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Review

# A Review on the toxicology and dietetic role of bacterial cellulose

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ABSTRACT

Bacterial cellulose (BC) is a biopolymer synthesized by certain acetic acid bacteria strains. The safety of BC regarding its potential use in food applications is here reviewed. The acute, sub-acute and subchronic oral toxicity assays showed that consumption of BC had no adverse effects in rats. Several studies demonstrated that BC is not genotoxic, did not induce chromosomal aberrations in CHO cells under both non-activating and metabolic activating conditions, is inactive in the *in vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay, had no reproductive toxicity in mice and exerted no embryotoxicity and teratogenicity effects in rats.

Several studies on the BC in biomedical applications further reinforces its safety: a primary eye and dermal irritation studies in the rabbit showed that BC was non-irritating. The inflammatory reaction to subcutaneously implanted BC has been evaluated in animal models and for different periods of time, demonstrating that BC is biocompatible and does not trigger a harsh inflammatory reaction.

Altogether, and considering its longstanding history of human consumption in Asian countries, as well as its utilization in biomedical devices, it may be concluded that BC is safe for applications in food technology.

## 1. Introduction

The determination of toxicants in foods/food substances has become increasingly important, to ensure that the benefits of the substances intended for use by humans, outweigh the risks from their use. Many countries have a well-established regulatory framework (and under constant revisions), to ensure the proper scientific evaluation of foods, food additives and ingredients, processing aids and food contacting substances, before their market approval. The toxicity tests that food operators are required to provide, for a pre-market approval of their products, depends on the type of substance, its intended use and on the regulations of a particular country. To this effect, several standard tests are available to evaluate different effects such as acute, sub-acute, subchronic and chronic toxicity, carcinogenicity, mutagenicity, reproductive and developmental toxicity, neurotoxicity, and several *in vitro* tests. Some products may require additional toxicity test such as irritancy and skin sensitization studies [1–7].

The safety of numerous kinds of plant cellulose and their derivative products has been extensively reviewed by national and international regulatory agencies such as the US Food and Drug Administration (FDA), The European Food Safety Authority (EFSA), the Joint FAO/WHO Expert Committee on Food Additives (JEFCA), the Select Committee on Generally Recognized as Safe (GRAS) Substances (SCOGS). The information provided below includes a comprehensive review on the toxicological data available for bacterial cellulose.

Bacterial cellulose (BC) is a pure cellulose exopolysaccharide produced by certain strains of acetic acid bacteria, such as those of the *Komagataeibacter* genus. The cellulose synthesized by these strains is identical to that of plants, regarding its molecular formula and polymeric structure. However, BC presents in general, a higher crystallinity. Also, BC is chemically pure, i.e. it is free of lignin, hemicelluloses and other biogenic compounds. Under static culture conditions, the synthetized BC, is presented as a gelatinous film consisting of a 3D nanofibrilar arrangement of pure cellulosic fibres (Fig. 1). These randomly assembled ribbon-shaped fibrils are less than 100 nm wide and composed of elementary nanofibrils, aggregated in bundles with lateral size of 7–8 nm; these fibrils have several micrometres in length [8–12]. The taxonomy of these bacteria [13], the BC biosynthesis [14] and potential applications in food [15–17], have been extensively reviewed.

## 2. Dietetic properties and human consumption

In Asian countries, BC is already produced at large scale and has a long history of use, being marketed under the trade name “nata de coco” [18–20]. Ever since its discovery in the eighteenth century, nata de coco gained widespread popularity in Asian countries, being first produced in large scale in the Philippines [21]. Philippines and Indonesia are the major producers and exporters of nata de coco products for human consumption. Thailand, Vietnam and Malaysia are also among the most representatives commercial producers (Phisalaphong

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**Fig. 1.** Scanning electron microscopy of cellulose pellicles and cells from G. xylinum IFO 13693, after 10 days of static culture. Reprinted from Chávez-Pacheco et al., 2005 with permission from John Wiley & Sons.

and Chiaoprakobkij 2013). Among non-traditional coconut export products in 2009, nata de coco was the second largest, earning US $6034 million from the sale of 6051 MT. The greatest market for nata de coco was Japan (77.8%) and the second largest market was the USA. From 2009–2011, the volume of nata exports from the Philippines has been within a calculated and steady average range of 6000 MT which corresponded to a market value of US$6 million [22,23]. These figures demonstrate the long-standing human consumption of nata de coco/bacterial cellulose. There are, to our knowledge, no reported cases of health related issues associated to the human consumption of BC or nata de coco, thus unequivocally supporting the claim on its safety. In fact, several studies demonstrate beneficial effects from the dietetic point of view, as observed bellow.

A study by Chau et al. [24] investigated and compared the hypolipidemic and hypocholesterolemic effects of plant cellulose and BC, specifically, the absorption and excretion of lipids and cholesterol in Golden Syrian hamsters' diets (Table 1). Three types of diets were prepared, the difference between them being the addition of bacterial cellulose on the "bacterial cellulose diet", plant cellulose on the "cellulose diet", and the "fibre-free diet" (a control diet, without fibre). All diets were supplemented with cholesterol (2.0 g/kg of diet) to induce the alimentary hypercholesterolemia in hamsters; also the insoluble fibre content was standardized to 50 g fibre/kg of diet in the diets containing fibre. For 30 days, food and drinking water were supplied *ad libitum*.

