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## Re-evaluation of celluloses E 460(i), E 460(ii), E 461, E 462, E 463, E 464, E 465, E 466, E 468 and E 469 as food additives

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS),  
Maged Younes, Peter Aggett, Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico,  
Birgit Dusemund, Metka Filipič, Maria Jose Frutos, Pierre Galtier, David Gott,  
Ursula Gundert-Remy, Gunter Georg Kuhnle, Claude Lambré, Jean-Charles Leblanc,  
Inger Therese Lillegaard, Peter Moldeus, Alicja Mortensen, Agneta Oskarsson, Ivan Stankovic,  
Paul Tobback, Ine Waalkens-Berendsen, Matthew Wright, Alexandra Tard,  
Stavroula Tasiopoulou and Rudolf Antonius Woutersen

### Abstract

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), enzymatically hydrolysed carboxy methyl cellulose (E 469) and cross-linked carboxy methyl cellulose (E 468) as food additives. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF) established an acceptable daily intake (ADI) 'not specified' for unmodified and modified celluloses. Celluloses are not absorbed and are excreted intact in the faeces; in addition, microcrystalline cellulose, powdered and modified celluloses could be fermented by the intestinal flora in animals and humans. Specific toxicity data were not always available for all the celluloses evaluated in the present opinion and for all endpoints. Given their structural, physicochemical and biological similarities, the Panel considered it possible to read-across between all the celluloses. The acute toxicity of celluloses was low and there was no genotoxic concern. Short-term and subchronic dietary toxicity studies performed with E 460(i), E 461, E 462, E 463, E 464, E 466 and E 469 at levels up to 10% did not indicate specific treatment related adverse effects. In chronic toxicity studies performed with E 460(i), E 461, E 463, E 464, E 465 and E 466, the no observed adverse effect level (NOAEL) values reported ranged up to 9,000 mg/kg body weight (bw) per day. No carcinogenic properties were detected for microcrystalline cellulose and modified celluloses. Adverse effects on reproductive performance or developmental effects were not observed with celluloses at doses greater than 1,000 mg/kg bw by gavage (often the highest dose tested). The combined exposure to celluloses (E 460–466, E 468 and E 469) at 95th percentile of the refined (brand-loyal) exposure assessment for the general population was up to 506 mg/kg bw per day. The Panel concluded that there was no need for a numerical ADI and that there would be no safety concern at the reported uses and use levels for the unmodified and modified celluloses (E 460(i); E 460(ii); E 461–466; E 468 and E 469). The Panel considered an indicative total exposure of around 660–900 mg/kg bw per day for microcrystalline, powdered and modified celluloses.

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**Keywords:** Microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked carboxy methyl cellulose (E 468), enzymatically hydrolysed carboxy methyl cellulose (E 469)

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**Correspondence:** [fip@efsa.europa.eu](mailto:fip@efsa.europa.eu)

**Panel members:** Peter Aggett, Fernando Aguilar, Riccardo Crebelli, Birgit Dusemund, Metka Filipič, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Gunter Georg Kuhnle, Claude Lambré, Jean-Charles Leblanc, Inger Therese Lillegaard, Peter Moldeus, Alicja Mortensen, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes.

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## Summary

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), enzymatically hydrolysed carboxy methyl cellulose (E 469) and cross-linked carboxy methyl cellulose (E 468) as food additives. These celluloses are authorised as food additives in accordance with Annex II and Annex III of Regulation (EC) No 1333/2008.

Cellulose is a linear glucose homopolymer consisting of glucopyranose units linked by  $\beta$ -1,4-glycosidic bonds; its molecular formula is  $(C_6H_{10}O_5)_m$ , with the degree of polymerisation (DP) dependent on the origin of the cellulolytic material. Cellulose molecular weight has been calculated to be approximately in the range 50,000–2,500,000. In modified celluloses, the chemical and physical characteristics of the native substances are modified in order to confer different technological properties for particular food applications.

Microcrystalline cellulose is purified, partially depolymerised cellulose prepared by treating  $\alpha$ -cellulose, obtained as a pulp from strains of fibrous plant material, while powdered cellulose is purified, mechanically disintegrated cellulose prepared by processing  $\alpha$ -cellulose.

Methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464) and ethyl methyl cellulose (E 465) are celluloses obtained synthetically from fibrous plant material. Each of the celluloses is partially etherified with methyl groups, ethyl groups, hydroxypropyl groups and contains a small degree of hydroxypropyl substitution, and methyl and ethyl groups, respectively.

Microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) have been previously evaluated by the Scientific Committee on Food (SCF), the most recent evaluation dating in 1999. In 1999, the SCF assessed additional toxicological data and confirmed the 'ADI not specified', established in 1978. As a matter of precaution, the Committee repeated the advice given in 1995, according to which, the particle size should not be lower than 5  $\mu$ m with a tolerance of 10% by the number of particles.

The latest evaluation of methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), methyl ethyl cellulose (E 465), carboxy methyl cellulose (E 466) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) was done in 1989 (JECFA, 1990), where an acceptable daily intake (ADI) 'not specified' was established for each modified cellulose. Latest evaluation of enzymatically hydrolysed carboxy methyl cellulose (E 469) was done in 1998 (JECFA, 1999a,b) and an ADI 'not specified' was established. The ADI for cross-linked sodium carboxy methyl cellulose previously established by JECFA (2003) is 'not specified' based on the substance being poorly absorbed and of low toxicity, with which is in agreement with the known low toxicity of other modified celluloses.

Animal and human data clearly demonstrated that microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) are not absorbed intact in the gastrointestinal tract and could be fermented during their passage through the large intestine by strains of bacteria found in the human colon. Data for methyl cellulose (E 461), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) demonstrated that these modified celluloses are not absorbed intact, not fermented and are excreted intact via the faeces. The Panel noted that microcrystalline, powdered and modified celluloses would not be absorbed intact and would be less fermented than other polysaccharides such as gums, starches or pectins.

Specific toxicity data were not always available for all the celluloses evaluated in the present opinion and for all endpoints. In general, the most complete data sets were available for microcrystalline cellulose (E 460(i)) and sodium carboxy methyl cellulose (E 466). However, given their structural, physicochemical and biological similarities, the Panel considered it possible to read-across between all the celluloses.

Data on acute oral toxicity are available for microcrystalline cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose and sodium carboxy methyl cellulose. These indicate a low oral acute toxicity.

Short-term and subchronic toxicity studies have been performed with microcrystalline cellulose (E 460(i)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), sodium carboxy methyl cellulose (E 466) and enzymatically

hydrolysed carboxy methyl cellulose (E 469). In the majority of studies, animals were dosed via diet at levels up to 10%. Effects on body weight at the highest dose tested (10%) were reported in some, but not all studies, which may reflect nutritional constraints rather than toxicity. No adverse effects were reported with most of the tested celluloses, except for local effects on caecal size due to the presence of undigested fibre. Groups of 20 Albino Wistar outbred rats per sex were dosed with carboxy methyl cellulose (E 466) or enzymatically hydrolysed carboxy methyl cellulose (E 469) via diet with 0%, 2.5%, 5% and 10% (up to 6,800 mg/kg body weight (bw) per day) for up to 102 days. Effects on caecal weight, urothelial hyperplasia, pelvic nephrocalcinosis, corticomedullary nephrocalcinosis and increased incidence of diffuse epithelial hyperplasia in the urinary bladder were observed. However, the findings in kidneys and urinary bladder were attributed to the concentration of sodium, which was up to fourfold higher in the test diet compared with the basal diet. The Panel noted that this was a plausible explanation for the reported findings.

Avicel<sup>®</sup> RCN-15 (a mixture of 85% microcrystalline cellulose with 15% guar gum) did not induce mutagenic effects in the presence or absence of a metabolic activation system in bacterial reverse mutation assays (Batt, 1992), in a gene mutation assay in mouse lymphoma cells (at thymidine kinase locus) (Cifone, 1992), in an *in vitro* test for unscheduled DNA synthesis (McKeon, 1992) and in the mouse bone marrow micronucleus assays (Murli, 1992). Negative results were also reported with other microcrystalline cellulose preparations in other unpublished studies (Documentation provided to EFSA no. 34, 35, 36, 37). Overall, the Panel concluded that microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)), which only differs for polymerisation degree, do not raise concern for genotoxicity.

Concerning methyl cellulose and sodium carboxy methyl cellulose, both substances were negative in Ames tests with different *Salmonella* Typhimurium strains, both with and without metabolic activation (Litton Bionetics Inc., 1975, 1980, Blevins and Taylor, 1982; Ishidate et al., 1984). Negative results were also obtained in a chromosomal aberration assay in Chinese hamster fibroblasts (CHL) (Ishidate et al., 1984), only performed without metabolic activation, and in host-mediated assays with yeast and bacteria (Litton Bionetics Inc., 1974, 1975).

Methyl cellulose was also tested with negative results in an *in vitro* chromosomal aberration assay in human embryonic lung cells (WI-38), and *in vivo* in a chromosome aberration assay in rat bone marrow and in the dominant lethal assay in male rats (Litton Bionetics Inc., 1974).

Therefore, the Panel concluded that methyl cellulose and sodium carboxy methyl cellulose do not raise concern for genotoxicity.

The Panel also considered that read-across from methyl cellulose (E 461) to the other modified celluloses bearing similar simple substituents (E 462, E 463, E 464, E 466) was scientifically justified, and supported by the *in silico* analysis, which did not highlight additional structural alerts for genotoxicity, and concluded that either these modified celluloses did not raise genotoxic concern. Similarly, the Panel considered scientifically justified the read-across from sodium carboxy methyl cellulose (E 466) to its products of enzymatic hydrolysis (E 469) or cross-linking (E 468), which do not bear additional structural determinants of genotoxicity. Overall, the Panel concluded that microcrystalline and powdered cellulose (E 460(i) and E 460(ii)) and modified celluloses (E 461–E 469) do not raise concern for genotoxicity.

Chronic toxicity studies have been performed with microcrystalline cellulose (E 460(i)), methyl cellulose (E 461), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465) and sodium carboxy methyl cellulose (E 466). Although there were some inconsistencies in the data, the main effects seen were decreases in body weight gain at the highest dose, which are likely to be due to the amount/bulk of celluloses in the diet leading to nutritional imbalance. Furthermore, in a chronic feeding study with microcrystalline cellulose (E 460(i)), some dystrophic calcification of renal tubules was observed in the high dose group (15,000 mg/kg bw per day). The no observed adverse effect level (NOAEL) values reported ranged up to 9,000 mg/kg bw per day. The Panel concluded that microcrystalline cellulose and modified celluloses have no carcinogenic properties and that there was no reason to expect carcinogenic properties with powdered cellulose (E 460(ii)).

Concerning reproductive and developmental toxicity, data are available for microcrystalline cellulose (E 460(i)), methyl cellulose (E 461), hydroxypropyl cellulose (E 463) and sodium carboxy methyl cellulose (E 466). The substances were tested in mice, rats, hamsters and/or rabbits with oral dosing via gavage (FDLI, 1973, 1975; Ferch, 1973a,b; Cannon Labs, 1975, 1977; Kitagawa et al., 1978a,b; Fritz and Becker, 1981; Freeman, 1992b). Adverse effects on reproductive performance or



developmental effects were not observed with modified and unmodified celluloses at doses greater than 1,000 mg/kg bw by gavage (often the highest dose tested).

Specific toxicity data were not always available for all the celluloses for all endpoints. In general, the most complete data sets were available for microcrystalline cellulose (E 460(i)) and sodium carboxy methyl cellulose (E 466). Given the similarities in their structure, relevant physicochemical, metabolic and toxicological properties, the Panel considered it possible to read-across between all the celluloses.

In addition, the Panel noted that methyl cellulose (E 461) and sodium carboxy methyl cellulose (E 466) were frequently used in the formulations for administration of xenobiotics by gavage in chronic, reproductive and developmental toxicity and carcinogenicity studies. In these studies, there should be a negative control group receiving the formulation alone. Although modified cellulose levels were usually only up to 2%, given the number of studies and group sizes in these studies, the overall number of animals tested would be very large. The Panel considered that the absence of reported adverse effects from such vehicle control groups provided additional evidence of the lack of safety concern for modified celluloses at levels up to 2% in the vehicle.

There was evidence that repeated doses up to 35 g/person of microcrystalline cellulose or powdered cellulose did not adversely affect clinical chemistry and haematological parameters and had no effect on the absorption and/or the metabolism of dietary constituents.

Some modified celluloses have been used in patients suffering from diarrhoea or constipation. In general, it can be concluded that an oral ingestion of up to 6,000 mg/person per day for 8 months was well tolerated.

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 (Chassaing et al., 2015). In several studies, other emulsifiers have been reported to alter the gut microbiota, promote gut inflammation, promote obesity and to impair glycaemic control in experimental studies with animals (Swidsinski et al., 2009a,b; Renz et al., 2012; Merga et al., 2014; Cani and Everard, 2015; Chassaing et al., 2015; Romano-Keeler and Weitkamp, 2015; Lecomte et al., 2016; Shah et al., 2017).

Some of the effects associated with emulsifiers are not systematically studied as specific endpoints in toxicity studies performed according to current toxicity testing guidelines; therefore, they would have to be investigated on a case-by-case basis if indicated by the results of the general toxicity testing, as recommended in the Guidance for submission of food additives (EFSA ANS Panel, 2012).

The Panel considered that based on the animal data, the toxicity of microcrystalline, powdered and modified celluloses was low and that NOAELs were generally the highest dose tested (up to at least 9,000 mg/kg bw per day). In addition, the large cumulative group of exposed animals from use in control populations to 2% would indicate that there were no reasons why humans would be expected to be more sensitive than animals in toxicodynamics or. The available data in humans indicate that daily doses of up to 6,000 mg for around 8 months were not associated with adverse effects; however in line with many other dietary fibres, large bolus intakes of celluloses were occasionally associated with laxation, but there was a lack of dose–response data available.

*The Panel considered that in line with the conceptual framework, it would be useful if risk managers had an indicative total exposure (daily consumption value) for microcrystalline, powdered and modified celluloses used as food additives, which would not pose a health risk and uses up to this value would not require a further risk assessment. The Panel considered this could be based on all the reported NOAELs from subchronic and chronic toxicity studies (ranging from 2100 to more than 9000 mg/kg bw/day), human data and allowing for interindividual uncertainty. The Panel considered an indicative total exposure (daily consumption value) of around 660 to 900 mg/kg bw per day for microcrystalline, powdered and modified celluloses.*

To assess the dietary exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives, the combined exposure was calculated based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario brand-loyal and non-brand-loyal consumer scenario*).

Celluloses (E 460–466, E 468 and E 469) are authorised in a wide range of foods. The Panel did not identify brand loyalty to specific food categories in infants and toddlers (e.g. flavoured drinks). Further, the Panel considered that the non-brand-loyal scenario covering other population groups was appropriate and a realistic scenario for risk characterisation because it was assumed that the population would probably be exposed long-term to the food additive present at the mean reported use in processed food.

A refined estimated exposure assessment scenario taking into account the food for special medical purpose (FSMP) for infants and young children (Food category (FC) 13.1.5.1 dietary foods for infants for special medical purposes and special formulae for infants and 13.1.5.2 dietary foods for babies and young children for special medical purposes as defined by Commission Directive 1999/22/EC in which E 466 is authorised) was also performed to estimate exposure for infants and toddlers who may be on a specific diet. However, no reported use levels were made available by industry for these food categories. Thus, MPLs of E 466 for FSMP were used. The Panel noted that according to Mintel, very few baby foods were on the European market containing E 466. This was in line with the fact that no data were submitted for the food categories 13.1.5.1 and 13.1.5.2.

A refined estimated exposure assessment scenario taking into account the consumption of food supplements for consumers only, were also performed to estimate exposure for children, adolescents, adults and the elderly, as exposure via food supplements may deviate largely from that via food, and the number of food supplements consumers may be low depending on populations and surveys.

The refined estimates were based on 26 out of 84 food categories in which celluloses (E 460–466, E 468 and E 469) are authorised. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to celluloses (E 460–466, E 468 and E 469) as food additives in European countries for the refined scenario if it is considered that the food additives may not be used in food categories for which no usage data have been provided.

The Panel noted that given the information from the Mintel's Global New Products Database (GNPD), it may be assumed that celluloses (E 460–466, E 468 and E 469) are used in food categories for which no data have been provided by food industry. The main food categories, in terms of amount consumed, not taken into account were processed fermented milk products, cheeses (unripened, processed), fish and fishery products and breakfast cereals. However, according to the Mintel GNPD, in the European Union (EU) market, a small percentage (< 1%) of food products belonging to these food categories are labelled with celluloses (E 460–466, E 468 and E 469). Therefore, the Panel considered that if these uncertainties were confirmed, it would result in a slight underestimation of the exposure.

The Panel further noted that the exposure to celluloses (E 460–466, E 468 and E 469) from their use according the Annex III to Regulation (EC) No 1333/2008 was not considered in the exposure assessment.

The Panel also noted that the refined exposure estimates were based on information provided on the reported levels of use of celluloses (E 460–466, E 468 and E 469). If actual practice changes, this refined estimates may no longer be representative and should be updated.

