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# Opinion of the Scientific Committee on Food

# on

# Carrageenan

**(expressed on 5 March 2003)**

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# Opinion of the Scientific Committee on Food on Carrageenan

## Terms of reference

The Committee is asked to review the information contained in two articles published in the scientific literature (Tobacman, 2001; Tobacman et al., 2001) and to advise whether this information affects its earlier opinion on the safety of carrageenan.

## Background

In 1978, the Scientific Committee for Food (SCF) endorsed the Acceptable Daily Intake (ADI) of 0 - 75 mg/kg bw established for carrageenan by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1974; SCF, 1978). JECFA reviewed carrageenan again in 1984 and allocated an ADI "not specified" for refined non-degraded carrageenan (JECFA, 1984a). The SCF also reviewed carrageenan again in 1992, when further technological and toxicological information became available. At that time, the Committee concluded that it would be premature to adjust the ADI until the results of further studies then underway were available and it recommended that carrageenan should not be permitted in infant formulas which would constitute the entire food supply for the infant at that age period (SCF, 1994a).

The Committee has also considered various specific requests for the use of carrageenan in foods for infants and young children. The Committee concluded that the use of carrageenan in follow-on milks, which may be given from the age of 4 months onwards, was acceptable at levels up to 0.3g/l (SCF, 1983), but a subsequent request for use in infant formula in the category of foods for special medical purposes, for infants from birth onwards, was not approved (SCF, 1998). At that time the Committee noted its earlier advice (SCF, 1994b) that it wished to defer any opinion on the use of carrageenan in weaning foods pending a re-evaluation of the general food use of carrageenan and that it might wish to reconsider its earlier approval of the use of carrageenan in follow-on milks. The reasons for these reservations were because it could not be excluded that carrageenan might be absorbed by the immature gut and that absorbed material might affect the immune system in the infant.

## Scope of the present review

In this review the Committee has addressed several issues. The first issue discussed by Tobacman (2001) was the possibility of exposure to degraded carrageenan. Degraded carrageenan, also called poligeenan, has a weight average molecular weight of 20-30 kDa (Weiner, 1991). It is known to cause haemorrhage and ulceration of the large intestine in some laboratory animal species (guinea pig, rabbit and monkey), whereas undegraded food-grade carrageenan, with a weight average molecular weight of above 100 kDa, does not (SCF, 1978; JECFA, 1984b). Tobacman (2001) discussed the possible sources of

degraded carrageenan, i.e. contamination by lower molecular weight components in the additive as such, generation of degraded carrageenan during the processing of foods containing the additive, or digestion of the additive by acid hydrolysis or degradation by bacteria in the gut. The second issue raised by Tobacman (2001) was a possible tumour-promotion effect of undegraded carrageenan in the gut. In considering these issues, the Committee took into consideration the recent review of carrageenan by JECFA (2002).

The other paper of Tobacman et al. (2001) considered in this review proposes an hypothesis that the increasing incidence of breast cancer in the USA during the twentieth century may be related to consumption of carrageenan and possibly other water-soluble polymers.

The Committee also reconsidered the unresolved issue of whether carrageenan might be absorbed by the immature gut and have effects on immune function in the very young (SCF, 1994b).

**Specification for carrageenan**

A recent publication (Uno *et al*., 2001), which examined 29 samples of food-grade carrageenan, showed that the weight average molecular weight of these samples ranged from 453-652 kDa and that poligeenan (20-30kDa) was not detectable in any sample. The authors estimated that the limit of detection for poligeenan contamination was around 5%. While this study indicates that should any lower molecular weight fraction be present in food-grade carrageenan, it would represent only a small proportion of the material, the Committee noted that the specification for carrageenan does not exclude the possible presence of lower molecular weight material. In the current specification (JECFA, 1998), the presence of lower molecular weight material is limited only by the requirement for a minimum viscosity of 5 mPa s for a 1.5% solution measured at 75$^{o}$C. This is equivalent to a weight average molecular weight of 100 kDa. Thus a greater proportion of higher molecular weight chains could mask the presence of lower molecular weight material.

**Studies on tumour promotion and cell proliferation in rat colon**

Studies on tumour promotion, aberrant crypt cell formation and cell proliferation in the rat colon have been extensively summarised in the recent review by JECFA (2002) and are only briefly described here.

Two studies in rats treated with known colon tumour initiators, either azoxymethane or *N*-methyl-*N*-nitrosourea or 1,2-dimethylhydrazine, showed that co-administration of carrageenan at 15% or 6% in the diet increased the incidence of colon tumours compared to animals treated with initiator alone (Watanabe et al., 1978; Arakawa et al., 1986). A study conducted to a classical design for detection of tumour promotion, in which 4 weekly injections of the initiator dimethylhydrazine were followed by 32 weeks of feeding carrageenan at concentrations of 0, 1.25, 2.5 and 5.0% in the diet, did not find any increase in the incidence of tumours compared with animals treated with initiator alone (Hagiwara et al., 2001). The nominal 5% dietary level gave a calculated mean

intake of carrageenan during the study period of 3230 mg/kg bw/day. The Committee noted that this negative study was not included in the review by Tobacman (2001). The carrageenan used in all these studies was stated to be undegraded but was otherwise not defined with respect to the range or average molecular weights. The JECFA (2002) review pointed out that the two positive studies used higher dietary levels of carrageenan, while the negative study used lower dietary levels of carrageenan. The review further noted that since the carrageenan in the positive studies was fed before during and after the administration of the tumour initiator, the enhanced carcinogenesis may have resulted from promotion but could also have resulted from altered toxicokinetics or biotransformation of the carcinogen used as the initiator.