The results showed that the serum triglyceride concentrations in hamsters fed with BC and plant cellulose diets, were significantly reduced, by 55.5 and 46.6%, respectively, as compared to the fibre-free diet. No significant changes in the serum high-density lipoprotein (HDL) cholesterol concentration were observed among the three diet groups. However, significantly higher HDL/total cholesterol ratios were found for the plant cellulose and BC supplemented groups (0.60 and 0.65, respectively) versus the fibre-free group (0.50) suggesting the higher antiatherogenic potential of BC. Further, the administration of both types of cellulose to hamsters, effectively decreased the concentration of serum total cholesterol (by 17.4% with plant cellulose and by 27.9% with BC) and also decreased the serum low-density lipoprotein (LDL) cholesterol (plant cellulose: −41.9%; BC: −47.9%). The cation-exchange capacity of bacterial cellulose, 67.5 mequiv/Kg of fibre, was found to be 6-fold higher than that of plant cellulose. The higher cation-exchange capacity of BC was proposed to better entrap, destabilize and disintegrate a lipid emulsion, leading to a decrease in diffusion and absorption of cholesterol and lipids. By the end of the experiment, no significant variations in the mass of the hamsters' visceral organs including small intestine, cecal wall, colon plus rectum, liver and kidney, among the three types of diets, were observed. Chemical analysis of the hamsters' liver tissues revealed that the addition of BC to the diet was more effective in reducing the concentration of the liver total lipids (-10.3%) and liver cholesterol (-16.3%) than with plant cellulose (-6.5% and −11.8% respectively), as compared to the fibre-free diet. Analysis of the hamsters' faeces showed that, as compared to the "fibre-free diet" group, the group fed with plant cellulose and BC had an increase in the excretion of total lipids (plant cellulose: +44%; BC: +82%), cholesterol (plant cellulose: +36%; BC: +103%) and bile acids (plant cellulose: +159%; BC: +379%). Also, the faecal moisture content of hamsters fed with BC was higher than those fed the fibre-free and plant cellulose diets (+37% (BC) and +20% (plant cellulose)). With the addition of plant cellulose and BC to fibre-free diet, the faecal dry weight increased by +42% and +49%, respectively. No significant differences in the faecal dry weight were observed between the plant cellulose and BC groups. The results thus indicated that BC was able to incur a higher output of total lipids, cholesterol and bile acids in faeces than plant cellulose.

Okiyama et al. [25] studied the faecal excretion and transit time of BC in rats for up to 16 days (Table 1). Eight weeks old male Wistar rats were fed with a diet containing 5% of BC, or plant cellulose powder or guar gum. Feeding was provided twice a day and drinking water was supplied *ad libitum*.

Rats fed with BC-containing meals showed the greatest increase (+223%) in faecal weight. Addition of BC to the diet decreased the transit time by 50%, as compared to no fibre diet group. There were no differences on lipoprotein cholesterol levels in plasma (total cholesterol, HDL, and LDL fractions) between the dietary fibres group and fibre-free diet (control). The guar-meal gum group had significantly lower lipoprotein cholesterol levels, as compared to the dietary fibre groups. Both BC and guar decreased (-52%) neutral sterol excretion in faeces and increased (+106%) faecal bile acid excretion. The proportion of coprostanol to total neutral sterols in the cecum was not significantly different between rats fed with BC and those fed with the fibre-free diet.

Mesomya et al. [26] compared the serum triglyceride and the serum cholesterol lowering effect of five kinds of dietary fibre diet on weanling male Sprague-Dawley rats (Table 1). These diets had different fibre and nutrient proportions: diet 1 was had a total of 33% (m/m) dietary fibre from unpolished rice, mung bean, sweet corn and 22% BC; Diet 2 had 60% fibre from the same plant sources and 40% BC. Diets 3, 4 and 5 had 100% apple pectin, plant cellulose and casein respectively. Cholesterol content was of 13%, 11.4%, 14.2%, 14.1% and 13.5% mg/100 g in diets 1, 2, 3, 4 and 5, respectively. After four weeks of study, diet 2 gave the best lowering effect of serum triglyceride in rats, as compared with those fed with apple pectin (diet 3) and cellulose (diet 4), even though the total dietary fibre content in diet 2 (2.86%) was lower than that of apple pectin diet 3 (7.76%) and of plant cellulose diet 4 (10.39%). Diet 2 however, had no effect in lowering serum cholesterol levels.

Mesomya et al. [27] investigated the effects of the cereal and BC supplementation on the serum lipids of hyperlipidemic human subjects for a period of 24 weeks: 4 weeks without (as the control) and 20 weeks with supplementation (Table 1). The supplements (15 g) were given twice daily for these 20 weeks, and consisted of 40% (m/m) BC, 6% (m/m) unpolished rice, 36% (m/m) sweet corn and 18% (m/m) mung bean. After 20 weeks, the subjects who complied with the dietary assignment (≥90% of the time; 15 subjects) were classified as group A, and those with < 90% (7 subjects), as group B. During the first four weeks (control) the subjects showed no significant changes in serum lipid levels. Afterwards, Group A showed gradually decreasing levels of serum total triglyceride (TC). By week 16/20 under supplementation, the serum total cholesterol (TC) level decreased by 20%.

A summary of the above-mentioned studies is present in Table 1.

**Table 1**
Summary of the studies on the physiological role of bacterial cellulose (BC).

| Type of study | Animal model | Meal plan | Main results | Ref. |
|---|---|---|---|---|
| Hypolipidemic and hypocholesterolemic effect of BC | Golden Syrian hamsters | Meal incorporating:<br>– BC (50 g fibre/kg of diet), or<br>– Plant cellulose (50 g fibre/kg of diet), or<br>– No fibre (control)<br>– All diets were supplemented with cholesterol (2.0 g/kg of diet) | BC diet allowed the highest reduction of:<br>– serum triglyceride (-55.5%)<br>– serum total cholesterol (-27.9%)<br>– LDL cholesterol (-47.9%)<br>– liver total lipids (-10.3%)<br>– liver cholesterol (-16.3%)<br>BC diet allowed the highest faecal increase of:<br>– excretion of total lipids (+82%)<br>– cholesterol (+103%)<br>– bile acids (+379%)<br>– moisture (+37%)<br>Both BC and plant cellulose increased the faecal dry weight (+49%) | Chau et al. [24] |
| Effect of BC on faecal excretion and transit time | Wistar rats | Meal incorporating:<br>– BC, or<br>– Plant cellulose, or<br>– Guar gum<br>– No fibre (control) | BC diet allowed:<br>– the highest increase in faecal mass (+223%)<br>– the highest decreased in faecal transit time (-50%)<br>Both BC and guar decreased (-52%) neutral sterol excretion bile acid excretion in faeces and increased (+106%) faecal bile acid excretion Fibre-based diets had no effect on lipoprotein cholesterol levels in plasma (total cholesterol, HDL, and LDL fractions), as compared to the control | Okiyamaet al. [25] |
| Effect of BC on serum triglyceride and the serum cholesterol lowering effect | Sprague-Dawley rats | Meal incorporating:<br>– Diet 1: unpolished rice, mung bean, sweet corn and BC (22%), cholesterol (13%), or<br>– Diet 2: fibre from the same plant sources and BC (40%), cholesterol (11.4%), sucrose, or<br>– Diet 3: apple pectin, cholesterol (14.2%) or<br>– Diet 4: plant cellulose, cholesterol (14.1%)<br>– Diet 5: Casein, cholesterol (13.5%) (Control) | Diet 2 (40% BC) diet 2 gave the best lowering effect of serum triglyceride in rats, as compared other fibre-rich diets Diet 2 had no effect in lowering serum cholesterol levels | Mesomya et al. [26] |
| Effects of cereal and BC on serum lipids | Human subjects | Meal incorporating:<br>– No supplementation; 4 weeks (control)<br>– 15 g of cereal and BC; 20 weeks | Cereal and CB supplementation reduced the:<br>– Serum TG level (-20%)<br>in subjects who complied (> 90%) with the diet regimen | Mesomya et al. [27] |