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- their structural, physicochemical and biological similarities, allows for read-across between all the celluloses
- animal and human data demonstrate that microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) are not absorbed intact in the gastrointestinal tract but could be fermented by intestinal microbiota. Chemically modified celluloses are not absorbed intact, nor fermented, but are excreted intact via the faeces
- using the read-across approach, adequate data on short- and long-term toxicity and carcinogenicity and reproductive toxicity are available,
- despite the limitations of some of the studies, the available data do not indicate a genotoxic concern for microcrystalline cellulose, methyl cellulose and carboxy methyl cellulose, and by read-across, of the other modified and unmodified celluloses
- no adverse effects were reported after repeated doses up to 35 g/person of microcrystalline cellulose or powdered cellulose; oral ingestion of some modified celluloses up to 6,000 mg/person per day for 8 months in patients suffering from diarrhoea or constipation was well tolerated;
- adequate combined exposure data were available; in the general population, the highest 95th percentile refined exposure assessment estimates calculated based on the reported data from food industry was 506 mg/kg bw per day in toddlers (brand-loyal scenario)
- an indicative high refined exposure assessment of up to 448 mg/kg bw per day for the elderly has been calculated at the 95th percentile among the population classes consuming food supplements

The Panel concluded that there was no need for a numerical ADI and that there would be no safety concern at the reported uses and use levels for the unmodified and modified celluloses (E 460(i);

E 460(ii); E 461–466; E 468 and E 469). The Panel further suggested an indicative total exposure (daily consumption value) of 660–900 mg/kg bw per day where these conclusions would remain valid.

Concerning the use of sodium carboxy methyl cellulose (E 466) in 'dietary foods for special medical purposes and special formulae for infants' (FC 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (FC 13.1.5.2), and given that:

- for infants and toddlers consumers only of FSMP, the highest 95th percentile refined exposure estimate was 1,557 mg/kg bw per day in infants;
- 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;

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. The Panel noted that E 466 seemed not to be used in these food categories as no use or use levels were submitted by industry and only very few food belonging to these categories appeared to be labelled with E 466.

The Panel recommended that the European Commission considers lowering the maximum limits for the toxic elements arsenic, lead, mercury and cadmium present as impurities in the EU specifications for unmodified and modified celluloses re-evaluated in the present opinion (E 460(i), E 460(ii), E 461, E 462, E 463, E 464, E 465, E 466, E 468 and E 469) should be revised to ensure that these food additives will not be a significant source of exposure to these toxic elements in food, in particular for infants and children.

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## 1. Introduction

The present opinion deals with the re-evaluation of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) when used as food additives. These celluloses are authorised food additives in the EU according to Annex II and Annex III of Regulation (EC) No 1333/2008.

For the purpose of this opinion, the term modified cellulose is used for all the celluloses mentioned above.

### 1.1. Background and Terms of Reference as provided by the European Commission

#### 1.1.1. Background

Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010<sup>1</sup>. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU of 2001. The report 'Food additives in Europe 2000' submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of adoption of Regulation (EU) 257/2010 the 2003 Terms of References are replaced by those below.

#### 1.1.2. Terms of Reference

The Commission asks EFSA to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedures and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives.

#### 1.1.3. Interpretation of Terms of Reference

Specific toxicity data were not always available for all the celluloses for all endpoints. However, given their structural, physicochemical and biological similarities, the Panel considered it possible to read-across between all the celluloses.

The ANS Panel described its risk assessment paradigm in its Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012). This Guidance states, that in carrying out its risk assessments, the Panel sought to define a health-based guidance value e.g. an Acceptable Daily Intake (ADI) (IPCS, 2004) applicable to the general population. According to the definition above, the ADI as established for the general population does not apply to infants below 12 weeks of age (JECFA,

<sup>1</sup> Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 80, 26.3.2010, p. 19–27.

1978; SCF, 1998). In this context, the re-evaluation of the use of sodium carboxy methyl cellulose (E 466) in food for infants below 12 weeks represents a special case for which specific recommendations were given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1972, 1978), by the SCF (SCF, 1996, 1998) and by EFSA (EFSA Scientific Committee, 2017). The Panel endorsed these recommendations.

In the current EU legislation (Annex II of Regulation (EC) No 1333/2008<sup>1</sup>), use levels of additives in food for infants under the age of 12 weeks are included in categories 13.1.1 and 13.1.5.1.<sup>2</sup> The Panel considers that these uses would require a specific risk assessment in line with the recommendations given by JECFA and SCF and endorsed by the Panel in its current Guidance for submission for food additives evaluations (EFSA ANS Panel, 2012). Therefore, a risk assessment for the general population is not considered applicable for infants under the age of 12 weeks, and will be performed separately.

This re-evaluation refers exclusively to the uses of celluloses as food additives in food, including food supplements and does not include a safety assessment of other uses of celluloses.

## 1.2. Information on existing authorisations and evaluations

In 1978, the SCF endorsed the 'ADI not specified' established by JECFA for microcrystalline cellulose and powdered cellulose. The Committee did not give any details of the data considered (SCF, 1978). In 1995, the SCF evaluated the presumed persorption and advised: (1) that microcrystalline cellulose of any particle size should not be used in foods specially prepared for infants and young children and, (2) a particle size > 5 µm should be introduced in the specification (SCF, 1995). In 1999, the SCF assessed the additional toxicological data and confirmed the 'ADI not specified'. As a matter of precaution, the Committee repeated the advice that particle size should not be lower than 5 µm with a tolerance of 10% by the number of particles (SCF, 1999).

In 1976, an 'ADI not specified' was allocated by JECFA for powdered cellulose (JECFA, 1976). The Committee stated that the toxicological evaluation performed for microcrystalline cellulose (JECFA, 1975) should also apply to powdered cellulose (JECFA, 1976). In the latest evaluation (JECFA, 1998a,b, 1999a,b), the Committee concluded that the toxicological data from humans and animals provided no evidence for toxic effects after the ingestion of microcrystalline cellulose in food, and endorsed the previous evaluation 'ADI not specified'. However, the Committee stated that small particles of other materials may be persorbed and that the extent of persorption is greater with sub-micrometer particles. Despite the absence of any demonstrated persorption of microcrystalline cellulose in the recent animal studies, the Committee revised the specifications for microcrystalline cellulose to limit the content of particles less than 5 µm in diameter (see Section 2.2).

Microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) were also evaluated by the Nordic Council of Ministers (TemaNord, 2002). The Committee concluded that microcrystalline cellulose and powdered cellulose as defined by the specifications is covered by the toxicological evaluation, including restriction on particle size.

Microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) (PM Ref. 43280) are included in the Union list of authorised substances that may be intentionally used in the manufacture of plastic layers in plastic materials and articles (Annex I to Commission Regulation (EU) No 10/2011<sup>3</sup>). Furthermore, microcrystalline cellulose (E 460(i)) is permitted as an antioxidant in cosmetic products (European Commission database-CosIng). Microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) are included in the European Union Register of feed additives (Regulation (EC) No 1831/2003<sup>4</sup>).

Methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) are listed in Commission Regulation (EU) No 231/2012<sup>5</sup> as authorised food additives in the EU

<sup>2</sup> Food of category 13.1.1: Infant formulae as defined by Directive 2006/141/EC; Food of category 13.1.5.1: Dietary foods for infants for special medical purposes and special formulae for infants. This interpretation also applies to those food additives in food category 13.1.5.2 (Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC) for which exceptional uses in food for infants under the age of 12 weeks are indicated.

<sup>3</sup> Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, p. 1–89.

<sup>4</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>5</sup> Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

and are used as stabilisers, emulsifiers, thickeners, humectants, anticaking agents, foaming agents, bulking agents, gelling agents and glazing agents.

These substances have been previously evaluated by JECFA in 1989 (JECFA, 1990) and 1998 (JECFA, 1999a,b). An 'ADI not specified' was established for each modified cellulose E 461–E 466 and E 469 (JECFA, 1990, 1999a,b).

Toxicological data for methyl cellulose (E 461), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), methyl ethyl cellulose (E 465) and sodium carboxy methyl cellulose (E 466) were re-evaluated by the SCF in 1992 (SCF, 1994). The Committee allocated an ADI 'not specified' to the five modified celluloses. The other modified celluloses, ethyl cellulose (E 462) and enzymatically hydrolysed sodium carboxy methyl cellulose (E 469) were not evaluated by the SCF.

In 1998, the SCF accepted the use of sodium carboxy methyl cellulose (E 466) in foods for special medical purposes (FSMP) for infants and young children at levels up to 10 g/L in liquid foods and up to 10 g/kg in solid foods. The Committee earlier reserved its opinion on a request to use E 466 in weaning foods pending completion of its work on persorption of macromolecular additives, but noted that otherwise the toxicological data did not indicate any effects likely to be of concern for infants and young children over weaning age. However, the Committee has since been informed that sodium carboxy methyl cellulose in water is in colloidal form and hence is not likely to be persorbed.

Cross-linked sodium carboxy methyl cellulose (E 468) is listed in Commission Regulation (EU) No 231/2012 as an authorised food additive in the EU and has been previously evaluated by JECFA in 2003 (JECFA, 2003). At its 59th meeting, JECFA concluded that the ADI could not be specified (JECFA, 2003).

Cross-linked sodium carboxy methyl cellulose (E 468) is the sodium salt of thermally cross-linked partly O-carboxymethylated cellulose, the use of which is solely as a carrier for sweeteners.

The SCF had accepted the use of cross-linked sodium carboxy methyl cellulose (E 468) on the grounds of lack of toxicity in a limited data set and a history of safe use of the parent compound sodium carboxy methyl cellulose (SCF, 1996, 1998). In 1996, no ADI was established due to insufficient toxicological data, but the use of cross-linked sodium carboxy methyl cellulose was deemed acceptable as a disintegrant for sweetener tablets due to its limited use through this application. The most recent SCF opinion dating from 1998, refers to new data submitted to the Committee, and recommends the use of cross-linked sodium carboxy methyl cellulose (E 468) as a disintegrant at a level not exceeding 30 g/kg in dietary supplements. The Committee's recommendation was based on the newly available technical and toxicological data and the long history of safe use of the parent compound, sodium carboxy methyl cellulose. The parent compound is stated to be poorly absorbed in humans and animals, with a likely further reduction in absorption as a consequence of cross-linking.

Cross-linked sodium carboxy methyl cellulose was thereafter reviewed by JECFA in 2003. The evaluation set a new ADI of 'ADI not specified', describing the substance as 'poorly absorbed' and of 'low toxicity', and with consideration to the available toxicological data which were consistent with that of other modified celluloses.

In 2002, the Nordic Council reviewed cross-linked sodium carboxy methyl cellulose and concluded that there was no need for a re-evaluation (TemaNord, 2002).

Modified celluloses are included in the Union list of authorised substances that may be intentionally used in the manufacture of plastic layers in plastic materials and articles (Annex I to Commission Regulation (EU) No 10/2011) (methyl cellulose (E 461), PM Ref. 66240; ethyl cellulose (E 462), PM Ref. 53280; hydroxypropyl cellulose (E 463), PM Ref. 61680; hydroxypropyl methyl cellulose (E 464), PM Ref. 66700; ethyl methyl cellulose (E 465), PM Ref. 66640; sodium carboxy methyl cellulose (E 466); (hydrolysed) carboxy methyl cellulose (E 469), PM Ref. 42640).

Furthermore, in 2010, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) issued an opinion addressing the scientific substantiation of health claims in relation to hydroxypropyl methylcellulose (EFSA NDA Panel, 2010a,b). It was concluded that a cause and effect relationship has been established between the consumption of hydroxypropyl methylcellulose and a reduction of post-prandial glycaemic responses, as well as the maintenance of normal blood cholesterol concentrations.



## 2. Data and methodologies

### 2.1. Data

The Panel was not provided with a newly submitted dossier. EFSA launched public calls for data,<sup>6,7,8,9</sup> to collect relevant information from interested parties.

The Panel based its assessment on information submitted to EFSA following the public calls for data, information from previous evaluations and additional available literature up to the last Working Group (WG) meeting before the adoption of the opinion. Attempts were made at retrieving relevant original study reports on which previous evaluations or reviews were based, however not always these were available to the Panel.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database<sup>10</sup>) was used to estimate the dietary exposure.

The Mintel's Global New Products Database (GNPD) is an online database which was used for checking the labelling of products containing E 460(i), E 460(ii), E 461–466, E 468–469 within the EU's food products, as GNPD shows the compulsory ingredient information presented in the labelling of products.

### 2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

The ANS Panel assessed the safety of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) as food additives in line with the principles laid down in Regulation (EU) 257/2010 and in the relevant guidance documents: Guidance on submission for food additive evaluations by the Scientific Committee on Food (SCF, 2000) and taking into consideration the Guidance for submission for food additive evaluations in 2012 (EFSA ANS Panel, 2012).

When the test substance was administered in the feed or in the drinking water, but doses were not explicitly reported by the authors as mg/kg bw per day based on actual feed or water consumption, the daily intake was calculated by the Panel using the relevant default values as indicated in the EFSA Scientific Committee (2012) for studies in rodents or, in the case of other animal species, by JECFA (2000a,b,c). When in human studies in adults (aged above 18 years) the dose of the test substance administered was reported in mg/person per day, the dose in mg/kg bw per day was calculated by the Panel using a body weight of 70 kg as default for the adult population as described in the EFSA Scientific Committee Guidance document (EFSA Scientific Committee, 2012).

Dietary exposure of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) from their use as food additives was estimated combining the food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum permitted levels (MPLs) and reported use levels submitted to EFSA following a call for data. Different exposure scenarios were calculated. Uncertainties on the exposure assessment were identified and discussed with regard to their impact on the final exposure calculation.

<sup>6</sup> Call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Published: 22 November 2009. Available online: <http://www.efsa.europa.eu/en/dataclosed/call/ans091123>.

<sup>7</sup> Call for technical data on certain thickening agents permitted as food additives in the EU. Published: 19 December 2014. Available online: <http://www.efsa.europa.eu/it/data/call/141219>

<sup>8</sup> Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 12 October 2015. Available online: <http://www.efsa.europa.eu/en/data/call/151012>

<sup>9</sup> Call for technical data on certain starches and celluloses authorised as food additives in the EU. Published 11 February 2016. <http://www.efsa.europa.eu/sites/default/files/consultation/160211.pdf>

<sup>10</sup> Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

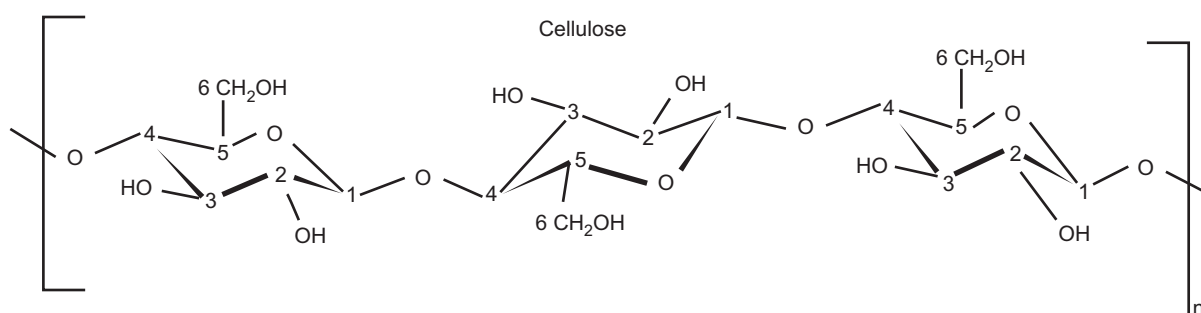
### 3. Assessment

#### 3.1. Technical data

##### 3.1.1. Identity of the substances

Cellulose is a linear homopolymer consisting of repeating  $\beta$ -D-glucopyranosyl units linked via (1,4) glycosidic bonds (Figure 1). It is the most abundant substance occurring in nature and an important structural component of the primary cell wall of green plants, many forms of algae and the oomycetes (Iijima and Takeo, 2000; Coffey et al., 2006).

Cellulose has the CAS Registry No 9004-34-6 and the EC (EINECS) Number 232-674-9. The molecular formula is  $(C_6H_{10}O_5)_m$ ; 'm' (degree of polymerisation (DP)) is dependent on the origin of the cellulolytic material. The molecular weight has been calculated to be approximately from 50,000 to 2,500,000, corresponding to (DP) 300–15,000 glucose units. As an example, for native cellulose the DP varies from 3,500 to 10,000 with molecular weights of 600,000–1,500,000 (Hamilton and Mitchell, 1964), although for cotton, the DP could be as high as 15,000 (Holtzapfle, 2003). According to Stepan et al., 2016, the cellulose of cotton linter had an average molecular weight of 253,770, which corresponds to a DP of 1,450.



**Figure 1:** Example of a general formula of a native cellulosic structure ('n' being variable, depending on the origin of the cellulolytic material)

Cellulose is described as a rigid material in which the molecules are associated, over extended regions, forming polycrystalline, fibrous bundles. The crystalline regions are held together by a large number of hydrogen bonds and are separated and connected by amorphous regions (BeMiller and Whistler, 1996).

Cellulose is insoluble in water because of strong hydrogen bonding in its crystal lattice. However, it is soluble in a number of solvents, including concentrated acids (e.g. 85% phosphoric, 72% sulfuric and 40% hydrochloric acid) and inorganic salt solutions (e.g. cuprammonium hydroxide, cadmium ethylenediamine solvent also known as cadoxen) (Holtzapfle, 2003).

Celluloses are subdivided into three classes:  $\alpha$ -,  $\beta$ - and  $\gamma$ -cellulose; all have the same chemical structure but differ in their DP:  $\alpha$ -cellulose DP > 200;  $\beta$ -cellulose DP 30–200;  $\gamma$ -cellulose DP 10–30 (Chen, 2014).

Celluloses differ in their solubility in 17.5% NaOH:  $\alpha$ -cellulose is the fraction that is not removed by treatment with 17.5% NaOH at 20°C; the fraction soluble in 17.5% NaOH contains  $\beta$ -cellulose and  $\gamma$ -cellulose; the subfraction precipitating after acidification of the alkaline liquor is  $\beta$ -cellulose; the acid-base soluble fraction that remains dispersed is  $\gamma$ -cellulose (Walter, 1998).

