present study, we examined this from a molecular, physiological, behavioral and also circulatory perspective without excluding it. In this context, it is a scientific mistake to consider a disorder that is thought to have a very complex mechanism such as obesity only at the digestive level.

5. Baran et al. mis-quoted another study (Bär et al.) by stating CMC "has some negative effects in mice and rats" when Bär et al. actually concluded "the results of the present study do not conflict with the general recognition of the human safety of CMC…"[13]

Bar et al. observed the following negative effects in animals treated with CMC in their study;

1. "Plasma alkaline phosphatase (AP) and alanine aminotransferase (GPT) activities were slightly higher in males of the high-dose CMC".

2. "Gross examination at autopsy revealed the presence of caecal enlargement and an increased liquid content in the intestinal tract, particularly in male and female rats of the high-dose CMC and CMC-ENZ group"

3. "On microscopic examination, an increased incidence of dilatation of the colon was observed in females of the high-dose CMC

4. "The renal observations included an increased occur- rence of pelvic urothelial hyperplasia and pelvic nephrocalcinosis in the males of some treatment group. Corticomedullary nephrocal- cinosis was noted more frequently in females than in males and was increased significantly in the high-dose CMC group. In the bladder of male rats, an increased incidence of epithelial hyperplasia was found in the high-dose CMC and CMC-ENZ groups".

And, the authors concluded that CMC was safe in the conclusion and established the following sentence: "the human consumption of sodium with an ordinary diet is not increased significantly by the use of CMC and CMC-ENZ". In other words, the reliability of this substance according to the study was linked to the amount of consumption at that time. Today, it can be questionable by both consumption analysis and global trade data whether the current situation has changed in favor of CMC.

More importantly, these comments written by the authors do not eliminate the fact that CMC has negative effects on these experimental animals.

Summary

Consequently, IPEC-Americas is calling attention to the Editorial Board of IJBM, the authors misinterpretation of scientific data to support a sensationalist title and erroneously inflate the importance of the reported findings. This article is not in line with the Journals publication policy to maintain the highest scientific standards.

One of the duties of us scientists is to contribute to the process by conducting scientific research within our means in order to enlighten the unenlightened mechanisms for a healthier society and healthier generations. Therefore, at this point, I would like to underline that our ultimate goal is to serve humanity. It is one of our most important scientific principles to interpret accurate and reliable data through literature information and to bring them to the literature with all their transparency. Because we are aware that this information can be used as a reference by other scientists or relevant commissions. In this article, we are happy to share the findings we obtained as a result of an 8-month laboratory study with all transparency with the scientific world. And we continue our work with the same excitement. As a principle in article writing, after discussing all the findings we have obtained, we complete our article with a title that will reflect our findings and our final result and present it to scientific evaluation. Therefore, discussing the data obtained for the sake of a sensational title with a really distant and exaggerated approach and thus trying to gain an advantage away from our ultimate goal is an approach that neither I nor other researchers in our team can accept. As a matter of fact, we sincerely believe that ours is a proper title for this study.

Finally, we put forward a hypothesis in the light of the scientific data we obtained; “CMC may be triggering obesity”. I think that it would be a more scientific approach to criticize our article, in which we present this hypothesis, not with a letter that contains information that does not reflect the truth and prepared with the literature of the 60s or even 40s, but with a scientific article in which our findings are proved to be the opposite.

King regards,

Prof. Dr. Saltuk Buğrahan CEYHUN

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