## 3. Acute and sub-acute oral toxicity

Schmitt et al. [28] tested the acute oral toxicity of a commercial product named Cellulon® fibre (dried BC:sucrose at a ratio of 1:1) in both sexes of Sprague-Dawley rats (Table 2). Rats were fed a single oral dosage of 2000 mg Cellulon/Kg of body weight (bw), via oral intubation

No deaths occurred during the study that lasted for 15 days. Clinical signs of gasping respiration and/or hunched posture were observed in two males through day 2. All males appeared normal from day 4 through day 14. The clinical observations appeared to be mechanical responses to the dosing regimen, rather than responses to the test material. However, a microscopic evaluation of tissues was not conducted. All female rats were normal throughout the study. No gross pathologic lesions were observed in any of the animals at necropsy.

Li-ming et al. [29] evaluated the acute oral toxicity of nata de coco (BC) in both sexes of Kunming mice, according to the Maximum Tolerated Dose (MTD) method (Table 2). A total dosage of 15.0 g/kg bw, equally split in two meals, was administered to the mice by gavage, at 4 and 6 h. In all mice, no abnormal symptoms or death were observed. Also, anatomical observation of the organs were normal. The maximum tolerance of 15.0 g/kg bw, was considered to be non-toxic.

Hagiwara et al. [30] evaluated the effect of BC sub-acute administration to both sexes of F344 rats for 28 days (Table 2). For this, a commercial product labelled “fermented cellulose” (composed of 60% BC, 20% carboxymethyl cellulose (CMC) and 20% sucrose) was incorporated at different proportions (0, 1.25, 2.5, and 5.0%) into the rat’s stock powdered diet.

No treatment-related deaths were observed during the 28 days of the experiment and no treatment-related clinical signs were noted in any of the treated animals. No significant variation from control body weights was noted in any of the treated groups. There were also no clear inter-group differences in food or water consumption. On urinalysis, a significant elevation of sodium was noted in males only of the 5.0% group; however, the values were within the historical control ranges. No treatment-related ophthalmological abnormalities were found in any animals of either control or treated groups. No treatment-related adverse effects were apparent from the haematology results. On blood biochemistry, statistically significant elevation of alanine aminotransferase was noted in males of the 2.5 and 5.0% groups. No other treatment-related changes were apparent from the blood biochemistry results. No treatment-related macroscopic changes were found in treated animals at necropsy. Statistically significant elevation of relative salivary gland weights was noted in both sexes of the 5.0% group and of relative kidney weights and relative adrenal weights in 5.0% females. Despite the statistically significant increases in cecum, salivary gland, kidney, and adrenal weights, observed in both sexes given 5.0%, these observations were not associated with any histopathological alterations. Increased cecum weights in this study was considered a physiological adaptation related to the ingestion of large amounts of modified starch, fibrous ingredients, or other carbohydrates which are poorly absorbed and have a high osmotic nature. Also, no histopathological findings related to test material treatment were observed in the other organs examined. The No Adverse Observed Effect Level (NOAEL) was set at the highest dose of 5.0% “fermented cellulose” in the feed, equivalent to 5331 mg/kg bw/day for males and 5230 mg/kg/day for females.

Li-ming et al. [29] also evaluated the sub-acute oral toxicity of nata de coco (BC) in Kunming mice, for 30 days (Table 2). The assay group was fed with 0 (control) 1.3, 2.5, and 5.0 g/kg bw. The animals in the dose group were given 1.3, 2.5 and 5.0% cocoa. During the experiment, no changes in the growth, development body weights was noted in any of the treated groups; also, no inter-group differences in food or water consumption was noted and no treatment-related deaths and clinical signs were observed. Haematological analysis showed that no inter-group differences were noted in haemoglobin, red blood cell count and leukocytes. The same observations were recorded for blood serum albumin, alanine aminotransferase, alanine aminotransferase, aspartate aminotransferase, creatinine, cholesterol, triglyceride, blood glucose and albumin. Histopathological examination of the various groups, showed no abnormal changes.

A summary of the above-mentioned studies is present in Table 2.

## 4. Sub-chronic toxicity

Schmitt et al. [28] studied the subchronic toxicity of BC (Cellulon® fibre) (Table 2). For 13 weeks, test and positive control animals (Sprague-Dawley rats) received meals containing either BC or microcrystalline cellulose (MCC), at levels of 0.5 (low dose group) or 10% (high dose group) in the diet. Control animals received the same diet without bacterial cellulose or MCC.

The results from this study revealed that there were no deaths attributable to treatment with Cellulon, MCC or control. Clinical observations noted during the course of the study (e.g., malocclusion, lacrimation, alopecia) were not indicative of toxic effects. No statistically significant differences were observed in mean body weight or mean body weight gain of male or female rats, when comparisons were made between test and control groups. Food consumption generally increased in animals fed with 15% (MCC) and 10% Cellulon), when compared with the control group. This increase in food consumption was expected as the animals adjusted for the altered nutritional value of the diet, as a result of the relatively high test article concentrations in the feed. However, there were no consistent differences in food consumption between groups fed with Cellulon and MCC. Cellulon intake by the 5% and 10% treatment groups was calculated to be of 3200 and 7000 mg/kg/day, respectively, for male rats and 4000 and 8500 mg/kg/day, respectively, for female rats. MCC intakes were similar to those of Cellulon. Statistically significant differences in haematology parameters included increased mean cell haemoglobin and haematocrit, platelets and monocytes. Evaluation of serum chemistry parameters revealed slight but significant decreases in total protein, albumin, total cholesterol, and calcium. None of the aforementioned significant differences were considered to be toxicologically important, due to a lack of dose-response relationships, relatively low magnitude of change, lack of differences between the control group and Cellulon-treated groups, and/or lack of important changes in related clinical parameters. There were no notable gross pathologic findings at necropsy. Cellulon and MCC treatment had no effect on organ weights. Microscopic evaluation of tissues revealed no unusual lesions or patterns of distribution that might suggest an effect of exposure to Cellulon or MCC. Furthermore, no histomorphologic alterations of the gastrointestinal tract were evident.