##### 3.1.1.1. Microcrystalline cellulose (E 460(i))

According to Commission Regulation (EU) No 231/2012, microcrystalline cellulose is purified, partially depolymerised cellulose prepared by treating  $\alpha$ -cellulose, obtained as a pulp from strains of fibrous plant material, with mineral acids. The DP is typically < 400; the molecular weight is about 36,000 g/mol.

Microcrystalline cellulose is purified, partially depolymerised cellulose with shorter crystalline polymer chains.

Synonyms: microcrystalline cellulose is known as cellulose gel (JECFA, 2009).

Microcrystalline cellulose is a fine, white, odourless, crystalline powder. The particles are insoluble but able to swell in water, in dilute alkali and acids, and in most organic solvents. The substance is soluble in NaOH solution (Klose and Glicksman, 1990; Coffey et al., 2006). At concentrations below

1%, microcrystalline cellulose forms colloidal solutions, and above 1%, thixotropic gels (Klose and Glicksman, 1990).

### 3.1.1.2. Powdered cellulose (E 460(ii))

According to the definition of Commission Regulation (EU) No 231/2012, powdered cellulose is purified, mechanically disintegrated cellulose prepared by processing  $\alpha$ -cellulose obtained as a pulp from strains of fibrous plant materials. The DP is predominantly  $\geq 1,000$  and greater, corresponding to a molecular weight of  $> 160,000$  (JECFA, 2006a).

In Commission Regulation (EU) No 231/2012, the substance is described as a white, odourless powder, which is insoluble in water, ethanol, ether and dilute mineral acids, but slightly soluble in sodium hydroxide solution.

Powdered cellulose is able to swell in water, dilute acid and most solvents, while alkali solutions lead to swelling and dissolution of the present hemicelluloses (Coffey et al., 2006).

### 3.1.1.3. Chemically modified celluloses (E 461–466, E 468 and E 469)

Methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464) and ethyl methyl cellulose (E 465) are celluloses obtained synthetically from fibrous plant material. Each of the celluloses is partially etherified with methyl groups, ethyl groups, hydroxypropyl groups and contains a small degree of hydroxypropyl substitution, and methyl and ethyl groups, respectively (Figure 2).

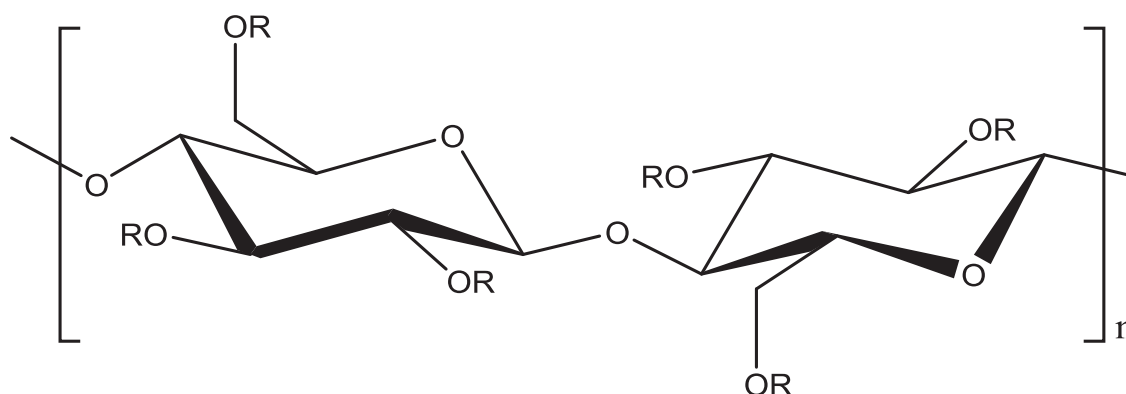
Sodium carboxy methyl cellulose (E 466) is the partial sodium salt of a carboxymethyl ether of cellulose.

Cross-linked sodium carboxy methyl cellulose (E 468) is obtained from carboxy methyl cellulose by acidifying and heating the aqueous suspension.

Enzymatically hydrolysed carboxy methyl cellulose sodium salt (E 469) is obtained from carboxy methyl cellulose by enzymatic digestion with a cellulase produced by *Trichoderma longibrachiatum* (formerly *Trichoderma reesei*).

All modified celluloses are derived from cellulose. They carry the cellulose backbone which consists of a polymer of  $\beta$ -(1 $\rightarrow$ 4) linked D-glucopyranose units (also called anhydroglucose unit (AGU)) (Freudenberg and Braun, 1928; Gardner and Blackwell, 1974). The hydroxyl groups at the carbon atoms C-2, C-3 and C-6 of the glucopyranose are more or less completely etherified depending on the used substitution reagent and conditions during manufacturing (Klemm et al., 1998; Murray, 2009; Qi et al., 2009).

The average number of hydroxyl groups substituted per glucopyranose unit is known as degree of substitution (DS). A complete substitution would provide a DS of 3. In the case of hydroxypropyl cellulose (E 463) and hydroxypropyl methyl cellulose (E 464), the DS can be higher than 3 because the hydroxypropyl group added also contains a hydroxyl group which can be etherified (Klemm et al., 1998; Desai et al., 2006).



E461 R= H or  $-\text{CH}_3$

E462 R= H or  $-\text{CH}_2\text{CH}_3$

E463 R= H or  $-\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$  or  $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OCH}_2\text{CH}(\text{OH})\text{CH}_3$

E464 R= H or  $-\text{CH}_3$  or  $-\text{CH}_2\text{CH}(\text{OH})\text{CH}_3$

E465 R= H or  $-\text{CH}_3$  or  $-\text{CH}_2\text{CH}_3$

E466 R= H or  $-\text{CH}_2\text{COONa}$  or  $-\text{CH}_2\text{COOH}$

E469 R= H or  $-\text{CH}_2\text{COONa}$  or  $-\text{CH}_2\text{COOH}$

**Figure 2:** General chemical structure of modified celluloses E 461–466 and E 468 and E 469, where R represents hydrogen or specific groups, as indicated. In this structure, 'n' represents the number of anhydrocellulose repeating units

The CAS Registry Numbers and EINECS numbers of the chemically modified celluloses (E 461–466, E 468 and E 469) are given in Table 1.

**Table 1:** CAS Registry Numbers and EINECS Numbers of chemically modified celluloses (Wüstenberg, 2015; ChemIdplus)

Substance	CAS registry number	EC (EINECS) <sup>(a)</sup> number	Molecular formula
Methyl cellulose (E 461)	9004-67-5	618-391-7	$\text{C}_6\text{H}_7\text{O}_2(\text{OH})_x(\text{OCH}_3)_y$ $x = 1.00\text{--}1.55$ ; $y = 2.00\text{--}1.45$ ; $x + y = 3.00$ ( $y = \text{degree of substitution}$ )
Ethyl cellulose (E 462)	9004-57-3	618-384-9	$\text{C}_2\text{H}_6\text{O}.x$ -unspecified
Hydroxypropyl cellulose (E 463)	9004-64-2	618-388-0	$[\text{C}_6\text{H}_7\text{O}_2(\text{OH})_x(\text{OCH}_2\text{CHOHCH}_3)_y(\text{OCH}_2\text{CH}[\text{R}_w]\text{CH}_3)_z]_n$ $x + y + z = 3$ ; $y + z(1 + w) \leq 4.6$ R: substituent with 'w' hydroxypropyl groups
Hydroxypropyl methyl cellulose (E 464)	9004-65-3	618-389-6	$[\text{C}_6\text{H}_7\text{O}_2(\text{OH})_x(\text{OCH}_3)_y(\text{OCH}_2\text{CHOHCH}_3)_z]_n$ $z = 0.07\text{--}0.34$ ; $y = 1.12\text{--}2.03$ ; $x = 3 - (z + y)$ , $(z + y) = \text{degrees of substitution}$
Ethyl methyl cellulose (E 465)	9004-59-5 <sup>(b)</sup>	–	$[\text{C}_6\text{H}_7\text{O}_2(\text{OH})_x(\text{OCH}_3)_y(\text{OC}_2\text{H}_5)_z]_n$ $z = 0.57\text{--}0.8$ ; $y = 0.2\text{--}0.4$ ; $x = 3 - (x + y)$ $(x + y) = \text{degrees of substitution}$
Sodium carboxy methyl cellulose (E 466)	9004-32-4	618-378-6	$[\text{C}_6\text{H}_7\text{O}_2(\text{OH})_x(\text{OCH}_2\text{COONa})_y]_n$ $n \geq 4$ $x = 1.50\text{--}2.80$ ; $y = 0.20\text{--}1.50$ ; $x + y = 3.0$ $(x + y) = \text{degree of substitution}$

Substance	CAS registry number	EC (EINECS) <sup>(a)</sup> number	Molecular formula
Cross-linked sodium carboxy methyl cellulose (E 468)	74811-65-7	–	Substituted anhydroglucose units with as general formula: C <sub>6</sub> H <sub>7</sub> O <sub>2</sub> (OR <sub>1</sub> )(OR <sub>2</sub> )(OR <sub>3</sub> ) where R <sub>1</sub> , R <sub>2</sub> and R <sub>3</sub> may be any of the following groups in varying proportions: –H –CH <sub>2</sub> –COONa –CH <sub>2</sub> –COOH  Degree of substitution: 0.60–0.85
Enzymatically hydrolysed carboxy methyl cellulose (E 469)	–	–	[C <sub>6</sub> H <sub>7</sub> O <sub>2</sub> (OH) <sub>x</sub> (OCH <sub>2</sub> COONa) <sub>y</sub> ] n ≥ 4 x = 1.50–2.80y = 0.2–1.50x + y = 3.0(x+y) = degree of substitution

CAS: Chemical Abstract Service; EINECS: European Inventory of Existing Commercial Chemical Substances.

(a): According to the ECHA database (ECHA, 2016), EC numbers with format 6xx-xxx-x have no official status and have no legal significance.

(b): The CAS Registry No 9004-69-7 has also been assigned to ethyl methyl cellulose (E 465).

The CAS Registry numbers and EC (or EINECS) numbers reported in Table 1 were subject to confirmatory steps to minimise the uncertainty of a possible equivocal identification. The Panel considered that modified celluloses are complex structures for which — likewise modified starches (EFSA ANS Panel, 2017a,b) — the CAS registration scheme may unintentionally allow that multiple, possibly erratic, or redundant entries exist for identification of a given substance. However, regardless of the potential uncertainty underlying CAS identifiers, the Panel noted that attributing CAS numbers, which are as reliable as possible to such structures, is probably the ‘best’ available way for a reasonably accurate identification of the chemicals.

Chemically modified celluloses are white or slightly yellowish, odourless and tasteless powders. However, the physical appearance of the modified celluloses depends on the type of substitution, the DP and the DS (Brandt, 2001; Murray, 2009).

All modified celluloses, except ethyl cellulose (E 462) and cross-linked sodium carboxy methyl cellulose (E 468), are water-soluble polymers. Ethyl cellulose (E 462) is the only modified cellulose not soluble in water but soluble in ethanol. Hydroxypropyl cellulose (E 463) and ethyl methyl cellulose (E 465) are both soluble in water and ethanol (Archer, 1991; Brandt, 2001; Cash and Caputo, 2010). However, the solubility of the modified celluloses is influenced by the DS, e.g. sodium carboxy methyl cellulose (CMC, E 466) with a DS < 0.3 is only soluble in alkali. As the DS approaches 0.7, CMC can be easily dissolved in water, above a DS of 1, CMC (E 466) is less water soluble (Coffey et al., 2006).

The Panel noted that the data provided by industry indicated that the majority of particles of individual modified celluloses were in the range of 10–100 µm. In addition, based on the known ability of cellulose particles to swell in water, the presence of nanoscale material after ingestion is highly unlikely.

Aqueous solutions of modified celluloses are highly viscous, depending on concentration, temperature, average chain length of the macromolecule (DP), and the presence of salts or other additives (Brandt, 2001; Cash and Caputo, 2010).

The rheological behaviour of a solution at a given concentration and temperature may be Newtonian, pseudoplastic, thixotropic or even gel-forming, depending on the chain length, the substituent distribution, and the nature of the ether group (Coffey et al., 2006). The viscosities of 2% neutral, aqueous solutions of modified celluloses at ambient temperature range from 5 to over 10<sup>5</sup> mPa.s (Brandt, 2001).

Aqueous solutions of methyl cellulose (E 461) and hydroxypropyl methyl cellulose (E 464) are characterised by thermal insolubility. Increases in temperature first lead to a minor decrease in viscosity. When the gelling temperature is reached, there is a sharp increase in viscosity. The viscosity or gel strength remains at a constant value. This gelation is a reversible process. Gel temperature and texture are dependent on the DS and substitution type (Haque and Morris, 1993; Haque et al., 1993; Klemm et al., 1998; Brandt, 2001; Coffey et al., 2006; Murray, 2009; Cash and Caputo, 2010).

The pH of aqueous extracts of E 461, E 463, E 464 and E 465 was found to be between 5 and 8, whereas E 462 was neutral. Due to their sodium content, E 466 and E 469 have slightly elevated pH values (between 5 and 8.5 and 6 and 8.5, respectively).



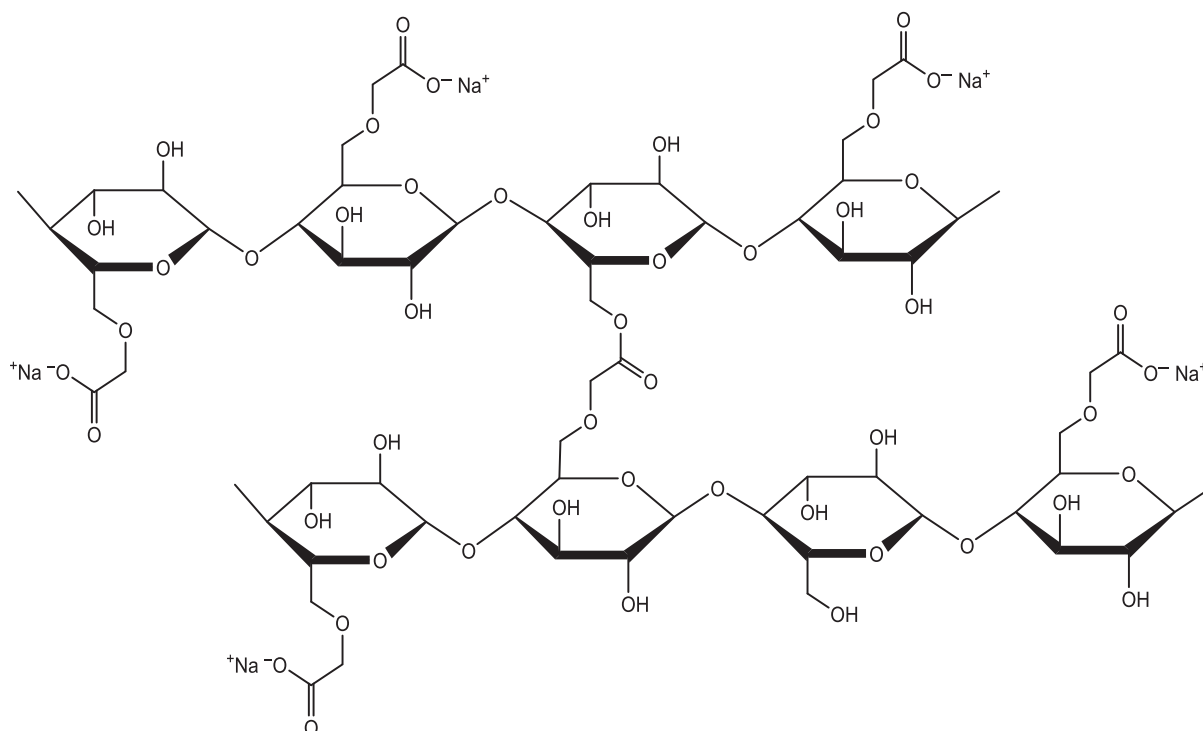
Infrared (IR) and nuclear magnetic resonance (NMR) spectra are available for all modified celluloses except for E 469 (Desai et al., 2006; Qi et al., 2009). Purity data are given by Nurkeeva et al. (2005).

### Cross-linked sodium carboxy methyl cellulose (E 468)

Cross-linked sodium carboxy methyl cellulose (E 468) is an internally linked polymer of carboxy methyl cellulose sodium (E 466). It is defined in Commission Regulation (EU) No 231/2012 as the sodium salt of thermally cross-linked partly O-carboxymethylated cellulose.

Cross-linked sodium carboxy methyl cellulose (E 468) has the CAS Registry Number 748-11-65-7. No EC (EINECS) Number has been attributed to E 468. The molecular formula is  $C_6H_7O_2(OR_1)(OR_2)(OR_3)$ , with  $R_1$ ,  $R_2$  and  $R_3$  groups in varying proportion ( $-H$ ,  $-CH_2COONa$ ,  $-CH_2COOH$ ).

A representative structural formula of cross-linked sodium carboxy methyl cellulose (E 468) is shown in Figure 3.



**Figure 3:** A representative structural formula of cross-linked sodium carboxy methyl cellulose (E 468)

Cross-linked sodium carboxy methyl cellulose is described as a white to off-white, slightly hygroscopic, odourless powder, containing not more than 10% water-soluble matter. The substance is insoluble in ether, alcohol and organic solvents. It is indicated that cross-linking makes the substance fibrous, hydrophilic, highly water-absorbing, which results in pronounced swelling and wicking properties (Mane and Vaidya, 2014).