Some studies have also investigated the occurrence of aberrant crypt foci in rat colon. Treatment with a tumour initiator was followed 7 days later by carrageenan given at 0.2 or 4 g/kg bw/day in the drinking water for 100 days (Corpet et al., 1997). The highest dose converted the drinking water into a gel, which, as JECFA (2002) commented, may have altered food and water intake and the animals lost weight compared with controls. There was a significant decrease in the number of aberrant crypt foci per colon at both carrageenan doses, while the higher carrageenan dose significantly increased the number of crypts per aberrant crypt focus, indicating that the size of the foci was increased. In a second study from the same laboratory (Taché et al., 2000), using conventional and germ-free rats, treatment with a tumour initiator was followed 7 days later by carrageenan given at 0.22 to 2.8 g/kg bw/day in the drinking water. The germ-free rats were inoculated with adapted microflora from faecal samples taken from healthy children given carrageenan-containing desserts 3 times a week for 3 weeks. The results from conventional rats were the same as in the first study, while in the germ-free rats, carrageenan did not have any effect on aberrant crypts. In a study from a different laboratory, in which carrageenan was given alone at 5% in the diet, equivalent to 3.2 g/kg bw/day, it had no effect on the number of aberrant crypt foci (Hagiwara et al., 2001). As JECFA (2002) has remarked, these results are difficult to interpret, not least because the relationship between aberrant crypts and colon tumorigenesis is unclear.

Conflicting evidence has also been obtained from studies on the effect of feeding carrageenan on faecal bile acids and cell proliferation in the rat colon (Glauert and Bennink, 1983; Arakawa et al., 1988; Calvert and Reicks, 1988; Calvert and Satchithanandam, 1992; Wilcox et al., 1992). Three of these studies indicated that cell proliferation, as measured indirectly by increases in thymidine kinase activity, occurred with the feeding of high doses of carrageenan at 2.6% or more in the diet for 28 or 91 days, but no effect was seen in the one study in which cell proliferation was assessed by a more robust method (autoradiography) following 7.4% carrageenan in the diet for 28 days. 1.5% carrageenan in the diet, equivalent to 750 mg/kg bw/day, was a no-effect level (NOEL) for increases in thymidine kinase activity.

**Carrageenan and breast cancer hypothesis**

The paper of Tobacman et al. (2001) presented a time-trend analysis of the relationship between the rising incidence of breast cancer in the USA from 1937-1996 and

consumption of carrageenan and other water-soluble gums estimated on a per capita basis with no adjustment for non-consumers. A statistically significant positive correlation was obtained for carrageenan after a lag time of 25 and 30 years, but not for shorter time periods. Per capita estimates of consumption of four other gums (agar, alginate, arabic and locust bean) also showed significant positive correlations with breast cancer incidence at sporadic time points.

**Absorption by the immature gut and effect on immune function**

No further data addressing the possibility that carrageenan may be absorbed by the immature gut or on possible influences on immune function have been published or submitted to the Committee.

**Estimates of intake**

JECFA (2002) summarised data on intake from Europe, Canada and the USA, noting that the resulting mean intake estimates were consistent, falling within a range of 30-50 mg/person/day from the use of carrageenan and processed *Eucheuma* seaweed as food additives.

**Discussion**

The Committee noted that the toxicological issues discussed by Tobacman (2001) were not new, that much of the data cited in the paper had been published before the 1992 SCF review and had been considered at that time by the Committee.

On the issue of degraded carrageenan, while there is no evidence of any adverse effects in humans from exposure to food-grade carrageenan, or that exposure to degraded carrageenan from use of food-grade carrageenan is occurring, the Committee nevertheless proposes the specification for food-grade carrageenan to be tightened in order to ensure that the presence of any degraded carrageenan is kept to a minimum. The Committee recognises that there may be technical difficulties in finding appropriate methods to ensure limitation of any lower molecular weight components in food-grade carrageenan, particularly if the methods are to be widely applicable. The Committee nevertheless considers that, if feasible, a limit of not >5% below 50 kDa should be introduced into the specification.

On the issue of undegraded carrageenan, the Committee agreed with the conclusions of the recent JECFA review that intakes of carrageenan and processed *Eucheuma* seaweed from their use as food additives were of no concern (JECFA, 2002). Mean human intakes, at 30-50 mg/person/day, equivalent to around 0.5-5 mg/kg bw/day for adults and children, are considerably below the NOEL of 750 mg/kg bw/day for increased thymidine kinase activity as a biomarker for increased cell proliferation in rat colon. The classical initiation-promotion study in rat colon with carrageenan intakes ranging up to 3230 mg/kg bw/day did not show any effect of carrageenan on tumour formation. In the two less well-designed rat studies that did show tumour promotion at high doses, the

intakes of carrageenan in the rats were higher than human intakes by 2-3 orders of magnitude or more.

The Committee considered that the data provided did not support the hypothesis that breast cancer may be causally related to intakes of carrageenan and other water-soluble polymers used as food additives. The Committee noted that such correlations might be found for any dietary component or chemical to which there has been increasing exposure during the twentieth century.

**Conclusion**

The Committee concluded that the information available since its last review of carrageenan as an additive for general food use did not provide any reason to alter the ADI of 0 - 75 mg/kg bw established previously. The Committee notes that intakes are considerably below the ADI.

The Committee does however consider that, if feasible, a molecular weight limit of not >5% below 50 kDa should be introduced into the specification, in order to ensure that the presence of any degraded carrageenan is kept to a minimum.

In the absence of any further information on possible absorption of carrageenan by the immature gut in the very young infant, the Committee reaffirms its earlier view (SCF, 1998) that it remains inadvisable to use carrageenan in infant formulae that are fed from birth, including those in the category of foods for special medical purposes. The Committee has no objection to the use of carrageenan in foods for older infants, such as follow-on milks (SCF, 1983) and weaning foods.

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