Li-ming et al. [29] evaluated the subchronic 30-day oral toxicity of BC on SD male and female rats (Table 2). The sample dosage was designed to be of 1.3, 2.5, 5.0 g BC/kg bw. The control group was fed with a normal diet. During the experiment, the growth and development of the animals in each group was normal; there were no death observed in any group. No clinical symptoms were deemed related to the feeding of BC. No difference among groups on organ weight and organ/body weight ratio were observed. There were no significant differences in the total weight gain, total food intake and total food consumption between male and female rats, as compared to the control group. Regarding haematological indicators, feeding BC had no obvious effect on rats’ haemoglobin, red blood cell count or white blood cell count. Also, rats fed with BC had similar values of serum albumin, alanine aminotransferase, alanine aminotransferase, aspartate aminotransferase, creatinine, cholesterol, triglyceride, blood glucose, albumin as that of the control group. Regarding the histopathological examination, no abnormal changes were found between the various groups. In the high dose group and the control group, vacuolization and hepatic blood stasis was observed. The liver serosa was intact, and the central vein, hepatic lobule and portal area were clear. The hepatocellular cord

**Table 2**
Summary of the acute, sub-acute and sub-chronic oral toxicity studies with bacterial cellulose.

| Type of study | Animal model | Dosages | Main results | Ref. |
|---|---|---|---|---|
| Acute oral toxicity | Sprague-Dawley rats | Single oral dosage of 2000 mg/Kg of body weight (bw), corresponding to 1000 mg/kg bw | After 15 days:<br>– No deaths occurred during the study;<br>– No gross pathologic lesions were observed in any of the animals at necropsy | Schmitt et al. [28] |
| | Kunming mice | Two oral dosages were fed at 4 and 6hr, totaling 15.0 g/kg bw | No deaths occurred during the study; Anatomical observation of the organs were normal | Li-ming et al. [29] |
| Sub-acute oral toxicity | F344 rats | Meals incorporating: 0, 1.25, 2.5, and 5.0% "fermented cellulose" (60% BC, 20% carboxymethyl cellulose (CMC) and 20% sucrose) | After 28 days: There were no<br>– treatment-related deaths and clinical signs;<br>– variation in any of the treated groups.<br>– inter-group differences in food or water consumption.<br>– treatment-related ophthalmological abnormalities<br>– treatment-related adverse effects were apparent from the haematology results.<br>On blood biochemistry, alanine aminotransferase was higher in males of the 2.5 and 5.0% groups;<br>Statistically significant elevation of relative salivary gland weights was noted in both sexes of the 5.0% group and of relative kidney weights and relative adrenal weights in 5.0% females;<br>The No Adverse Observed Effect Level (NOAEL) is set at the highest dose of 5.0% "fermented cellulose" in the feed, equivalent to 5331 mg/kg bw/day for males and 5230 mg/kg/day for females. | Hagiwara et al. [30] |
| | Kunming mice | The assay group was fed with 0 (control) 1.3, 2.5, and 5.0 g "fermented cellulose"/kg bw. The animals in the dose group were given 1.3, 2.5 and 5.0% cocoa. | After 30 days:<br>– No inter-group differences were noted in<br>– the growth, development and body weights<br>– food or water consumption<br>– deaths and clinical signs<br>– haemoglobin, red blood cell count and leukocytes.<br>– blood serum albumin, alanine aminotransferase, alanine aminotransferase, aspartate aminotransferase, creatinine, cholesterol, triglyceride, blood glucose and albumin<br>– histopathological examination related to BC consumption | Li-ming et al. [29] |
| Sub-chronic toxicity | Sprague-Dawley rats | 13 weeks assay Assay: meals containing either BC or microcrystalline cellulose (MCC), at levels of 0.5 (low dose group) or 10% (high dose group) in the diet. Control assay: same diet without BC or MCC. | No deaths attributable to treatment with BC, MCC or control; Malocclusion, lacrimation, alopecia were not indicative of toxic effects;<br>No differences were observed in the mean body weight or mean body weight gain of male or female rats, made between test and control groups;<br>No dose-response relationships were attributed to differences in cell haemoglobin and haematocrit, platelets and monocytes, between the assay and control groups;<br>No gross pathologic findings at necropsy, in all groups;<br>BC and MCC treatment had no effect on organ weights;<br>No lesions or patterns of distribution that might suggest an effect of exposure to BC or MCC or Cellulon;<br>No histomorphologic alterations of the gastrointestinal tract were evident between all groups | Schmitt et al. [28] |
| | Sprague-Dawley rats | 30 days assay<br><br>Assay: meals containing 1.3, 2.5, 5.0 g BC/kg bw<br><br>Control: no BC in feed | No deaths were observed in any group;<br><br>No clinical symptoms were deemed related to the feeding of BC; No difference among groups on organ weight and organ/body weight ratio were observed;<br>No significant differences in the total weight gain, total food intake, total food consumption between male and female rats, as compared to the control group;<br>Feeding BC had no obvious effect on rats' haemoglobin, red blood cell count or white blood cell count;<br>Rats fed with BC had similar values of serum albumin, alanine aminotransferase, alanine aminotransferase, aspartate aminotransferase, creatinine, cholesterol, triglyceride, blood glucose, albumin as that of the control group;<br>No abnormal changes were found between the various groups. In the high dose group and the control group, vacuolization and hepatic blood stasis was observed.<br>Liver serosa was intact, and the central vein, hepatic lobule and portal area were clear;<br>Hepatocellular cord arranged radially around the central vein;<br>The structure of the renal capsule was complete, the glomeruli of the cortex, the structure of the renal capsule was clear;<br>The structure of the gastrointestinal serosa, muscularis, mucosa, and submucosal layer was also clear;<br>The spleen capsule was complete. Testicular and ovarian albuginea integrity was maintained;<br>Visible levels of spermatogenic cells were also recorded | Li-ming et al. [29] |

arranged radially around the central vein. The structure of the renal capsule was complete, the glomeruli of the cortex, the structure of the renal capsule was clear; the structure of the gastrointestinal serosa, muscularis, mucosa, and submucosal layer was also clear; the spleen capsule was complete. Testicular and ovarian albuginea integrity was maintained. Visible levels of spermatogenic cells were also recorded.