Common synonyms for chemically modified celluloses are given in Table 2.

**Table 2:** Synonyms for chemically modified celluloses

Substance	Synonyms
Methyl cellulose (E 461)	Methyl ether of cellulose; Cellulose methyl ether; INS No. 461
Ethyl cellulose (E 462)	Ethyl ether of cellulose; INS No. 462
Hydroxypropyl cellulose (E 463)	Cellulose hydroxypropyl ether; hydroxypropyl ether of cellulose; modified cellulose; INS No. 463
Hydroxypropyl methyl cellulose (E 464)	2-Hydroxypropyl ether of methyl cellulose; INS No. 464; hypromellose (Ph. Eur.)
Ethyl methyl cellulose (E 465)	Ethyl methyl ether of cellulose; methyl ethyl cellulose; MEC; INS No. 465

Substance	Synonyms
Sodium carboxy methyl cellulose (E 466)	Sodium salt of the carboxymethyl ether of cellulose; sodium cellulose glycolate; Na CMC; CMC; cellulose gum; sodium CMC; INS No. 466, carmellose sodium (Ph. Eur.)
Cross-linked sodium carboxy methyl cellulose (E 468)	Cross-linked CMC, cross-linked sodium CMC, cross-linked carboxy methyl cellulose, croscarmellose sodium (Ph. Eur.)
Enzymatically hydrolysed carboxy methyl cellulose sodium (E 469)	Sodium carboxy methyl cellulose; enzymatically hydrolysed; Enzymatically hydrolysed carboxy methyl cellulose; CMC-ENZ; INS No. 469

### 3.1.2. Specifications

Specifications for microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), and modified celluloses E 461, E 462, E 463, E 464, E 465, E 466, E 468 and E 469 as defined in Commission Regulation (EU) No 231/2012 and by JECFA are listed in Tables 3–12.

#### 3.1.2.1. Microcrystalline cellulose (E 460(i))

**Table 3:** Specifications for microcrystalline cellulose (E 460(i)) according to Commission Regulation (EU) No 231/2012 and JECFA (2009)

	Commission Regulation (EU) No 231/2012	JECFA (2009)
Definition	Microcrystalline cellulose is purified, partially depolymerised cellulose prepared by treating $\alpha$ -cellulose, obtained as a pulp from natural strains of fibrous plant material, with mineral acids. The degree of polymerisation is typically less than 400	Purified, partially depolymerised cellulose prepared by treating $\alpha$ -cellulose, obtained as a pulp from fibrous plant material, with mineral acids. The degree of polymerisation is typically less than 400
Assay	Not less than 97% calculated as cellulose on the anhydrous basis	Not less than 97% of carbohydrate calculated as cellulose on the dry basis
Particle size	Not less than 5 $\mu$ m (not more than 10% of particles of less than 5 $\mu$ m)	Not more than 10% of the particles have a diameter below 5 $\mu$ m
Description	A fine white or almost white odourless powder	Fine, white or almost white, odourless, free flowing crystalline powder
<b>Identification</b>		
Solubility	Insoluble in water, ethanol, ether and dilute mineral acids Slightly soluble in sodium hydroxide solution	Insoluble in water, ethanol, ether and dilute mineral acids Slightly soluble in sodium hydroxide solution
Colour reaction	To 1 mg of the sample, add 1 mL of phosphoric acid and heat on a water bath for 30 min. Add 4 mL of a 1 in 4 solution of pyrocatechol in phosphoric acid and heat for 30 min. A red colour is produced	–
Infrared absorption spectroscopy	To be identified	The infrared absorption spectrum of a potassium bromide dispersion of the sample corresponds to the infrared spectrum below
Suspension test	Mix 30 g of the sample with 270 mL of water in a high-speed (12,000 rpm) power blender for 5 min. The resultant mixture will be either a free-flowing suspension or a heavy, lumpy suspension which flows poorly, if at all, settles only slightly and contains many trapped air bubbles. If a free-flowing suspension is obtained, transfer 100 mL into a 100-mL graduated cylinder and allow to stand for 1 h. The solids settles and a supernatant liquid appears	Mix 30 g of the sample with 270 mL of water in a high-speed (18,000 rpm) blender for 5 min. Transfer 100 mL of the mixture to a 100-mL graduated cylinder, and allow to stand for 3 h. A white, opaque, bubble-free dispersion that forms a supernatant is obtained

	<b>Commission Regulation (EU) No 231/2012</b>	<b>JECFA (2009)</b>
pH	The pH of the supernatant liquid is between 5.0 and 7.5 (10% suspension in water)	5.0–7.5 Shake 5 g of the sample with 40 mL of water for 20 min and centrifuge. Determine the pH of the supernatant
<b>Purity</b>		
Loss on drying	Not more than 7% (105°C, 3 h)	Not more than 7.0% (105°, 3 h)
Water-soluble matter	Not more than 0.24%	Not more than 0.24% Shake 5 g of the sample with approximately 80 mL of water for 10 min, filter through Whatman No. 42 or equivalent filter paper into a tared beaker, wash residue with 20 mL of water and evaporate to dryness on a steam bath. Dry at 105° for 1 h, cool, weigh and calculate as percentage
Sulfated ash	Not more than 0.5% determined at 800 ± 25°C	Not more than 0.05% Test 10 g of the sample (Method I)
Starch	Not detectable To 20 mL of the dispersion obtained in Identification, suspension test, add a few drops of iodine solution and mix. No purplish to blue or blue colour should be produced	Not detectable To 20 mL of the dispersion obtained in the identification test for starch, add a few drops of iodine TS and mix. No purplish to blue or blue colour should be obtained
Carboxyl groups	Not more than 1%	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an AAS/ICP-AES technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on principles of methods described in Volume 4 (under 'General Methods, Metallic Impurities')
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

JECFA: Joint FAO/WHO Expert Committee on Food Additives; AAS: atomic absorption spectroscopy; ICP-AES: inductively coupled plasma atomic emission spectroscopy.

In 2017, following a request from the European Commission, the ANS Panel provided a scientific opinion regarding the safety of an amendment of the specifications for microcrystalline cellulose (E 460 (i)) (EFSA ANS Panel, 2017a,b). The applicant proposed an amendment as regards the solubility of the food additive to 'practically insoluble or insoluble in sodium hydroxide solution'. The Panel concluded that the amendment proposed would not give rise to a safety concern. However, the Panel recommended that the concentration of sodium hydroxide solution to be used in the solubility test should be indicated in the EU specifications.

### 3.1.2.2. Powdered cellulose (E 460(ii))

**Table 4:** Specifications for powdered cellulose (E 460(ii)) according to Commission Regulation (EU) No 231/2012 and JECFA (2006a)

	Commission Regulation (EU) No 231/2012	JECFA (2006a)
<b>Definition</b>	Purified, mechanically disintegrated cellulose prepared by processing $\alpha$ -cellulose obtained as a pulp from natural strains of fibrous plant materials	Purified, mechanically disintegrated cellulose prepared by processing $\alpha$ - cellulose obtained as a pulp from fibrous plant materials; occurs as a white, odourless substance consisting of fibrous particles which may be compressed into self-binding tablets which disintegrate rapidly in water; exists in various grades exhibiting degrees of fineness ranging from a dense free flowing powder to a coarse, fluffy non-flowing material
Assay	Content not less than 92%	Not less than 92% (C <sub>12</sub> H <sub>20</sub> O <sub>10</sub> ) <sub>n</sub>
Particle size	Not less than 5 $\mu$ m (not more than 10% of particles of less than 5 $\mu$ m)	
<b>Description</b>	A white, odourless powder	–
<b>Identification</b>		
Solubility	Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution	Insoluble in water, ethanol, ether and dilute mineral acids. Slightly soluble in sodium hydroxide solution
Suspension test	Mix 30 g of the sample with 270 mL of water in a high-speed (12,000 rpm) power blender for 5 min. The resultant mixture will be either a free-flowing suspension or a heavy, lumpy suspension which flows poorly, if at all, settles only slightly and contains many trapped air bubbles. If a free-flowing suspension is obtained, transfer 100 mL into a 100-mL graduated cylinder and allow to stand for 1 h. The solids settle and a supernatant liquid appears	Mix 30 g of the sample with 270 mL of water in a high-speed (12,000 rpm) power blender for 5 min. The resultant mixture will be either a free-flowing suspension or a heavy, lumpy suspension which flows poorly, if at all, settles only slightly and contains many trapped air bubbles. If a free flowing suspension is obtained, transfer 100 mL into a 100-mL graduated cylinder and allow to stand for 1 h. The solids settle and a supernatant liquid appears
pH	The pH of the supernatant liquid is between 5.0 and 7.5 (10% suspension in water)	5.0–7.5 Mix 10 g of the dried sample, accurately weighed, with 90 mL water and allow to stand with occasional stirring for 1 h
<b>Purity</b>		
Loss on drying	Not more than 7% (105°C, 3 h)	Not more than 7% after drying (105°C, 3 h)
Water-soluble matter	Not more than 1.0%	Not more than 1.5% Mix about 6 g of the sample, previously dried, with 90 mL of recently boiled and cooled water and allow to stand with occasional stirring for 10 min. Filter, discard the first 10 mL of filtrate and pass the filtrate through the same filter a second time if necessary to obtain a clear filtrate. Evaporate a 15 mL portion of the filtrate to dryness in a tared evaporation dish on a steam bath, dry at 105° for 1 h. Not more than 15 mg of residue is obtained
Sulfated ash	Not more than 0.3% (800 $\pm$ 25°C)	Not more than 0.3% (at approximately 800°C to constant weight)
Starch	Not detectable To 20 mL of the dispersion obtained in Identification, suspension test, add a few drops of iodine solution and mix. No purplish to blue or blue colour should be produced	Not detectable To 20 mL of the mixture obtained in the Identification Test B add a few drops of iodine TS and mix. No purplish-to-blue or blue colour is produced

	Commission Regulation (EU) No 231/2012	JECFA (2006a)
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, 'Instrumental Methods'
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

The Panel noted that, according to the EU specifications for microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)), impurities of the toxic elements arsenic, cadmium, lead and mercury are accepted up concentrations of 3, 1, 2 and 1 mg/kg, respectively. Contamination at those levels could have a significant impact on the exposure to these metals, for which the intake is already close to the health-based guidance values established by EFSA (EFSA, 2009a,b; EFSA CONTAM Panel, 2009, 2010, 2012).

The Panel noted that there are monographs in the European Pharmacopoeia (Ph. Eur. 8th edition, 2014) on 'microcrystalline cellulose' and 'powdered cellulose'. In these monographs, limits for total anaerobic microbial count (TAMC) and total combined yeast and mould count (TYMC) are defined and absence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella* is required.

### 3.1.2.3. Methyl cellulose (E 461)

**Table 5:** Specifications for methyl cellulose (E 461) according to Commission Regulation (EU) No 231/2012 and JECFA (2006a)

	Commission Regulation (EU) No 231/2012	JECFA (2006a)
<b>Definition</b>	Methyl cellulose is cellulose obtained directly from strains of fibrous plant material and partially etherified with methyl groups	The methyl ether of cellulose, prepared from wood pulp or cotton by treatment with alkali and methylation of the alkali cellulose with methyl chloride. The article of commerce can be specified further by viscosity
Assay	Content not less than 25% and not more than 33% of methoxyl groups ( $-\text{OCH}_3$ ) and not more than 5% of hydroxyethoxyl groups ( $-\text{OCH}_2\text{CH}_2\text{OH}$ )	Not less than 25% and not more than 33% of methoxyl groups. (Some products of commerce designated 'methyl cellulose' also contain components substituted with small amounts (max. 5%) of hydroxyethyl and/or hydroxypropyl groups. Development of separate specifications for these products should be considered)
<b>Description</b>	Slightly hygroscopic white or slightly yellowish or greyish odourless and tasteless, granular or fibrous powder	Hygroscopic white or off-white, odourless fine granules, filaments or powder
<b>Identification</b>		
Solubility	Swelling in water, producing a clear to opalescent, viscous, colloidal solution Insoluble in ethanol, ether and chloroform Soluble in glacial acetic acid	Swelling in water, producing a clear to opalescent, viscous, colloidal solution; insoluble in ethanol, ether and chloroform. Soluble in glacial acetic acid
Foam test	–	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. (This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers)



	Commission Regulation (EU) No 231/2012	JECFA (2006a)
Precipitate formation	–	To 5 mL of a 0.5% solution of the sample, add 5 mL of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers)
<b>Purity</b>		
Loss on drying	Not more than 10% (105°C, 3 h)	Not more than 10% (105°C, 3 h)
Sulfated ash	Not more than 1.5% (800 ± 25°C)	Not more than 1.5%
pH	Not less than 5.0 and not more than 8.0 (1% colloidal solution)	5.0–8.0 (1 in 100 solution)
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

The Panel noted that in JECFA (2006a), descriptions are included for tests on determination of lead and determination of methoxyl groups. The Panel also noted that methyl cellulose specifications include methyl hydroxyethyl cellulose (MHEC) (up to 5% hydroxyethyl substitution is permitted).

#### 3.1.2.4. Ethyl cellulose (E 462)

**Table 6:** Specifications for ethyl cellulose (E 462) according to Commission Regulation (EU) No 231/2012 and JECFA (2012)

	Commission Regulation (EU) No 231/2012	JECFA (2012)
<b>Definition</b>	Ethyl cellulose is cellulose obtained directly fibrous plant material and partially etherified with ethyl groups	Ethyl ether of cellulose, prepared from wood pulp or cotton by treatment with alkali and ethylation of the alkali cellulose with ethyl chloride. The article of commerce can be specified further by viscosity. Antioxidants permitted for use in food may be added for stabilisation purposes
Assay	Content not less than 44% and not more than 50% of ethoxyl groups ( $-OC_2H_5$ ) on the dried basis (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit)	Not less than 44% and not more than 50% of ethoxyl groups ( $-OC_2H_5$ ) on the dried basis (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit)
<b>Description</b>	Slightly hygroscopic white to off-white, odourless and tasteless powder	Free flowing, white to light tan powder
<b>Identification</b>		
Solubility	Practically insoluble in water, in glycerol and in propane-1,2-diol but soluble in varying proportions in certain organic solvents depending upon the ethoxyl content. Ethyl cellulose containing less than 46–48% of ethoxyl groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform and in aromatic hydrocarbon ethanol mixtures. Ethyl cellulose containing 46–48% or more of ethoxyl groups is freely soluble in ethanol, in methanol, in toluene, in chloroform and in ethyl acetate	Practically insoluble in water, in glycerol, and in propane-1,2-diol, but soluble in varying proportions in certain organic solvents, depending upon the ethoxyl content. Ethyl cellulose containing less than 46–48% of ethoxyl groups is freely soluble in tetrahydrofuran, methyl acetate and aromatic hydrocarbon ethanol mixtures. Ethyl cellulose containing 46–48% or more of ethoxyl groups is freely soluble in ethanol, methanol, toluene and ethyl acetate

	<b>Commission Regulation (EU) No 231/2012</b>	<b>JECFA (2012)</b>
Film forming test	Dissolve 5 g of the sample in 95 g of an 80:20 (w/w) mixture of toluene ethanol. A clear, stable, slightly yellow solution is formed. Pour a few mL of the solution onto a glass plate and allow the solvent to evaporate. A thick, tough, continuous, clear film remains. The film is flammable	Dissolve 5 g of the sample in 95 g of an 80:20 (w/w) mixture of toluene-ethanol. A clear, stable, slightly yellow solution is formed. Pour a few mL of the solution onto a glass plate, and allow the solvent to evaporate. A thick, tough continuous, clear film remains. The film is flammable.
<b>Purity</b>		
Loss on drying	Not more than 3% (105°C, 2 h)	Not more than 3% (105°C, 2 h)
Sulfated ash	Not more than 0.4%	Not more than 0.4%
pH	Neutral to litmus (1% colloidal solution)	Neutral to litmus (1 in 20 suspension)
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

The Panel noted that in JECFA (2012) descriptions are included for tests on determination of lead and determination of the ethoxyl content. Furthermore, the Panel noted that in the European Pharmacopeia the concentration of acetaldehyde is limited to 100 ppm (Ph. Eur. 8th edition, 2014).

### 3.1.2.5. Hydroxypropyl cellulose (E 463)

**Table 7:** Specifications for hydroxypropyl cellulose (E 463) according to Commission Regulation (EU) No 231/2012 and JECFA (2006b)

	<b>Commission Regulation (EU) No 231/2012</b>	<b>JECFA (2006b)</b>
<b>Definition</b>	Hydroxypropyl cellulose is cellulose obtained directly from strains of fibrous plant material and partially etherified with hydroxypropyl groups	An ether of cellulose containing hydroxypropyl substitution prepared from cellulose by treatment with alkali and propylene oxide. The article of commerce can be specified further by viscosity
Assay	Content not more than 80.5% of hydroxypropoxyl groups ( $-OCH_2CHOHCH_3$ ) equivalent to not more than 4,6 hydroxypropyl groups per anhydroglucose unit on the anhydrous basis	Not more than 80.5% of hydroxypropoxy groups equivalent to not more than 4.6 hydroxypropyl groups per anhydroglucose unit on the dried basis
<b>Description</b>	Slightly hygroscopic white or slightly yellowish or greyish odourless and tasteless, granular or fibrous powder	Slightly hygroscopic, white or off-white, almost odourless, granular or fibrous powder
<b>Identification</b>		
Solubility	Swelling in water, producing a clear to opalescent, viscous, colloidal solution. Soluble in ethanol. Insoluble in ether	Swells in water, producing a clear to opalescent, viscous colloidal solution; insoluble in ethanol; insoluble in ether
Gas chromatography	Determine the substituents by gas chromatography	
Foam formation	–	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers

	Commission Regulation (EU) No 231/2012	JECFA (2006b)
Precipitate formation	–	To 5 mL of a 0.5% solution of the sample, add 5 mL of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers
<b>Purity</b>		
Loss on drying	Not more than 10% (105°C, 3 h)	Not more than 10% (105°C to constant weight)
Sulfated ash	Not more than 0.5% (800 ± 25°C)	Not more than 0.5%. Test 1 g of the sample
pH	Not less than 5.0 and not more than 8.0 (1% colloidal solution)	Not less than 5.0 and not more than 8.0 (1 in 100 solution)
Propylene chlorohydrins	Not more than 0.1 mg/kg	Not more than 0.1 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

The Panel noted that JECFA and EU specifications state different solubility properties for hydroxypropyl cellulose (E 463) in ethanol (soluble according to Commission Regulation, insoluble according to JECFA).