A summary of the above-mentioned studies is present in Table 2.

## 5. Genotoxicity & reproductive and developmental toxicology

### 5.1. Single cell gel electrophoresis assay (comet assay)

Moreira et al. [31] studied the *in vitro* genotoxicity of BC (Table 3). For this, the DNA integrity of Chinese hamster ovary (CHO) cells, grown in the presence of different BC concentrations (0.1, 0.5 or 1 mg/ml), was evaluated by alkaline single cell gel assay (also known as comet assay). Cells were incubated with BC suspensions for 48 h. Hydrogen peroxide (100 mM) and water were used as positive and negative controls, respectively. Damage to DNA was evaluated by image analysis using the "Comet Assay IV version 4.2" image analysis system. Cells grown on a BC membrane were also tested as a control. The results showed that the DNA damages in the presence of BC fibres are similar to the negative control for each BC concentration. Around 95% of cells showed none or insignificant DNA damage (comet class 0 and 1). Regarding the comet parameters obtained from image analyses, (tail length, tail migration, percent tail DNA and tail moment), BC fibres did not induce DNA damages under the concentrations tested, as compared to the negative and positive control. The results from visual scoring and image analysis overall showed that, under the range of tested conditions, BC was not genotoxic.

### 5.2. Salmonella/microsome mutagenicity assay ("Ames test")

Schmitt et al. [28] studied the potential of BC in Cellulon to induce gene mutations in a bacterial reverse mutation test with *Salmonella typhimurium* strains TA 89, TA 100, TA 1535, TA 1537, and TA 1538 in the absence and presence of a metabolic activation system (Table 3). Cellulon, a mixture of BC:sucrose (1:1), was suspended in deionized water and tested in a standard incorporation assay at 0, 66.7, 100, 333, 667, 1000, and 2500 μg/plate in each tested strain, with and without metabolic activation. The maximum dose tested was limited by the viscosity of BC. The results indicate that BC did not cause an increase in the number of histidine revertants (mutations) per plate in any bacterial strain, either in the presence or absence of S9 microsomal enzymes.

Moreira et al. [31] studied the mutagenic potential of BC nanofibers using the bacterial reverse mutation assay, using four strains *Salmonella tryphimurium* (TA97a, TA98, TA100 and TA102) (Table 3). The test was conducted in the presence or absence of a S9 mixture, using 0.1, 0.5 or 1.0 mg/ml of a bacterial cellulose suspension. The mutagenicity of BC was evaluated according to the following parameters: the maximum number of revertants in the presence of BC should be 2-fold or more relative to the negative control; a dose-dependent increase in the number of revertants should be observed. The results obtained, in the presence of BC without S9 mixture, correspond to the spontaneous reversion for each strain and are similar to those obtained to negative control. In the presence of S9 mixture, an increase of revertant colonies per plate, for the TA98 and TA100 strains, was detected as compared with control; however, the increases were in each case $<$ 2-fold and did not appear to be dose-related. It was concluded that, under the conditions tested, BC does not present a mutagenic behaviour [31].

Hagiwara et al. [30] evaluated the mutagenic potential of nata de coco (BC) in mutant strains of *S. typhimurium* (TA97, TA98, TA100, TA102), according to the norm GB 15193-2003 (Table 3). For this, SPF-grade Sprague-Dawley (SD) rats' liver S9 mixture was used as the exogenous metabolic activation system. Five control groups (at 8, 40, 200, 1000 and 5000 μg CB/dish) were set up. The criteria for a positive response were a $\geq$ two-fold increase in the average plate count compared with the solvent control for at least one concentration level and a dose response over the range of tested concentrations in at least one strain with or without S9. Results showed that the numbers of colonies of each group at any BC dose, with or without S9 did, did not exceed twice of those of spontaneous reverse mutation group. Reversion mutation colonies did not grow with increasing dosages of BC, when compared to the solvent plates, indicating that no dose-response relationship was reflected. BC did not show any mutagenic activity under the experimental conditions.

### 5.3. Cytogenetic assay measuring chromosomal aberration frequencies

Schmitt et al. [28] also performed cytogenetic assays with BC from Cellulon (Table 3). For this, CHO cells were grown in a McCoy's 5a culture medium. The assays were conducted with and without metabolic activation. Target concentrations of 0.333 μg/ml to 10,000 μg/ml Cellulon in McCoy's Sa culture medium, in a half-log series were tested in range-finding assays. Cytotoxicity and cell cycle kinetics were evaluated, and the results were used to determine the dose levels in the chromosomal aberrations assay. Results from this studied showed that no significant increase in cells with chromosomal aberrations was observed at the Cellulon's concentrations analysed. The BC in Cellulon was considered negative for inducing chromosomal aberrations in CHO cells under both non-activation and metabolic activation conditions.

### 5.4. Unscheduled DNA synthesis (UDS) assay

Unscheduled DNA Synthesis assay was performed by Schmitt et al. (1991) with BC from Cellulon, using rat primary hepatocytes. The UDS assay was initiated by replacing the media in the culture dishes with 2,5 mL WMEI containing about 10 μCi/ml 3H-thymidine (50 Ci/mmol) and Cellulon at concentrations of 501, 1000, 2000, 3010, 4010, and 5010 μg/ml in WMEI culture medium). BC from Cellulon was shown not to induce significant changes in the nuclear labelling of rat primary hepatocytes within the range of tested concentrations. None of the criteria used to indicate UDS were approached by any of the analysed treatments and no dose-related response was observed. However, the assay system was demonstrated to be highly responsive to the positive control, 2-acetylanunofluorene which provided conclusive evidence of the validity of the assay and the lack of UDS induction by BC from Cellulon. In summary, BC was evaluated as inactive in the in vitro Rat Primary Hepatocyte UDS Assay.

### 5.5. CHO/HGPRT forward mutation assay

Schmitt et al. [28] performed a Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl-transferase Forward Mutation Assay for the detection of mutagens in CHO-KI-BH4 cells (Table 3). BC from Cellulon was not cytotoxic in either mutation assay (with or without S9 metabolic activation) within the range of tested concentrations. The mutant frequencies of treated cultures varied randomly with Cellulon dose, within the range acceptable for background mutant frequencies which is less than $15 \times 10^{-6}$. Of the 14 cultures treated with Cellulon, only one culture, in the activation mutation assay, had a mutant frequency that was statistically elevated over the mutant frequencies of the concurrent vehicle control cultures. This observation is consistent with normal variation in background mutant frequency in independent cultures. Therefore, BC was considered negative for inducing forward mutations at the HGPRT locus in CHO cells under both nonactivation and S9 metabolic activation conditions.

### 5.6. Limulus amebocyte iysate (LAL) assay

Schmitt et al. [28] assayed the pyrogenicity of BC in Cellulon by using the Limulus amebocyte Iysate (LAL) assay (Table 3). As negative

**Table 3**
Summary of the genotoxicity & reproductive toxicology studies with bacterial cellulose.

| Type of study | Cell line/animal model | Dosages | Main results | Ref. |
|---|---|---|---|---|
| *In vitro* | | | | |
| Comet assay | Chinese hamster ovary (CHO) cells | Assay: 0.1, 0.5 or 1 mg BC/ml<br>Positive control: hydrogen peroxide (100 mM)<br>Negative control: water | DNA damages in the presence of BC fibres are similar to the negative control for each BC concentration;<br>Around 95% of cells showed none or insignificant DNA damage (comet class 0 and 1) | Moreira et al. [31] |
| Ames test | *Salmonella typhimurium* (TA 89, TA 100, TA 1535, TA 1537, TA 1538) with and without metabolic activation | Assay: 0, 66.7, 100, 333, 667, 1000, and 2500 μg/plate<br>Positive controls used without metabolic activation:<br>– 2-nitrofluorene (TA 98, TA 1538)<br>– sodium azide (TA 100, TA 1535)<br>– ICR-191 with TA 1537<br>– Positive controls with metabolic activation:<br>– 2- aminoanthracene was used with all strains | BC did not cause an increase in the number of histidine revertants (mutations) per plate in any bacterial strain, either in the presence or absence of S9 microsomal enzymes | Schmitt et al. [28] |
| | *Salmonella tryphimurium* (TA97a, TA98, TA100 and TA102) | Assay, with and without S9 mixture: 0.1, 0.5 or 1.0 mg BC/ml<br>Negative control: distilled water<br>Positive controls:<br>– 0.1 μg/plate 4-nitroquinoline 1-oxide (TA97a, TA98)<br>– 5.0 μg/plate sodium azide (TA100)<br>– 0.5 μg/plate mytomicyn C (TA102) | The results obtained, in the presence of BC without S9 mixture, correspond to spontaneous reversion for each strain and are similar to those obtained to negative control;<br>In the presence of S9 mixture, an increase of revertant colonies per plate, for the TA98 and TA100 strains, was detected as compared with control; however, the increases were in each case < 2-fold and did not appear to be dose-related | Moreira et al. [31] |
| | *S. typhimurium* (TA97, TA98, TA100, TA102) | Assay: 8, 40, 200, 1000 and 5000 μg CB/dish<br>Positive controls without S9: 9-fluorenone, sodium azide, mitomycin C;<br>Positive controls with S9: 1,8-dihydroxy anthraquinone, 2-amino fluorine | The numbers of colonies of each group at any BC dose, with or without S9 did, did not exceed twice of those of spontaneous reverse mutation group;<br>Reversion mutation colonies did not grow with increasing dosages of BC, when compared to the solvent plates, indicating that no dose-response relationship was reflected. | Hagiwara et al. [30] |
| Cytogenetic Assay | CHO cells | Assay: 0.333 μg/ml to 10,000 μg/ml Cellulon in McCoy's Sa culture medium<br>Positive controls: mitomycin C, nonactivation series; cyclophosphamide, metabolic activation series | No significant increase in cells with chromosomal aberrations was observed at the concentrations analysed;<br>BC was considered negative for inducing chromosomal aberrations in CHO cells under both non-activation and metabolic activation conditions | [28] |
| UDS[a] Assay | Rat primary hepatocytes | Assay: replacement of the culture media with<br>– 2,5 mL WMEI with 10 μCi/ml 3H-thymidine (50 Ci/mmol), BC (501, 1000, 2000, 3010, 4010, and 5010 μg/ml)<br>Positive controls: (2- acetylaminofluorene)<br>Negative control:<br>– WMEI with 10 pCi/ml 3H-TdR,<br>– WMEI with sucrose | BC did not to induce significant changes in the nuclear labelling of rat primary hepatocytes within the range of tested concentrations;<br>None of the criteria used to indicate UDS were approached by any of the analysed treatments and no dose-related response was observed | |
| CHO/HGPRT[b] Forward Mutation Assay | CHO-KI-BH4 cells | Assay with and without S9 metabolic activation: BC at 0.098-5.0 mg/ml, in F12 culture medium<br>Negative control: Sucrose<br>Positive control: (nonactivation assay, 5-bromo-2′ deoxyuridine (BrdU)<br>Metabolic activation: 3-methylcholanthrene | BC was considered negative for inducing forward mutations at the HGPRT locus in CHO cells under both nonactivation and S9 metabolic activation conditions | |

(*continued on next page*)