### 3.1.2.6. Hydroxypropyl methyl cellulose (E 464)

**Table 8:** Specifications for hydroxypropyl methyl cellulose (E 464) according to Commission Regulation (EU) No 231/2012 and JECFA (2011a)

	Commission Regulation (EU) No 231/2012	JECFA (2011a)
<b>Definition</b>	Hydroxypropyl methyl cellulose is cellulose obtained directly from strains of fibrous plant material and partially etherified with methyl groups and containing a small degree of hydroxypropyl substitution	Hydroxypropylmethyl cellulose is a methyl cellulose modified by treatment with alkali and propylene oxide by which a small number of 2-hydroxypropyl groups are attached through ether links to the anhydroglucose units of the cellulose. The article in commerce may be further specified by viscosity
Assay	Content not less than 19% and not more than 30% methoxyl groups (–OCH <sub>3</sub> ) and not less than 3% and not more than 12% hydroxypropoxyl groups (–OCH <sub>2</sub> CHOHCH <sub>3</sub> ), on the anhydrous basis	Not less than 19% and not more than 30% of methoxy groups (–OCH <sub>3</sub> ) and not less than 3% and not more than 12% hydroxypropoxy groups (–OCH <sub>2</sub> CHOHCH <sub>3</sub> ), on the dried basis
<b>Description</b>	Slightly hygroscopic white or slightly yellowish or greyish odourless and tasteless, granular or fibrous powder	Hygroscopic white or off-white powder, or granules or fine fibres
<b>Identification</b>		
Solubility	Swelling in water, producing a clear to opalescent, viscous, colloidal solution. Insoluble in ethanol	Swells in water, producing a clear to opalescent, viscous colloidal solution; insoluble in ethanol
Gas chromatography	Determine the substituents by gas chromatography	–
Foam formation	–	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers

	Commission Regulation (EU) No 231/2012	JECFA (2011a)
Precipitate formation	–	To 5 mL of a 0.5% solution of the sample, add 5 mL of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers
<b>Purity</b>		
Loss on drying	Not more than 10% (105°C, 3 h)	Not more than 10% (105°C to constant weight)
Sulfated ash	Not more than 1.5% for products with viscosities of 50 mPa.s or above Not more than 3% for products with viscosities below 50 mPa.s	Not more than 1.5% for products with viscosities of 50 centipoise or above, and not more than 3% for products with viscosities below 50 centipoise
pH	–	Not less than 5.0 and not more than 8.0 (1 in 100 solution)
Propylene chlorohydrins	Not more than 0.1 mg/kg	Not more than 1 mg/kg
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

### 3.1.2.7. Ethyl methyl cellulose (E 465)

**Table 9:** Specifications for ethyl methyl cellulose (E 465) according to Commission Regulation (EU) No 231/2012 and JECFA (2006c)

	Commission Regulation (EU) No 231/2012	JECFA (2006c)
<b>Definition</b>	Ethyl methyl cellulose is cellulose obtained directly from strains of fibrous plant material and partially etherified with methyl and ethyl groups	A mixed ether of cellulose, prepared from cellulose by treatment with alkali, dimethyl sulfate and ethyl chloride; both the methyl and ethyl groups are attached to the anhydroglucose units by ether linkages. The article of commerce can be specified further by viscosity
Assay	Content on the anhydrous basis not less than 3.5% and not more than 6.5% of methoxyl groups ( $-\text{OCH}_3$ ) and not less than 14.5% and not more than 19% of ethoxyl groups ( $-\text{OCH}_2\text{CH}_3$ ), and not less than 13.2% and not more than 19.6% of total alkoxy groups, calculated as methoxyl	Methyl ethyl cellulose contains, on the dried basis, not less than 3.5% and not more than 6.5% of methoxyl groups ( $-\text{OCH}_3$ ), not less than 14.5% and not more than 19.0% of ethoxyl groups ( $-\text{OCH}_2\text{CH}_3$ ), and not less than 13.2% and not more than 19.6% of total alkoxy groups, calculated as methoxyl (on the dry basis)
<b>Description</b>	Slightly hygroscopic white or slightly yellowish or greyish odourless and tasteless, granular or fibrous powder	Hygroscopic and slightly yellowish odourless fibre or powder
<b>Identification</b>		
Solubility	Swelling in water, producing a clear to opalescent, viscous, colloidal solution. Soluble in ethanol. Insoluble in ether	Swelling in water, producing a clear to opalescent, viscous, colloidal solution; insoluble in ethanol
Foam test	–	A 0.1% solution of the sample is shaken vigorously. A layer of foam appears. (This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ether and alginates and natural gums)

	Commission Regulation (EU) No 231/2012	JECFA (2006c)
Precipitate formation	–	To 5 mL of a 0.5% solution of the sample add 5 mL of a 5% solution of copper sulfate or of aluminium sulfate. No precipitate appears. (This test permits the distinction of cellulose ethers from sodium carboxy methyl cellulose, gelatine, carob bean gum and tragacanth gum)
Substituents	–	Determine the substituents by gas chromatography
<b>Purity</b>		
Loss on drying	Not more than 15% for the fibrous form, and not more than 10% for the powdered form (105°C to constant weight)	Not more than 15% for the fibrous form, and not more than 10% for the powdered form, after drying to constant weight
Sulfated ash	Not more than 0.6%	Not more than 0.6%
pH	Not less than 5.0 and not more than 8.0 (1% colloidal solution)	
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–

The Panel noted that JECFA and EU specifications state different solubility properties for E 465 in ethanol (soluble according to Commission Regulation, insoluble according to JECFA).

### 3.1.2.8. Sodium carboxy methyl cellulose (E 466)

**Table 10:** Specifications for sodium carboxy methyl cellulose (E 466) according to Commission Regulation (EU) No 231/2012 and JECFA (2011b)

	Commission Regulation (EU) No 231/2012	JECFA (2011b)
<b>Definition</b>	carboxy methyl cellulose is the partial sodium salt of a carboxymethyl ether of cellulose, the cellulose being obtained directly from strains of fibrous plant material	Prepared from cellulose by treatment with alkali and monochloro-acetic acid or its sodium salt. The article of commerce can be specified further by viscosity
Assay	Content on the anhydrous basis not less than 99.5%	Not less than 99.5% of sodium carboxy methyl cellulose, calculated on the dried basis
<b>Description</b>	Slightly hygroscopic white or slightly yellowish or greyish odourless and tasteless, granular or fibrous powder	White or slightly yellowish, almost odourless hygroscopic granules, powder or fine fibres
<b>Identification</b>		
Solubility	Yields a viscous colloidal solution with water. Insoluble in ethanol	Yield viscous colloidal solution with water; insoluble in ethanol
Foam test	A 0.1% solution of the sample is shaken vigorously. No layer of foam appears. (This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers)	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxy methyl cellulose from other cellulose ethers and from alginates and natural gums
Precipitate formation	To 5 mL of a 0.5% solution of the sample, add 5 mL of 5% solution of copper sulfate or of aluminium sulfate. A precipitate appears. (This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers and from gelatine, locust bean gum and tragacanth)	To 5 mL of a 0.5% solution of the sample add 5 mL of a 5% solution of copper sulfate or of aluminium sulfate. A precipitate appears. (This test permits the distinction of sodium carboxy methyl cellulose from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum)



	Commission Regulation (EU) No 231/2012	JECFA (2011b)
Colour reaction	Add 0.5 g powdered carboxy methyl cellulose sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced, and use the solution for the following test: To 1 mg of the sample, diluted with an equal volume of water, in a small test tube, add 5 drops of 1-naphthol solution. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface	Add 0.5 g of powdered carboxy methyl cellulose sodium to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. To 1 mL of the solution, diluted with an equal volume of water, in a small test tube, add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface
<b>Purity</b>		
Degree of substitution	Not less than 0.2 and not more than 1.5 carboxymethyl groups ( $-\text{CH}_2\text{COOH}$ ) per anhydroglucose unit	Not less than 0.20 and not more than 1.50
Loss on drying	Not more than 12% (105°C to constant weight)	Not more than 12% after drying (105°, to constant weight)
pH	Not less than 5.0 and not more than 8.5 (1% colloidal solution)	6.0–8.5 (1 in 100 solution)
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg	–
Cadmium	Not more than 1 mg/kg	–
Total glycolate	Not more than 0.4%, calculated as sodium glycolate on the anhydrous basis	Not more than 0.4% calculated as sodium glycolate on the dried basis
Sodium	Not more than 12.4% on the anhydrous basis	Not more than 12.4% on the dried basis
Sodium chloride	–	Not more than 0.5% on the dried basis

The European Pharmacopoeia distinguishes two monographs of sodium carboxy methyl cellulose: Carmellose sodium (Ph. Eur. 8th edition, 3rd supplement 2015), with a sodium content of not less than 6.5% and not more than 10.8% on the anhydrous basis; carmellose sodium, low-substituted (Ph. Eur. 8th edition, 2014), with a sodium content of not less than 2% and not more than 4.5% on the anhydrous basis. Both monographs differ in their criteria for sulfated ash, which are not included in Commission Regulation (EU) No 231/2012 and in the JECFA specifications.

### 3.1.2.9. Cross-linked sodium carboxy methyl cellulose (E 468)

**Table 11:** Specifications for cross-linked sodium carboxy methyl cellulose (E 468) according to Commission Regulation (EU) No 231/2012 and JECFA (2006a–g)

	Commission Regulation (EU) No 231/2012	JECFA (2006a–g)
<b>Assay</b>		
<b>Description</b>	Slightly hygroscopic, white to off white, odourless powder	A slightly hygroscopic, white to greyish-white, odourless powder
<b>Identification</b>		
Precipitate formation	Shake 1 g with 100 mL of a solution containing 4 mg/kg methylene blue and allow to settle. The substance to be examined absorbs the methylene blue and settles as a blue, fibrous mass	Mix 1 g of the powdered sample with 100 mL of solution containing 4 mg/kg of methylene blue in water and allow to settle. The substance absorbs methylene blue and settles as a blue, fibrous mass

	Commission Regulation (EU) No 231/2012	JECFA (2006a–g)
Colour reaction	Shake 1 g with 50 mL of water. Transfer 1 mL of the mixture to a test tube, add 1 mL water and 0.05 mL of freshly prepared 40 g/l solution of $\alpha$ -naphthol in methanol. Incline the test tube and add carefully 2 mL of sulfuric acid down the side so that it forms a lower layer. A reddish-violet colour develops at the interface	Add 1 g of the powdered sample to 50 mL water, while stirring to produce a uniform dispersion. Dilute 1 mL of this mixture with 1 mL of water in a small test tube and add 5 drops of 1-naphthol TS. Incline the test tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface
Test for sodium	Passes test	–
pH	Not less than 5.0 and more than 7.0 (1% solution)	Not less than 5.0 and not more than 7.0 (1 in 100 suspension in water)
Solubility	–	Practically insoluble in acetone, in ethanol and in toluene
<b>Purity</b>		
Loss on drying	Not more than 6% (105°C, 3 h)	Not more than 6% (105°C, 3 h)
Water soluble matter	Not more than 10%	Not more than 10%
Degree of substitution	Not less than 0.2 and not more than 1.5 carboxymethyl groups per anhydroglucose unit	Not less than 0.2 and not more than 1.5 carboxymethyl groups ( $-\text{CH}_2\text{COOH}$ ) per anhydroglucose unit on the dried basis.
Sodium content	Not more than 12.4% on anhydrous basis	–
Arsenic	Not more than 3 mg/kg	–
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Cadmium	Not more than 1 mg/kg	–
Mercury	Not more than 1 mg/kg	–
Sulfated ash	–	Not less than 14.0% and not more than 28.0% on the dried basis (2 g of sample)
Sodium chloride and sodium glycolate	–	Not more than 0.5% (sum of sodium chloride and sodium glycolate) on the dried basis

JECFA and the European Pharmacopoeia (Ph. Eur. 8th edition, 2014) include criteria for solubility, sulfated ash and the combined content of sodium chloride and sodium glycolate, which are not included in Commission Regulation (EU) No 231/2012. In addition, the European Pharmacopoeia defines limits for TAMC and TYMC, and requires the absence of *E. coli*.

### 3.1.2.10. Enzymatically hydrolysed carboxy methyl cellulose (E 469)

**Table 12:** Specifications for enzymatically hydrolysed carboxy methyl cellulose (E 469) according to Commission Regulation (EU) No 231/2012 and JECFA (2006d)

	Commission Regulation (EU) No 231/2012	JECFA (2006d)
<b>Definition</b>	Enzymatically hydrolysed carboxy methyl cellulose is obtained from carboxy methyl cellulose by enzymatic digestion with a cellulase produced by <i>Trichoderma longibrachiatum</i> (formerly <i>T. reesei</i> )	The product is the sodium salt of a carboxymethyl ether of cellulose, which has been partially hydrolysed by enzymatic treatment with food-grade <i>Trichoderma reesei</i> cellulase. The total content of mono- and disaccharides does not exceed about 7.5%
Assay	Not less than 99.5%, including mono- and disaccharides, on the dried basis	Not less than 99.5%, including mono- and disaccharides, on the dried basis

	<b>Commission Regulation (EU) No 231/2012</b>	<b>JECFA (2006d)</b>
<b>Description</b>	White or slightly yellowish or greyish, odourless, slightly hygroscopic granular or fibrous powder	White or slightly yellowish or greyish, odourless, slightly hygroscopic granular or fibrous powder
<b>Identification</b>		
Solubility	Soluble in water, insoluble in ethanol	Soluble in water; insoluble in ethanol
Foam test	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxy methyl cellulose, whether hydrolysed or not, from other cellulose ethers and from alginates and natural gums	Vigorously shake a 0.1% solution of the sample. No layer of foam appears. This test distinguishes sodium carboxy methyl cellulose, whether hydrolysed or not, from other cellulose ethers and from alginates and natural gums.
Precipitate formation	To 5 mL of a 0.5% solution of the sample add 5 mL of a 5% solution of copper or aluminium sulfate. A precipitate appears. This test distinguishes sodium carboxy methyl cellulose, whether hydrolysed or not, from other cellulose ethers and from gelatine, carob bean gum and tragacanth gum	To 5 mL of a 0.5% solution of the sample add 5 mL of a 5% solution of copper or aluminium sulfate. A precipitate appears. This test distinguishes sodium carboxy methyl cellulose, whether hydrolysed or not, from other cellulose ethers, and from gelatine, carob bean gum and tragacanth gum
Colour reaction	Add 0.5 g of the powdered sample to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. Dilute 1 mL of the solution with 1 mL of water in a small test tube. Add 5 drops of 1-naphthol TS. Incline the tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface	Add 0.5 g of the powdered sample to 50 mL of water, while stirring to produce a uniform dispersion. Continue the stirring until a clear solution is produced. Dilute 1 mL of the solution with 1 mL of water in a small test tube. Add 5 drops of 1-naphthol TS. Incline the tube, and carefully introduce down the side of the tube 2 mL of sulfuric acid so that it forms a lower layer. A red-purple colour develops at the interface
Viscosity (60% solids)	Not less than 2,500 kg m <sup>-1</sup> s <sup>-1</sup> at 25 °C corresponding to an average molecule weight of 5,000 Da	Not less than 2,500 mPa.s corresponding to an average molecular weight of 5,000 Da. This test also distinguishes enzymatically hydrolysed CMC from non-hydrolysed CMC since it is not possible to make a 60% solution of ordinary CMC
<b>Purity</b>		
Degree of substitution	Not less than 0.2 and not more than 1.5 carboxymethyl groups per anhydroglucose unit on the dried basis	Not less than 0.2 and not more than 1.50 carboxymethyl groups (CH <sub>2</sub> COOH) per anhydroglucose unit on the dried basis
Loss on drying	Not more than 12% (105°C to constant weight)	Not more than 12% (105°C to constant weight)
pH	Not less than 6.0 and not more than 8.5 (1% colloidal solution)	Not less than 6.0 and not more than 8.5 (1 in 100 solution)
Lead	Not more than 2 mg/kg	Not more than 2 mg/kg
Sodium chloride and sodium glycolate	Not more than 0.5% singly or in combination	Not more than 0.5%, singly or in combination
Residual enzyme activity	Passes test. No change in viscosity of test solution occurs, which indicates hydrolysis of the sodium carboxy methyl cellulose	Passes test

The Panel noted that the content of the tables is not consistent in that heavy metals are not consistently listed at the end of the tables.

### 3.1.3. Manufacturing process

The raw materials for the production of the different types of celluloses are mainly wood chips. Chemical wood pulping involves the extraction of cellulose from wood by dissolving the lignin that binds the cellulose fibres together. Several processes used in chemical pulping have been described, among these, 'kraft pulping' and 'sulfite pulping' being the most important. The choice of a pulping process is determined by the desired product, the wood species available, and by economic considerations.