**Table 3** (*continued*)

| Type of study | Cell line/animal model | Dosages | Main results | Ref. |
|---|---|---|---|---|
| LAL[c] assay | | Assay: BC (0.5% Cellulon fibre, 99.5% water)<br>Negative: sterile water and endotoxin | BC was negative for the presence of gram-negative bacterial endotoxin (< 0,25 EU/ml) | |
| Mouse sperm abnormality test | Kunming male mice | Assay: BC meals with 1.3, 2.5, 5.0 g/Kg bw, through oral gavage;<br>Negative control: 1% CMC<br>Positive control: cyclophosphamide, (40 mg/kg bw) | No significant differences in the rate of sperm abnormality between each BC dosage group and the solvent control group (CMC);<br>There was a significant difference between the positive control group (cyclophosphamide) and the solvent control group | Li-ming et al. [29] |
| Teratogenicity test | Fertilized SD rats | Assay: during gestation (7–16 days), dosage groups received oral gavage of 1.0, 2.0, 4.0 g BC/kg;<br>Control group: 10.0 mL/kg bw of 1% CMC | No deaths and no gross anatomical abnormalities were observed to any pregnant rat in all groups;<br>No abnormalities in the anatomy of the rats in each dose group;<br>No significant differences in the:<br>– weight and weight gain of pregnant rats,<br>– placental weight,<br>– incidence of foetal and stillbirth in pregnant rats,<br>– foetal body length and tail length,<br>– absorption rate (0–5.8%), rate of stillbirth (0%), rate of malformation (0%), rate of visceral deformity (0%),<br>– litter size and skeletal deformities,<br>between each dose group and the control group | |
| In vivo | | | | |
| Mouse bone marrow micronucleus assay | Kunming mice | Assay: oral gavage of 1.3, 2.5, 5.0 g BC/Kg bw<br>Negative control: 1% CMC<br>Positive control: cyclophosphamide, at 40 mg/kg bw | No significant differences in the incidence of micronucleus in the bone marrow of female and male mice in each dose group, as compared to the solvent control group (CMC);<br>The micronucleus rate in the positive control group (cyclophosphamide) was significantly higher than that of the CMC group | Hagiwara et al. [30] |

[a] Unscheduled DNA Synthesis.
[b] Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl-transferase.
[c] Limulus amebocyte Iysate.

control, sterile water and endotoxin dilutions, that labelled sensitivity of the lysate, were used. BC was negative for the presence of gram-negative bacterial endotoxin (< 0,25 EU/ml).

In this paper, no details were provided on the purification procedure for Cellulon. Lipopolysaccharide (LPS) is a characteristic pathogen-associated molecular pattern (PAMP) present in the outer membrane of all Gram-negative bacteria. LPS is widely known for triggering an extremely violent and uncontrolled immune response. Once LPS is recognized in the blood stream, it causes severe cytokine-mediated damage, ultimately leading to death [32]. Along with native bacteria removal, remnants from the culture media, BC purification methods must ensure the absence of LPS in the final product, if it is to be used in food and biomedical applications. Usually this is achieved through the use of alkali solutions, sodium dodecylsulphate, high temperature and sterilization [33–35].

### 5.7. Mouse sperm abnormality test

Li-ming et al. [29] performed the mouse sperm abnormality test, to detect reproductive toxicity, on 25 Kunming male mice, divided into 5 groups (Table 3). Dose design was as follows: BC concentrations were 1.3, 2.5, 5.0 g/Kg bw, through oral gavage; the negative control group was fed with 1% CMC and the positive control group, cyclophosphamide, at 40 mg/kg bw. Results from this test showed that, after 35 days, there were no significant differences in the rate of sperm abnormality between each BC dosage group and the solvent control group (CMC), but there was a significant difference between the positive control group (cyclophosphamide) and the solvent control group. These results suggest that BC did not induce any reproductive toxicity, under the conditions tested.

### 5.8. Bone marrow cell micronucleus test

Li-ming et al. [29] performed the mouse bone marrow micronucleus assay (Table 3). For this, Kunming mice were divided in groups 3 groups of 10 each (in each group, half male and half female). Three BC concentrations were tested: 1.3, 2.5, 5.0 g/Kg bw, through oral gavage. The negative control group was fed with 1% CMC, whereas cyclophosphamide, at 40 mg/kg bw, was fed to positive control group. Mice were fed twice, with a 24 h interval between meals, at a dosage of 20 mL/kg bw. Six hours after the last administration, the animals were euthanized. Results from the bone marrow micronucleus test showed that there were no significant differences in the incidence of micronucleus in the bone marrow of female and male mice in each dose group, as compared to the solvent control group (CMC), but the micronucleus rate in the positive control group (cyclophosphamide) was significantly higher than that of the CMC group. These results indicated that the dry powder of BC was not mutagenic for mice.

### 5.9. Teratogenicity test

Li-ming et al. [29] performed the teratogenicity tests on fertilized SD rats, according to the norm GB 15193-2003 (Table 3). Rats were randomly divided into four groups of 12. In the gestation of 7–16 days, the dosage groups received oral gavage of 10.0 mL/kg bw at 1.0, 2.0, 4.0 g BC/kg, while the control group, received 10.0 mL/kg bw of 1% CMC. During the gestation, no deaths and no gross anatomical abnormalities were observed to any pregnant rat in all groups. There were no abnormalities in the anatomy of the rats in each dose group. There were no significant differences in the weight of pregnant rats, placental weight, weight gain, on the incidence of foetal and stillbirth in pregnant rats compared with solvent control group. There were also no significant differences in foetal body length and tail length, between each dose group and control group. There were no significant differences in the absorption rate (0–5.8%), the rate of stillbirth (0%), the rate of malformation (0%), the rate of visceral deformity (0%), litter size and skeletal deformities, between each dose group and the control group. The results obtained indicated that BC (nata de coco) showed no embryotoxicity and teratogenicity.

A summary of the above-mentioned studies is present in Table 3.

### 5.10. Lung, eye and dermal toxicity

Schmitt et al. [28] evaluated the primary eye and dermal irritation of Cellulon, on adult female New Zealand White rabbits. Fifty mg of the Cellulon powder was placed in the conjunctival sac of the left eye of each rabbit. The upper and lower lids were gently held together for 1 s following instillation, to prevent the loss of material and then released. The right eye of the rabbit remained untreated and served as the control. All rabbits survived to study termination. Minor conjunctival irritation was observed in several animals after 1 h post-treatment. The observable irritation (in 4 out of 6 animals) was characterized as Grade 1 for redness and Grade 1 for chemosis (2 out of 6 animals). The redness subsided in all but one animal at 24 h post-dose observation and in all animals after 72-h observation. Chemosis persisted in one animal at 24 h post-dose observation, but was absent at 48 h evaluation. No irritation was noted in the cornea or iris. Due to the dry, granular form of Cellulon fibre, the resultant irritation was considered to be of mechanical nature.

For the primary dermal irritation study, Cellulon fibre (0.5 g in 0.5 mL of tap water) was applied to an area (approximately 2 × 2 inches) of the dorsal surface of the intact, shaved skin of rabbits and held in place with a taped gauze patch and non-absorbent binding, for 4 h. The patch was then removed, and at certain intervals, the degree of erythema and edema was evaluated according to the Draize method. As above, all rabbits survived to study termination. No erythema, edema, or other dermal effects were noted throughout the study. Under the conditions tested, Cellulon fibre, did not to induce a dermal irritation in New Zealand white rabbits.

No other studies on the dermal sensitization and dermal toxicity of BC were found in the literature. However, there have been several publications on the successful use of BC in humans, has a wound dressing. A commercial product from BC, called Biofill®, has been used for several skin injury treatments such as basal cell carcinoma/skin graft, severe body burns, facial peeling, sutures, dermabrasions, skin lesions, chronic ulcers, and both donor and receptor sites in skin grafts [36]. The clinical applications of BC as a wound dressing in humans have been reviewed [37–41]. BC has also been used in cosmetic applications, mostly as a facial masks (BC thin and hydrated membrane), facial scrub (BC dispersion) and proposed also to be used has a transdermal drug delivery agent (BC thin and hydrated membrane) [42,41,43–45]. From the above, it is unlikely that BC will induce any dermal irritation on humans.

No studies were found addressing the pulmonary toxicity of BC, but there are a few studies with plant celluloses. As pilot scale productions of plant nanocelluloses (nanofibrillar, nanocrystals) are emerging, and due to the higher aspect ratio of these nano-scalar fibres, occupational exposure studies to have also been receiving increasingly more attention. Still, studies on the effects of exposure of “nano” celluloses by inhalation routes are still scarce [46–58].

## 6. Inflammatory response

BC has long been explored for use as a biomaterial, such as artificial skin/wound dressing, artificial blood vessels, artificial cornea, heart valve prosthesis, artificial urethra, artificial bone, artificial cartilage, artificial porcine knee menisci, to name a few examples. BC has also been explored for the delivery of drugs, hormones and proteins. The biocompatibility and hemocompatibilty of BC has been demonstrated *in vitro* and *in vivo*. In general, BC can be considered to be broadly biocompatible, invoking only moderate (if any) foreign body response *in vivo* [42,59,38,60–63]. After 12 weeks following subcutaneous BC

implantation in female Wistar rats, Helenius et al. [64] found that no fibrotic capsule or giant cells were detectable by microscopy, indicating that no foreign body reaction occurred. Also, macroscopically, no redness, swelling, or exudate developed around the implantation sites was observed. Klemm et al. [65] demonstrated the *in vivo* (white rat (Han:WIST)) biocompatibility of BC implants, using BASYC® (Bacterial Synthesized Cellulose) tubes, for use as artificial blood vessels and on micronerve surgery. Schumann et al. [66] and Wippermann et al. [67] also studied *in vivo* the potential of BC, for use in tissue-engineered blood vessels, using pigs as animal models. Andrade et al. [68] investigated the biocompatibility of BC and peptide (Arg-Gly-Asp)-modified BC membranes subcutaneously implanted in white merino sheep, for up to 32 weeks. Compared with negative control samples (expanded polytetrafluoroethylene (ePTFE)), peptide-modified BC membranes were only mildly irritating to the tissue, with no significant differences in the inflammation degree. In another study, by Pertile et al. [69] the *in vivo* biocompatibility of BC was evaluated, through histological analysis of long-term (up to 12 months) subcutaneous implants in male BALB/c mice. BC implants caused a mild and benign inflammatory reaction that decreased with time and did not elicit a foreign body reaction. Moreover, no differences were observed between the controls (without implants) and BC implanted animals, in thymocyte populations and in B lymphocyte precursors and myeloid cells in the bone marrow. Svensson et al. [70] studied *in vitro*, the potential of BC as a scaffold for cartilage repair. In this study, BC did not induce significant activation of pro-inflammatory cytokine production during in vitro macrophage screening. Xu et al. [71] explored the use of BC as an artificial dura mater and examined the histocompatibility and inflammatory effects of this BC implant in New Zealand rabbits. There were seldom inflammatory cells surrounding the BC membrane, during early postoperative period. The expression of inflammatory cytokines IL-1β, IL-6 and TNF-α as well as iNOS and COX-2 were lower in the BC group compared to the control group (with NormalGEN® membrane (Biological Dural Repairing Patch)) for up to 21 days after implantation. BC was observed to allow the repairing of dural defects in rabbit and had a decreased inflammatory response compared to traditional materials. Panerari et al. [72] studied *in vivo*, using rabbits as animal models, the use of a commercial product from BC (Bionext®) as a dressing to prevent scarring tissue formation, following tracheal stenosis surgery. Bionext dressings were observed not to induce acute inflammatory response, up to 180 days following scarification. Andrade et al. [73] studied the *in vitro* hemocompatibility of BC and BC-based biomaterials. It was reported that native BC and peptide (Arg-Gly-Asp)-modified BC membranes both preserved original conformational structures and exhibited a favourable interaction (non-activation) with platelets, which were indicative that BC and modified BC could be considered hemocompatible materials. As a matter of fact, several companies have medical devices based on BC in the market (e.g. dura mater allografts from DePuy Synthes from *Johnson & Johnson*, wound dressings from Bowil Biotech), meaning that purified BC can meet the strict regulatory requirements for this class of products.

## 7. Conclusion

In response to legislation, scientific developments, and public concerns, toxicity-testing methods have been implemented, to generate information on the potential hazards or risks posed to humans by the use of several agents. Due to primary concerns on the toxicity, cancer and reproductive development of human food substances, their placement on the market is often dependant on regulatory approval. This has become increasingly important, due to the growing interest in the use of nano-sized biopolymers in the food sector, among them, nanocelluloses. Bacterial cellulose (BC) is a biopolymer synthesized by *Komagataeibacter* strains. The unique properties of this biopolymer have allowed its exploitation in the development of numerous bio-based products and applications. Several food applications of BC have been proposed, due to its potential as a gelling, thickening, suspending and stabilizing agent, along with its potential role as a source of dietary fibre. This manuscript addressed the safety of BC for human food applications. Information here reviewed on the toxicological data of BC, in animals and *in vitro*, strongly suggest that BC is not genotoxic, carcinogenic, tumour promoter, pyrogenic or a developmental or reproductive toxicant and thus it is not expected to pose any adverse side effects when used in human foods. In addition, the data available supports the general conclusion that BC is non-toxic on ingestion, skin contact or on inhalation, or elicit any other inflammatory or oxidative stress responses at the cellular level.

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