In 'kraft pulping' wood chips are digested at elevated temperature and pressure in 'white liquor', being an aqueous solution of sodium sulfide ( $\text{Na}_2\text{S}$ ) and sodium hydroxide ( $\text{NaOH}$ ). During digestion the lignin fraction is dissolved. When cooking is complete, the content of the digester is transferred to an atmospheric tank where the spent cooking liquor is separated from the pulp. The pulp then proceeds through various stages of washing, and possibly bleaching, after which it is pressed and dried into the finished product (wood pulp).

In 'sulfite pulping' wood chips are digested under high pressure in the presence of sulfurous acid. To buffer the cooking solution, either sodium bisulfite ( $\text{NaHSO}_3$ ), magnesium bisulfite ( $\text{Mg}(\text{HSO}_3)_2$ ), calcium bisulfite ( $\text{Ca}(\text{HSO}_3)_2$ ) or ammonium bisulfite ( $(\text{NH}_4)\text{HSO}_3$ ) is used. Afterwards, the pulp is treated in a similar way as in 'kraft pulping' (Someswar and Pinkerton, 1992, available at: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.592.5534&rep=rep1&type=pdf>).

In the patent literature, processes have been described to produce  $\alpha$ -cellulose. In these processes, wood chips are digested, at high temperature ( $> 100^\circ\text{C}$ ), by treatment with a mixture of sodium hydroxide ( $\text{NaOH}$ ), sodium sulfide ( $\text{Na}_2\text{S}$ ) and sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) in various concentrations. During this treatment, the lignin fraction is removed. The product so obtained is extracted washed, concentrated and bleached by treatment with chlorine dioxide ( $\text{ClO}_2$ ). After bleaching, the extract is subjected to a second digestion with sodium hydroxide at lower temperature ( $< 50^\circ\text{C}$ ). The resulting product is centrifuged and washed with oxalic acid to neutralise the residual alkali and to facilitate the extraction of other residuals and colouring impurities. Finally, the extract consists of  $\alpha$ -cellulose at a concentration of  $> 97\%$  (Durchman, 1930; Wayman et al., 1959).

#### 3.1.3.1. Microcrystalline cellulose (E 460(i))

Microcrystalline cellulose (E 460(i)) is prepared by the controlled hydrolysis of highly purified  $\alpha$ -cellulose. Hydrolysis is performed with a dilute mineral acid, e.g. hydrochloric acid. Cellulose is thereby converted into an acid-soluble fraction and an acid-insoluble crystalline material. The amorphous regions of the cellulose are completely hydrolysed. Resultant water-soluble cello-oligosaccharides and glucose are removed by subsequent rinsing and filtration. The remaining wet cake contains only pure crystalline regions of natural cellulose. Mechanical shearing in a water slurry is used to free the microcrystals from their fibrous, packed structure, in the form of short, rod-like particles. These are dried and ground to a fine, white, free-flowing crystalline powder (Klose and Glicksman, 1990; Iijima and Takeo, 2000).

Haafiz et al. (2010) described a method for the isolation of microcrystalline cellulose from oil palm empty fruit pulp (OPEFB-pulp). The pulp was hydrolysed with 2.5 N HCl (ratio pulp-liquor 1:20) at  $105^\circ\text{C}$  for 30 min under constant agitation. The reaction mixture was filtered and washed repeatedly first with distilled water and subsequently with 5% diluted  $\text{NH}_4\text{OH}$  solution. Finally, distilled water was used to rinse the mixture until it was free from acid. The microcrystalline cellulose so obtained was dried at  $105^\circ\text{C}$  under vacuum until constant weight, and further ground into a fine powder, snowy-white in appearance.

#### 3.1.3.2. Powdered cellulose (E 460(ii))

According to Commission Regulation (EU) No 231/2012, powdered cellulose is obtained by purification and mechanical disintegration of  $\alpha$ -cellulose.

JECFA (2006a) stated that purified, mechanically disintegrated cellulose is prepared by processing  $\alpha$ -cellulose obtained as a pulp from fibrous plant materials; it occurs as a white, odourless substance consisting of fibrous particles which may be compressed into self-binding tablets which disintegrate rapidly in water; it exists in various grades exhibiting degrees of fineness ranging from a dense free-flowing powder to a coarse, fluffy non-flowing material.

In the patent literature, powdered cellulose is described as a product obtained from cellulose, primarily by mechanical procedures such as grinding. It is stated to be a purified, partially depolymerised cellulose, white (white to grey colour scale), odourless, tasteless powder. It is indicated that various methods for obtaining powdered cellulose of different grades have been described. In all

these methods, the cellulose chains are partially degraded, enzymatically or thermally, or by means of using chemical reagents. The amorphous portions of the cellulose are hydrolysed with these treatments and removed. Methods for preparing powdered cellulose are described in patents by Durian et al. (2011) and Yamasaki et al. (2006).

### 3.1.3.3. Chemically modified celluloses

Chemically modified celluloses (E 461–466), except enzymatically hydrolysed carboxy methyl cellulose (E 469) and cross-linked sodium carboxy methyl cellulose (E 468), are obtained from cellulose as raw material. The base material for E 468 and E 469 is carboxy methyl cellulose (E 466).

The manufacturing processes of modified celluloses are detailed in literature. In general terms, cellulose pulp is dispersed in alkali solution (generally sodium hydroxide, 5–50%) to form alkali cellulose. Alkali celluloses are compounds with given stoichiometric relations between alkali and cellulose (Sjöström, 1993). Subsequently, alkali cellulose is treated with appropriate reagents, under strictly controlled conditions, to substitute the anhydroglucose monomers of the cellulose chain. In this reaction step, the hydroxy groups of the anhydroglucose monomers of the cellulose chain are etherified according to a nucleophilic substitution reaction ( $S_N2$  reaction; Williamson synthesis), a bimolecular reaction with simultaneous a bond-making and a bond-breaking step.

The appropriate etherifying reagents are: (i) in case of methyl cellulose (E 461), methyl chloride; (ii) in case of ethyl cellulose (E 462), ethyl chloride; (iii) in case of ethyl methyl cellulose (E 465) a mixture of ethyl and methyl chloride; (iv) in case of hydroxypropyl cellulose (E 463), propylene oxide; (v) in case of hydroxypropyl methyl cellulose (E 464), a mixture of propylene oxide and methyl chloride.

In the patent literature (Engleman et al., 2014), it is indicated that in the course of the manufacturing of hydroxypropyl methyl cellulose (E 464), propylene chlorohydrin (PCH) can be formed during the process that is aimed to reduce the viscosity of hydroxypropyl methyl cellulose (by adding HCl), via the reaction of HCl with propylene oxide. It is further indicated that PCH can also be formed in the substitution reactor, when propylene oxide and salt react with each other as a secondary reaction, next to the intended reaction of propylene oxide and methyl chloride with alkali cellulose to achieve the methoxy and hydroxypropyl substitutions on the cellulose.

Engleman et al. (2014) described a method for the manufacturing of low molecular weight hydroxyalkyl alkyl cellulose with a reduced concentration of such propylene chlorohydrin or other alkylene halogenohydrins. It is described that alkylene halogenohydrin could be efficiently removed from cellulose ether if extra water was added to the cellulose ether prior to drying, or if steam or a steam mixture was used instead of air, nitrogen or vacuum as the drying medium.

For the manufacturing of additives E 463 and E 464, the hydroxyalkylation of alkali cellulose is not limited to the hydroxyl groups originally present in the system; hydroxyalkylation can proceed at the newly formed hydroxyl groups resulting in hydroxyalkyl chains of varying length and complexity (Klemm et al., 1998; Murray, 2009).

Sodium carboxy methyl cellulose (E 466) is obtained by etherification of alkali cellulose with sodium monochloroacetate (up to 30%) in an alcohol–water medium. During the substitution process, the mixture of alkali cellulose and reagent is heated (50–75°C) and stirred. The DS of the resulting modified cellulose can be controlled by the reaction conditions and use of additives such as organic solvents (isopropanol). Side reactions (e.g. formation of sodium glycolate) can occur, and consume a certain amount of the aqueous alkali. The substitution reaction is followed by purification and washing stages to remove by-products and to achieve the purity levels specified for food additives (Feddersen and Thorp, 1993; Dönges, 1997; Klemm et al., 1998; Mann et al., 1998; Murray, 2009; Heydarzadeh et al., 2009).

According to JECFA (2002), cross-linked sodium carboxy methyl cellulose (E 468) is manufactured by acidifying an aqueous suspension of sodium carboxy methyl cellulose and heating the suspension to achieve cross-linking. The product is then washed and dried. It can also be produced during the manufacture of sodium carboxy methyl cellulose by lowering the pH and heating to produce cross-linking.

According to Swarbrick and Boylan (1990), cross-linked sodium carboxy methyl cellulose (E 468) is made by soaking crude cellulose in sodium hydroxide, and reacting the cellulose with sodium monochloroacetate to form sodium carboxy methyl cellulose. Excess sodium monochloroacetate slowly hydrolyses to glycolic acid and the glycolic acid catalyses the cross-linkage to form cross-linked sodium carboxy methyl cellulose.



Enzymatically hydrolysed carboxy methyl cellulose (E 469) is manufactured by hydrolysis (at 50°C) of carboxy methyl cellulose by an extracellular cellulase enzyme produced by *T. reesei* (now *T. longibrachiatum*). The enzymatic hydrolysis results in the breakdown of cellulose to D-glucose. The enzymatic hydrolysis depends on DS; hydrolysis decreases with increasing DS. However, treatment of carboxy methyl cellulose with a DS of 0.7 results in complete hydrolysis (Melo and Kennedy, 1993).

#### 3.1.4. Methods of analysis in food

Methods specifically used for analysing celluloses in food are available only for methyl cellulose (E 461) and hydroxypropyl methyl cellulose (E 464) (Harfmann et al., 2007; Turowski et al., 2007). The dried foods were subjected to sequential enzymatic digestion with the use of heat-stable  $\alpha$ -amylase, protease, and amyloglycosidase in order to remove starch and proteins. After enzymatic digestion, the solutions were refrigerated for full hydrolysis of the methyl cellulose. The chilled solutions were then filtered and analysed by size-exclusion liquid chromatography (AOAC, 2011).

For the other celluloses (E 460(i), E 460(ii), E 462, E 463, E 465, E 466, E 468, E 469), no food specific analytical methods are available.

In literature, methods are described for the determination of total dietary fibre.

In 1985, the Association of Official Analytical Chemists (AOAC) introduced a standard method for the determination of total dietary fibre (AOAC Official Method 985.29); this method was based on a method described by Prosky et al. (1985). In 1991, AOAC described an extended and optimised gravimetric method for the measurement of total dietary fibre in food (AOAC Official Method 991.43). Both methods were based on the enzymatic removal of starch and protein of the samples by amylase and protease treatment at 90°C and 60°C, respectively. Insoluble dietary fibres are then separated by filtration and high-molecular weight soluble dietary fibres (HMWSDF) precipitated by 78% ethanol and collected by filtration. Both fibre fractions are dried and weighted and collectively give the total dietary fibre content of the sample.

McCleary (2007, 2010); McCleary et al., (2010) elaborated an 'integrated' method (AOAC Method 2009.01) in which the sample was incubated first with  $\alpha$ -amylase at 37°C, and then protein was digested at 60°C by protease; subsequently, insoluble and high molecular weight soluble dietary fibres were precipitated at 78% ethanol and finally determined gravimetrically. Non-digestible oligosaccharides (NDO) were measured in the ethanol filtrate by high-performance liquid chromatography (HPLC).

In McCleary et al. (2012) described a new, AOAC-validated, enzymatic-gravimetric method for the analysis of insoluble, soluble and total dietary fibre, inclusive resistant starch and water:alcohol-soluble NDO and polysaccharides with a DP  $\geq$  3. This method was a combination of the Official Methods of Analysis AOAC 985.29 (and its extensions 991.42 and 993.19), 991.43, 2001.03 and 2002.02. In this method, the test substance is treated with pancreatic  $\alpha$ -amylase and amyloglucosidase for 16 h at 37°C in a sealed container under agitation. The reaction is terminated by pH adjustment and temporary heating. Protein is then digested with proteinase.

#### 3.1.5. Reaction and fate in food

Proteins and hydrocolloids are known to interact in a variety of ways. Hydrocolloids in general may carry positive or negative charges or be uncharged. Few hydrocolloids carry positive charges and most are negatively charged or uncharged. Environmental conditions such as pH, temperature and ionic strength also influence the interaction (Tolstoguzov, 1991). Cellulose, for example, is a non-ionic polymer, and since it does not carry a charge, the changes in pH do not affect the polymer. Carboxy methyl cellulose, however, contains a percentage of anionically charged with a pKa value of 4.0; therefore above pH 4, it is negatively charged. The charge of meat proteins depends strongly upon the pH and they carry an overall positive charge below their isoelectric point (5.0–5.1) and negative charge above. Thus, one can expect no electrostatic interaction between cellulose and meat proteins regardless of the pH, and electrostatic interaction between CMC and meat proteins below a pH of 5.0.

CMC and microcrystalline cellulose (MCC) can be used as fibres in meat batters. Therefore, they can have an impact on the structural characteristics of emulsified sausages that contain large concentrations of proteins that may exceed 15%. Schuh et al. (2013) studied the molecular interactions of meat protein with celluloses using a formulation of Lyoner-style sausages with 0.3–2% CMC/MCC. The addition of CMC (> 0.7%) led to destabilisation of the batter, interfering in the protein network; however, MCC was compatible with the protein matrix and improved firmness without affecting the water binding capacity.

For modified celluloses, different substitutions on the cellulose backbone, as anionic groups in CMC or certain hydrophobicity in hydroxypropyl methyl cellulose (HPMC), lead to macromolecules with different properties compared to native cellulose.

Modified celluloses affect gluten network conformation, depending on the interactions on the type of hydrocolloid and NaCl addition.

Correa and Cristina Ferrero (2014) studied the interaction of modified celluloses (MCC, CMC, HPMC) and pectins with gluten proteins. The modified celluloses were employed at 1.5% (flour basis) of wheat bread dough making. They have reported that they can induce changes on gluten network during their formation, affecting strongly the secondary conformation of the proteins, the CMC dough showing the smallest percentage of  $\alpha$ -helix conformation and the highest unfolded structures. CMC is an anionic molecule that could interact with gluten proteins through electrostatic forces, leading to a less cross-linked structure. While MCC samples showed similar structural characteristics to the control, samples with CMC and HPMC led to a more filamentous and oriented gluten network. CMC, as a negatively charged molecule, could establish electrostatic interactions in the absence of NaCl, while for HPMC, the addition of NaCl favoured the hydrophobic interactions.

CMC is widely used as a stabiliser and thickener in the food industry. Bayarri and Costell, 2010, reported the behaviour of CMC in dairy desserts, which are complex systems where CMC interactions with carbohydrates and milk proteins can be studied. The influence of CMC concentration (0.75%, 1.00%, 1.25% and 1.50% w/w) and type of dispersing media (aqueous solution, skimmed milk and whole milk) on the viscoelastic properties of aqueous and milk systems were studied. Both parameters affected the viscoelastic behaviour, which ranged from fluid-like to weak gel. Concentrations of CMC below 0.75% w/w did not have any effect. The highest CMC concentration (1.5% w/w) the most affected systems were the whole milk samples with higher values for the viscoelastic parameters and the aqueous solutions the less affected, suggesting some substantial interaction between the protein adsorbed in oil droplet of the milk and CMC.

Xue and Ngadi (2009) studied the functionality of methyl cellulose (MC) and CMC in batter systems during thermal processing, and the synergistic effects of the hydrocolloids and different flour blend combinations on the thermal properties of the different batter systems. For this study, three flour blends were prepared with rice, wheat and corn flours, with 2.5% salt and 3.1% leavening agent (1.78% sodium acid pyrophosphate/1.32% sodium bicarbonate). The MC and CMC powders were first dispersed and mixed in with the total amount of cold distilled water required for the batter. Afterwards, the hydrocolloid was totally dissolved, and the dry ingredients (flour, salt and leavening) were added to the hydrocolloid solution and manually mixed thoroughly until the batter was uniform and free of lumps. Addition of these hydrocolloids increased the gelatinisation temperatures but depressed the glass transition temperatures of the resulting batters. Batters with MC showed increased DHm (melting enthalpy) for all the thermal processes, whereas batters with CMC only showed significant effect on total melting enthalpies DHm for cooked samples. To simulate processing of battered products that are first frozen before they are cooked (namely freezing-cooking (FC) process), the samples were first rapidly cooled to 50°C at the rate of 20°C/min, then heated to 120°C at 10°C/min.

The effect of these hydrocolloids on glass transition temperature was more pronounced in raw samples (FC process) than in cooked samples and increased with increasing levels of CMC and MC used in the formulations.

Floury et al. (2003) observed, when studying the effect of high-pressure homogenisation (at 350 mPa) on methyl cellulose as a food emulsifier, that microphase separation occurred at homogenisation temperatures > 63°C. At these temperatures, methyl cellulose lost a great part of its stabilising properties and precipitated. The phenomenon was explained by the fact that methyl cellulose was subject to thermoreversible gelation upon increasing the temperature due to the fact that the polymer its phase-transition temperature (Chevillard and Axelos, 1997).

### 3.2. Authorised uses and use levels

Maximum levels of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) have been defined in Annex II to Regulation (EC) No 1333/2008 on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

Currently, microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466) and enzymatically hydrolysed carboxy methyl cellulose (E 469) are authorised food additives in the EU at *quantum satis* (QS), in almost all authorised food categories listed in Table 13. They are also included in Group I of food additives authorised at QS. Cross-linked sodium carboxy methyl cellulose (E 468) is an authorised food additive in the EU at levels of 30,000–50,000 mg/kg in three food categories.

Table 13 summarises foods that are permitted to contain E 460–466 and E 468 and E 469 and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008.

**Table 13:** MPLs of E 460–466 and E 468 and E 469 in foods according to the Annex II to Regulation (EC) No 1333/2008

Food category number	Food category name	E-number	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
01.3	Unflavoured fermented milk products, heat-treated after fermentation	Group I		<i>Quantum satis</i>
01.4	Flavoured fermented milk products including heat-treated products	Group I		<i>Quantum satis</i>
01.6.1	Unflavoured pasteurised cream (excluding reduced fat creams)	E 466		<i>Quantum satis</i>
01.6.2	Unflavoured live fermented cream products and substitute products with a fat content of less than 20%	E 460, E 466		<i>Quantum satis</i>
01.6.3	Other creams	Group I		<i>Quantum satis</i>
01.7.1	Unripened cheese excluding products falling in category 16	E 460(ii)	Only grated and sliced mozzarella	<i>Quantum satis</i>
01.7.1	Unripened cheese excluding products falling in category 16	Group I	Except mozzarella	<i>Quantum satis</i>
01.7.2	Ripened cheese	E 460	Only sliced and grated ripened cheese	<i>Quantum satis</i>
01.7.4	Whey cheese	E 460(ii)	Only grated and sliced cheese	<i>Quantum satis</i>
01.7.5	Processed cheese	Group I		<i>Quantum satis</i>
01.7.6	Cheese products (excluding products falling in category 16)	Group I		<i>Quantum satis</i>
01.7.6	Cheese products (excluding products falling in category 16)	E 460	Only grated and sliced ripened products and unripened products	<i>Quantum satis</i>
01.8	Dairy analogues, including beverage whiteners	Group I		<i>Quantum satis</i>
02.2.2	Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions	Group I		<i>Quantum satis</i>
02.3	Vegetable oil pan spray	Group I		<i>Quantum satis</i>
03	Edible ices	Group I		<i>Quantum satis</i>

Food category number	Food category name	E-number	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
04.1.1	Entire fresh fruit and vegetables	E 464	Only for citrus fruit, melons and pomegranates in order to: <ul style="list-style-type: none"> <li>• repeat all or some of the mandatory information particulars required by the Union legislation and/or national law and/or</li> <li>• provide on a voluntary basis brand name, production method, PLU-code, QR-code and/or barcode</li> </ul>	10
04.2.1	Dried fruit and vegetables	Group I		<i>Quantum satis</i>
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Group I		<i>Quantum satis</i>
04.2.4.1	Fruit and vegetable preparations excluding compote	Group I		<i>Quantum satis</i>
04.2.5.4	Nut butters and nut spreads	Group I		<i>Quantum satis</i>
04.2.6	Processed potato products	Group I		<i>Quantum satis</i>
05.1	Cocoa and Chocolate products as covered by Directive 2000/36/EC	Group I	Only energy-reduced or with no added sugar	<i>Quantum satis</i>
05.2	Other confectionery including breath freshening microsweets	Group I		<i>Quantum satis</i>
05.3	Chewing gum	Group I		<i>Quantum satis</i>
05.4	Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4	Group I		<i>Quantum satis</i>
06.2.2	Starches	Group I		<i>Quantum satis</i>
06.3	Breakfast cereals	Group I		<i>Quantum satis</i>
06.4.2	Dry pasta	Group I	Only gluten free and/or pasta intended for hypoproteic diets in accordance with Directive 2009/39/EC	<i>Quantum satis</i>
06.4.4	Potato gnocchi	Group I	Except fresh refrigerated potato gnocchi	<i>Quantum satis</i>
06.4.5	Fillings of stuffed pasta (ravioli and similar)	Group I		<i>Quantum satis</i>
06.5	Noodles	Group I		<i>Quantum satis</i>
06.6	Batters	Group I		<i>Quantum satis</i>
06.7	Pre-cooked or processed cereals	Group I		<i>Quantum satis</i>
07.1	Bread and rolls	Group I	Except products in 7.1.1 and 7.1.2	<i>Quantum satis</i>
07.2	Fine bakery wares	Group I		<i>Quantum satis</i>
08.3.1	Non-heat-treated meat products	Group I		<i>Quantum satis</i>
08.3.2	Heat-treated meat products	Group I	Except <i>foie gras</i> , <i>foie gras entier</i> , <i>blocs de foie gras</i> , <i>Libamáj</i> , <i>libamáj egészben</i> , <i>libamáj tömbben</i>	<i>Quantum satis</i>
08.3.3	Casings and coatings and decorations for meat	Group I		<i>Quantum satis</i>

Food category number	Food category name	E-number	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
09.2	Processed fish and fishery products including molluscs and crustaceans	Group I		<i>Quantum satis</i>
09.3	Fish roe	Group I	Only processed fish roe	<i>Quantum satis</i>
10.2	Processed eggs and egg products	Group I		<i>Quantum satis</i>
11.2	Other sugars and syrups	Group I		<i>Quantum satis</i>
11.4.1	Table-top sweeteners in liquid form	E 460(i), E 463, E 464, E 465, E 466		<i>Quantum satis</i>
11.4.2	Table-top sweeteners in powder form	E 460, E 461, E 463, E 464, E 465, E 466		<i>Quantum satis</i>
11.4.2	Table-top sweeteners in powder form	E 468		50,000
11.4.3	Table-top sweeteners in tablets	E 460, E 461, E 463, E 464, E 465, E 466		<i>Quantum satis</i>
11.4.3	Table-top sweeteners in tablets	E 468		50,000
12.1.2	Salt substitutes	Group I		<i>Quantum satis</i>
12.2.1	Herbs and spices	E 460	Only when dried	
12.2.2	Seasonings and condiments	Group I		<i>Quantum satis</i>
12.3	Vinegars	Group I		<i>Quantum satis</i>
12.4	Mustard	Group I		<i>Quantum satis</i>
12.5	Soups and broths	Group I		<i>Quantum satis</i>
12.6	Sauces	Group I		<i>Quantum satis</i>
12.7	Salads and savoury-based sandwich spreads	Group I		<i>Quantum satis</i>
12.8	Yeast and yeast products	Group I		<i>Quantum satis</i>
12.9	Protein products, excluding products covered in category 1.8	Group I		<i>Quantum satis</i>
13.1.5.1	Dietary foods for infants for special medical purposes and special formulae for infants	E 466	From birth onwards in products for the dietary management of metabolic disorders	10,000
13.1.5.2	Dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC	E 466	From birth onwards in products for the dietary management of metabolic disorders	10,000
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group I		<i>Quantum satis</i>
13.3	Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)	Group I		<i>Quantum satis</i>



Food category number	Food category name	E-number	Restrictions/exceptions	MPL (mg/L or mg/kg as appropriate)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group I	Including dry pasta	<i>Quantum satis</i>
14.1.2	Fruit juices as defined by Directive 2001/112/EC and vegetable juices	Group I	Only vegetable juices	<i>Quantum satis</i>
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	Group I	Only vegetable nectars	<i>Quantum satis</i>
14.1.3	Fruit nectars as defined by Directive 2001/112/EC and vegetable nectars and similar products	E 466	Only traditional Swedish and Finnish fruit syrups from citrus	<i>Quantum satis</i>
14.1.4	Flavoured drinks	Group I		<i>Quantum satis</i>
14.1.5.2	Other	Group I	Excluding unflavoured leaf tea; including flavoured instant coffee	<i>Quantum satis</i>
14.2.3	Cider and perry	Group I		<i>Quantum satis</i>
14.2.4	Fruit wine and made wine	Group I		<i>Quantum satis</i>
14.2.5	Mead	Group I		<i>Quantum satis</i>
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group I	Except whisky or whiskey	<i>Quantum satis</i>
14.2.7.1	Aromatised wines	Group I		<i>Quantum satis</i>
14.2.7.2	Aromatised wine-based drinks	Group I		<i>Quantum satis</i>
14.2.7.3	Aromatised wine-product cocktails	Group I		<i>Quantum satis</i>
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol	Group I		<i>Quantum satis</i>
15.1	Potato-, cereal-, flour- or starch-based snacks	Group I		<i>Quantum satis</i>
15.2	Processed nuts	Group I		<i>Quantum satis</i>
16	Desserts excluding products covered in category 1, 3 and 4	Group I		<i>Quantum satis</i>
17.1 <sup>(a)</sup>	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group I		<i>Quantum satis</i>
17.1 <sup>(a)</sup>	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	E 468		30,000
17.2 <sup>(a)</sup>	Food supplements supplied in a liquid form	Group I		<i>Quantum satis</i>
17.3 <sup>(a)</sup>	Food supplements supplied in a syrup-type or chewable form	Group I		<i>Quantum satis</i>
18	Processed foods not covered by categories 1–17, excluding foods for infants and young children	Group I		<i>Quantum satis</i>

MPL: maximum permitted level.

(a): FCS 17 refers to food supplements as defined in Directive 2002/46/EC of the European Parliament and of the Council excluding food supplements for infants and young children.

According to Annex III, Part 1 of Regulation (EC) No 1333/2008, E 460–466 are also authorised as carriers in all food additives at QS; E 468 is authorised as a carrier in sweeteners at QS and E 469 is authorised as a carrier in all food additives at QS.

According to Annex III, Part 2 of Regulation (EC) No 1333/2008, E 460, E 461, E 462, E 463, E 464, E 465, E 466 and E 469 are authorised as food additives other than carriers at QS, in all food additive preparations.

According to Annex III, Part 3 of Regulation (EC) No 1333/2008, E 460, E 461, E 463, E 464 and E 466 are authorised as food additives including carriers in food enzymes at QS, while E 462, E 465 and E 469 are authorised as food additives at QS levels but cannot be used as carriers.

According to Annex III, Part 4 of Regulation (EC) No 1333/2008, E 460, E 461, E 462, E 463, E 464, E 465, E 466 and E 469 are authorised as food additives including carriers in all food flavourings at QS.

In addition, according to Annex III, Part 5, Section A of Regulation (EC) No 1333/2008, E 460–466 and E 469 are authorised to be used as food additives including carriers in all nutrients at QS except for nutrients intended to be used in foodstuffs for infants and young children listed in point 13.1 of Part E of Annex II.

Finally, according to Annex III, Part 5, Section B of Regulation (EC) No 1333/2008, E 466 is authorised as a food additive for uses in nutrient preparations under the condition that the maximum level in foods mentioned in point 13.1 of Part E of Annex II is not exceeded,<sup>11</sup> in all nutrients in dietary foods for infants and young children for special medical purposes as defined in Directive 1999/21/EC, nutrients intended to be used in foodstuffs for infants and young children listed in point 13.1 of Part E of Annex II.

### 3.3. Exposure data

#### 3.3.1. Reported use levels or data on analytical levels

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued public calls,<sup>12,13</sup> for occurrence data (usage level and/or concentration data) on microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), methyl cellulose (E 464), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469). In response to this public call, updated information on the actual use levels of celluloses (E 460–466, E 468 and E 469) in foods was made available to EFSA by industry. One analytical data on the concentration of these food additives in foods was made available by Member State (MS).

##### 3.3.1.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels ( $n = 1,177$ ) of celluloses (E 460–466, E 468 and E 469) in foods for 57 out of the 84 food categories in which celluloses (E 460–466, E 468 and E 469) are authorised.

Updated information on the actual use levels of celluloses (E 460–466, E 468 and E 469) in foods was made available to EFSA by the Association of the European Self-Medication Industry (AESGP), Aviko, Dr Loges Naturheilkunde neu entdecken, the European Dairy Association (EDA), the European Federation of Associations of Health Products Manufacturers (EHPM), FoodDrinkEurope (FDE), Food Supplements Europe (FSE), the International Chewing Gum Association (ICGA), Kruger GmbH & Co and the Organisation des Fabricants de produits Cellulosiques Alimentaires (OFCA) [Documentation provided to EFSA, n. 1–10].

The Panel noted that some data providers (e.g. OFCA) are not food industry using celluloses in their food products but food additive producers. Usage levels reported by food additive producers should not be considered at the same level as those provided by food industry. Food additive producers might recommend usage levels to the food industry but the final levels might, ultimately, differ, unless food additive producers confirm that these levels are used by food industry. In all other

<sup>11</sup> 10,000 mg/kg.

<sup>12</sup> <http://www.efsa.europa.eu/sites/default/files/consultation/151012.pdf>

<sup>13</sup> <http://www.efsa.europa.eu/sites/default/files/consultation/ans091123.pdf>

cases, data from food additive producers will only be used in the MPL scenario in case of QS authorisation and no data are available from food industry or MSs in order to have the most complete exposure estimates. In any case, all usage data provided will be acknowledged in the appendices.

Appendix A provides data on the use levels of celluloses (E 460–466, E 468 and E 469) in foods as reported by industry.

### 3.3.1.2. Summarised data on concentration levels in foods submitted by Member States

In total, one analytical result was reported to EFSA by Germany, on a meat product sampled and analysed in 2013, with a level of 3,700 mg/kg. This meat preparation was not taken into account in the exposure estimates.

Complete information on the methods of analysis (e.g. validation) was not made available to EFSA, but the sample was derived from accredited laboratory.

### 3.3.2. Summarised data extracted from the Mintel Global New Products Database

The Mintel GNPD is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU's food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.<sup>14</sup>

For the purpose of this Scientific Opinion, the Mintel GNPD<sup>15</sup> was used for checking the labelling of products containing E 460–466, E 468 and E 469 within the EU's food products as the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel, celluloses (E 460–466, E 468 and E 469) are labelled on more than 18,000 products out of which around 11,500 between 2012 and 2017.

Appendix B presents the percentage of the food products labelled with celluloses (E 460–466, E 468 and E 469) out of the total number of food products per food subcategory according to the Mintel GNPD food classification. The percentages ranged from less than 0.1% in many food subcategories to 27.7% in Mintel's GNPD subcategory 'Vitamins and dietary supplements'. The average percentage of foods labelled to contain celluloses (E 460–466, E 468–469) was 2.3%.

### 3.3.3. Food consumption data used for exposure assessment

#### EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys<sup>16</sup> added in the Comprehensive database were also taken into account in this assessment.<sup>10</sup>

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible subjects' underreporting and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 14).

<sup>14</sup> Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.

<sup>15</sup> <http://www.gnpd.com/sinatra/home/> accessed on 31/08/2017.

<sup>16</sup> Available online: <http://www.efsa.europa.eu/en/press/news/150428.htm>

**Table 14:** Population groups considered for the exposure estimates of celluloses

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 12 weeks up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers <sup>(a)</sup>	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, the Netherlands, Spain, UK
Children <sup>(b)</sup>	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly <sup>(b)</sup>	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Netherlands, Romania, Sweden, UK

(a): 'Toddlers' in the EFSA Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.

(b): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge of 'elderly' and 'very elderly' in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, FoodEx food codes were matched to the FCS food categories.

#### **Food categories selected for the exposure assessment of celluloses (E 460–466, E 468 and E 469)**

The food categories in which the use of celluloses is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This is the case for 12 food categories and may result in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 01.7.6 Cheese products;
- 02.3 Vegetable oil pan spray;
- 06.6 Batters;
- 06.7 Pre-cooked or processed cereals;
- 08.3.3 Casings and coatings and decorations for meat;
- 12.1.2 Salt substitutes;
- 14.1.3 Fruit nectars, only vegetable nectars;
- 14.1.3 Fruit nectars, only traditional Swedish and Finnish fruit syrups from citrus;
- 14.2.4 Fruit wine and made wine;
- 14.2.5 Mead;
- 14.2.7.2. Aromatised wine-based drinks;
- 14.2.7.3. Aromatised wine-product cocktails.

For the following food categories, the restrictions/exceptions which apply to the use of celluloses (E 460–466, E 468 and E 469) could not be taken into account, and therefore the whole food category was considered in the exposure assessment. This is the case for three food categories and may result in an overestimation of the exposure:

- 07.1 Bread and rolls, except products in 7.1.1 and 7.1.2;
- 09.3 Fish roe, only processed fish roe;

Celluloses (E 460–466, E 468 and E 469) are also allowed in FC 13.2, 13.3, 13.4 and 18. Food items under food categories 13.2, 13.3 and 13.4 consumed by population groups – children, adolescents, adults and the elderly – may be very diverse and, in addition, there is very limited information on their consumption. Therefore, eating occasions belonging to the food categories 13.2, 13.3 and 13.4 were reclassified under food categories in accordance to their main component. The use levels available for food categories 13.2, 13.3 and 13.4 were not considered for the exposure assessment.

For all scenarios, additional food categories were not taken into account because no concentration data were provided for these food categories to EFSA (Appendix C). Overall, for the maximum level exposure scenario, 48 food categories were included, while for the refined scenarios, 26 food categories were included in the present exposure assessment to celluloses (E 460–466, E 468 and E 469). For the remaining food categories, the refinements considering the restrictions/exceptions as set in Annex II to Regulation No 1333/2008 were applied (Appendix C).

### 3.4. Exposure estimate(s)

#### 3.4.1. Exposure to celluloses from their use as food additives

The Panel estimated chronic exposure to celluloses (E 460–466, E 468 and E 469) for the following population groups: infants, toddlers, children, adolescents, adults and the elderly. Dietary exposure to celluloses was calculated by multiplying celluloses concentrations for each food category (Appendix C) with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Dietary surveys with only one day per subject were excluded, as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 3). Based on these distributions, the mean and 95th percentile of exposure were calculated per survey for the total population and per population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in the present assessment, high levels of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment to celluloses (E 460–466, E 468 and E 469) was carried out by the ANS Panel based on (1) maximum levels of data provided to EFSA (defined as the maximum level exposure assessment scenario), and (2) reported use levels (defined as the refined exposure assessment scenario) as provided by industry. These two scenarios are discussed in detail below.

These scenarios do not consider the consumption of food supplements (FC 17.1, FC 17.2 and FC 17.3), nor the consumption of foods for special medical purposes (FSMP), which are covered in additional refined exposure scenarios detailed below (*food supplements consumers only scenario* and *food for special medical purposes consumer only scenario*).

Considering that the food category 18 (Processed foods not covered by categories 1–17, excluding foods for infants and young children) is extremely unspecific (e.g. composite foods), processed foods, prepared, or composite dishes belonging to the food category 18 were reclassified under food categories in accordance to their main component. Therefore, food category 18 is not taken into account as contributor to the total exposure estimates.

Concerning the uses of celluloses (E 460–466, E 468 and E 469) as carriers, there might be food categories where celluloses are used according to Annex III and not to Annex II. These food categories can only be addressed by analytical data or limits set in the Regulation (EC) No 1333/2008. According to Annex III, Parts 1, 2, 3, 4 and 5 of Regulation (EC) No 1333/2008, celluloses (E 460–466, E 468 and E 469) are also authorised at QS; as no data were made available to EFSA, this added dietary exposure could not be taken into account in the any of the exposure scenarios.

##### 3.4.1.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As celluloses (E 460–466, E 468 and E 469) are authorised according to QS in almost all food categories, a 'maximum level exposure assessment' scenario was



estimated based on the maximum reported use levels provided by industry (food industry and food additive producers) as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

A possible additional exposure from the use of celluloses (E 460–466, E 468 and E 469) in accordance with Annex III to Regulation (EC) No 1333/2008 (Part 1, 3, 5) was not considered in the maximum level exposure assessment scenario. Despite this, the Panel considers the exposure estimates derived following this scenario as the most conservative, as it is assumed that the population group will be exposed to celluloses (E 460–466, E 468 and E 469) present in food at maximum reported use levels over a longer period of time.

#### 3.4.1.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on use levels reported by food industry. This exposure scenario can consider only food categories for which the above data were available to the Panel.

Appendix C summarises the concentration levels of celluloses (E 460–466, E 468 and E 469) used in the refined exposure assessment scenario. Based on the available data set, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to celluloses (E 460–466, E 468 and E 469) present at the maximum reported use level for one food category. This exposure estimate was calculated as follows:
  - Combining food consumption with the maximum of the reported use levels, for the main contributing food category at the individual level.
  - Using the mean of the typical reported use levels, for the remaining food categories.
- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to celluloses (E 460–466, E 468 and E 469) present at the mean reported use level in food. This exposure estimate was calculated using the mean of the typical reported use levels for all food categories.

#### 3.4.1.3. Specific exposure assessment scenario

- *Food supplements consumers only* scenario: celluloses (E 460–466, E 468 and E 469) are authorised in the food category 17 (i.e. FC 17.1, 17.2, 17.3) (Table 13) Food supplements as defined in Directive 2002/46/EC excluding food supplements for infants and young children. As exposure via food supplements may deviate largely from the one via food, and the number of food supplement consumers may be low depending on populations and surveys, an additional scenario was calculated in order to reflect additional exposure to food additives from food supplements compared to exposure to food additives excluding these sources. This scenario was estimated as follow:
  - Consumers only of food supplements were assumed to be exposed to a food additive present at the maximum reported usage on a daily basis via consumption of food supplements. For the remaining food categories, the mean of the typical reported use levels was used.

As food category 17 does not consider food supplements for infants and toddlers as defined in the legislation, exposure to celluloses (E 460–466, E 468 and E 469) from food supplements is not estimated for these two population groups.

- *FSMP scenario consumers only*: as E 466 is also authorised in the FC 13.1.5 (13.1.5.1 and 13.1.5.2), an additional exposure assessment scenario taking into account this food category was performed to estimate the exposure of infants and toddlers who may eat and drink these FSMP.

The consumption of these foods under FC 13.1.5 is not reported in the EFSA Comprehensive database. To consider the exposure to food additives via consumption of these foods, the Panel assumed that the amount of FSMP consumed by infants and toddlers resembles that of comparable foods for infants and toddlers from the general population. Thus, the consumption of FSMP categorised as food category 13.1.5 was assumed to equal that of formulae and food products categorised as food categories 13.1.1, 13.1.2, 13.1.3 and 13.1.4.

The Panel noted that no data were submitted for the food categories 13.1.5.1 and 13.1.5.2, thus, the FSMP exposure assessment scenario for consumers only was performed using the

MPLs of E 466 for these two food categories; for the remaining food categories, the mean of the typical reported use levels was used.

Certain FSMP consumed by other population groups (children, adolescents, adults and the elderly) may be very diverse; they cannot be considered because of very limited information on consumption. Eating occasions belonging to the food categories 13.2, 13.3, 13.4 were therefore reclassified under food categories in accordance to their main component.

#### 3.4.1.4. Dietary exposure to celluloses (E 460–466, E 468 and E 469)

Table 15 summarises the estimated exposure to celluloses from their use as food additives in six population groups (Table 14) according to the different exposure scenarios (Section 3.4.1). Detailed results per population group and survey are presented in Appendix D.

**Table 15:** Summary of anticipated exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure scenarios, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	<b>Infants (12 weeks– 11 months)</b>	<b>Toddlers (12–35 months)</b>	<b>Children (3–9 years)</b>	<b>Adolescents (10–17 years)</b>	<b>Adults (18–64 years)</b>	<b>The elderly (≥ 65 years)</b>
<b>Maximum level exposure assessment scenario</b>						
Mean	22–135	103–546	138–410	65–260	58–157	55–116
High level (95th percentile)	60–394	259–718	270–745	136–460	120–303	109–190
<b>Refined estimated exposure assessment scenario</b>						
<b>Brand-loyal scenario</b>						
Mean	5–50	15–190	44–177	27–126	20–67	18–37
High level (95th percentile)	30–186	42–506	112–387	86–274	47–180	37–80
<b>Non-brand-loyal scenario</b>						
Mean	2–18	5–64	11–58	7–40	7–23	7–16
High level (95th percentile)	13–55	14–111	30–103	21–73	16–48	14–29

bw: body weight.

From the *maximum level exposure assessment scenario*, mean exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives ranged from 22 mg/kg body weight (bw) per day in infants to 546 mg/kg bw per day in toddlers. The 95th percentile of exposure to celluloses (E 460–466, E 468 and E 469) ranged from 60 mg/kg bw per day in infants to 745 mg/kg bw per day in children.

From the *refined estimated exposure scenario*, in the *brand-loyal scenario*, mean exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives ranged from 5 mg/kg bw per day in infants to 190 mg/kg bw per day in toddlers. The high exposure to celluloses (E 460–466, E 468 and E 469) ranged from 30 mg/kg bw per day in infants to 506 mg/kg bw per day in toddlers. In the *non-brand-loyal scenario*, mean exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives ranged from 2 mg/kg bw per day in infants to 64 mg/kg bw per day in toddlers. The 95th percentile of exposure to celluloses (E 460–466, E 468 and E 469) ranged from 13 mg/kg bw per day in infants to 111 mg/kg bw per day in toddlers.

From the refined estimated exposure scenario taking into account FSMP (FC 13.1.5.1 and 13.1.5.2) in which E 466 is authorised, consumers only, mean exposure to celluloses from their uses as food additives ranged for infants between 4 and 508 mg/kg bw per day and between 6 and 154 mg/kg bw per day for toddlers. The 95th percentile of exposure ranged for infants between 14 and 1,557 mg/kg bw per day, and for toddlers between 14 and 557 mg/kg bw per day.

For the food supplement consumers only, mean exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives ranged between 23 and 332 mg/kg bw per day across the different population classes of children, adolescents, adults and the elderly. The 95th percentile of exposure to celluloses (E 460–466, E 468 and E 469) ranged between 78 and 448 mg/kg bw per day across the same population groups.

### 3.4.1.5 Main food categories contributing to exposure to celluloses (E 460–466, E 468–469)

#### Main food categories contributing to exposure to celluloses (E 460–466, E 468–469) using the maximum level exposure assessment scenario

From the *maximum level exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates were breakfast cereals and fine bakery wares for infants and bread and rolls, flavoured fermented milk products and flavoured drinks for toddlers. For children, adolescents and adults, the main contributing food categories were bread and rolls, fine bakery wares and flavoured drinks; while, for the elderly, the main contributing food category was bread and rolls (see Appendix E for more details).

#### Main food categories contributing to exposure to celluloses (E 460–466, E 468–469) using the refined exposure assessment scenario

The main contributing food categories for the *refined estimated exposure scenario, brand-loyal scenario* were bread and rolls, sauces and processed fruits and vegetables for infants; bread and rolls, fine bakery wares and flavoured drinks were the main contributing food categories for the other population groups: toddlers; children, adolescents, adults and the elderly. In the *non-brand-loyal scenario*, the main contributing food categories were bread and rolls, processed fruits and vegetables and fine bakery wares for infants and toddlers. For children, adolescents and adults, the main contributing food categories were bread and rolls, fine bakery wares and flavoured drinks; while for the elderly, the main contributing food categories were bread and rolls and processed fruits and vegetables (see Appendix E for more details).

Appendix E can be found in the online version of this output ('Supporting information' section): <https://doi.org/10.2903/j.efsa.2018.5047>

### 3.4.1.6. Uncertainty analysis

Uncertainties in the exposure assessment of celluloses have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and summarised in Table 16.

**Table 16:** Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction <sup>(a)</sup>
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/-
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n = 12/84 food categories)	-
Food categories selected for the exposure assessment: inclusion of one food category without considering the restriction/exception	+
Food categories included in the exposure assessment: n = 48 food categories for max scenario, and 26 for refined scenario, as no data available for certain food categories which were therefore not considered in the exposure estimates	-
Concentration data: <ul style="list-style-type: none"> <li>use levels considered applicable for all foods within the entire food category, whereas on average, 2.3% of the foods, belonging to food categories with foods labelled with celluloses, was labelled with the additives.</li> </ul>	+/-
Maximum level exposure assessment scenario: <ul style="list-style-type: none"> <li>authorisation according to Annex III to Regulation (EC) No 1333/2008 not considered</li> <li>exposure calculations based on the maximum reported use levels from industries</li> </ul>	- +

Sources of uncertainties	Direction <sup>(a)</sup>
Refined exposure assessment scenarios: <ul style="list-style-type: none"> <li>scenario including only the food categories according to Annex II to Regulation (EC) No 1333/2008</li> <li>exposure calculations based on the maximum or mean levels (reported use from industries)</li> </ul>	– +/-
Food supplements consumers only scenario: <ul style="list-style-type: none"> <li>exposure calculations based on consumers only</li> <li>exposure calculations based on the maximum levels for food supplements and the mean levels for all other food categories</li> <li>foods which may contain the food additive according to Annex III to Regulation (EC) No 1333/2008 not taken into account</li> </ul>	+ +/- –
FSMP consumers only scenario: <ul style="list-style-type: none"> <li>exposure calculations based on consumers only</li> <li>exposure calculations based on the MPL levels for the FSMP and mean levels for all other food categories</li> <li>foods which may contain the food additive according to Annex III to Regulation (EC) No 1333/2008 not taken into account</li> </ul>	+ + –
Uncertainty in possible national differences in use levels of food categories	+/-

(a): +, uncertainty with potential to cause overestimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would, in general, result in an overestimation of the real exposure to celluloses (E 460–466, E 468 and E 469) as food additives in European countries considered in the EFSA European database for the maximum level exposure scenario and for the refined scenario, if it was considered that the food additives may not be used in food categories for which no usage data have been provided.

This assumption of non-use was supported by the observation that celluloses (E 460–466, E 468 and E 469) are authorised as Group I food additives in the majority of food categories (Table 13). Since all these food categories correspond to the general Group I food additives authorisation, celluloses (E 460–466, E 468 and E 469) may not necessarily be used in some of these food categories. It may thus be explained why reported use levels adequate for the refined exposure scenario of celluloses (E 460–466, E 468 and E 469) were only available for 26 food categories. The Panel noted that the information from the Mintel GNPD supported the observation that due to its Group I authorisation, celluloses (E 460–466, E 468 and E 469) may not be used in all food categories in which these are authorised (Section 3.3.2). For the 26 food categories considered for the refined exposure assessment, the products labelled with celluloses (E 460–466, E 468 and E 469) were also reported in the Mintel GNPD.

Regarding *food supplements consumers only scenario*, the Panel considered that the uncertainties would result in an overestimation of the exposure to celluloses (E 460–466, E 468 and E 469) as food additives, given that the calculations were based on consumers only of food supplements and assuming a long-term brand loyal consumption of these food products on a daily basis.

Regarding *FSMP consumers only scenario*, the Panel considered that the uncertainties would also result in an overestimation of the exposure, given that the calculations were based on consumers only of FSMP, and no data from industry were provided; therefore, only the MPLs were used.

In none of the exposure scenarios, the use of celluloses (E 460–466, E 468 and E 469) according to Annex III to Regulation (EC) No 1333/2008 was considered. Neglecting this source of exposure may have resulted in an underestimation of exposure to celluloses (E 460–466, E 468–469) in all scenarios.

### 3.4.2. Exposure via the regular diet

In 2010, the NDA Panel published a scientific opinion on the Dietary Reference Values for carbohydrates and dietary fibre (EFSA NDA Panel, 2010a,b). In this opinion, the intakes of carbohydrates and dietary fibre among several population groups (toddlers, children, adolescents and adults) in EU countries are presented. For adults (19–65 years old), the mean intake for dietary fibre is reported to be in the range from 15.7 to 29.7 g/day. At high levels, the dietary fibre intakes are up to 39 g/day (p95).

Considering that the mean body weight for this population group equals 70 kg (EFSA Scientific Committee, 2012), the highest intake of dietary fibre would be around 560 mg/kg bw per day.

### 3.4.3. Exposure from all sources

Based on the data from the EFSA NDA opinion on Dietary Reference Values for carbohydrates and dietary fibre (EFSA NDA Panel, 2010a,b), mean intakes of dietary fibre coming from natural sources were estimated in mg/kg bw using default body weight values (EFSA Scientific Committee, 2012). Mean intake from all sources and estimated exposure from food additives (non-brand-loyal scenario) were added in order to give an overall intake estimate of fibre.

Table 17 summarises the estimated exposure to celluloses from their use as food additives and from natural sources.

**Table 17:** Estimated exposure to celluloses from their use as food additives and from natural sources

	<b>Infants (12 weeks– 11 months)</b>	<b>Toddlers (12–35 months)</b>	<b>Children (3–9 years)</b>	<b>Adolescents (10–17 years)</b>	<b>Adults (18–64 years)</b>	<b>The elderly (≥ 65 years)</b>
<b>Food additive refined exposure, non-brand-loyal scenario (mg/kg bw per day)</b>						
Mean	10	35	35	24	15	11
[range]	[2–18]	[5–64]	[11–58]	[7–40]	[7–23]	[7–16]
<b>Natural sources of dietary fibre (EFSA NDA Panel, 2010)</b>						
Mean (g)	NA	12	15	23	23	NA
[range] (g)	NA	[9.0–15.0]	[9.4–20.2]	[12.0–33.0]	[15.7–29.7]	NA
Mean (mg/kg bw)	NA	1008	649	431	311	NA
<b>% of food additives exposure out of all sources</b>						
Mean	–	3%	5%	6%	5%	–

NA, Not available; bw: body weight (default values from EFSA, 2012).

Celluloses intake as food additives would correspond to 6% (non-brand-loyal scenario) of the overall dietary mean intake in the adolescent population.

### 3.4.4. Exposure via other sources

Exposure to celluloses due to the following uses was not considered in this opinion.

#### Celluloses as ingredients in foods

Cause and effect relationships have been established between the consumption of hydroxypropyl methylcellulose and a reduction of post-prandial glycaemic responses and maintenance of normal blood cholesterol concentrations. In order to obtain these physiological effects, 4 g of hydroxypropyl methylcellulose per meal or 5 g/day of hydroxypropyl methylcellulose consumed in two or more servings, respectively, are required (EFSA NDA Panel, 2010a,b).

#### Pharmaceutical uses

Celluloses are used as active ingredients and excipients in medicinal products. As excipients, mostly in medicinal products that come in various types of tablet forms (hard, chewable, film-coated, prolonged-release tablets), in capsules, in granules/powders for oral suspensions, injections, syrups (E 466) and in gels. Their function as excipients is described as disintegrants, diluents/fillers, binders, thickeners, taste maskers, compression aids, stabilising and swelling agents (Guo et al., 1998; Kadajji and Betageri, 2011; Shokri and Adibkia, 2013; Martindale, 2016).

From data provided by the European Medicines Agency (EMA), information about the current medicinal usage of celluloses and their usage as active ingredients and excipients was retrieved (Documentation provided to EFSA n. 11).

In a medicinal product on the European market, methylcellulose is used for the control of consistency of the intestinal content after colostomy, ileostomy and diarrhoea, in the management of diverticular disease and ulcerative colitis, in the management of constipation and as an aid to appetite control and the treatment of obesity. The daily dose used was up to 6 g daily. It was pointed out that



adequate fluid intake should be maintained to avoid intestinal obstruction. Furthermore, it was stated that bulk laxatives such as oral methylcellulose lower the transit time through the gut and could affect the absorption of other drugs. Contraindications are hypersensitivity to methylcellulose, imminent or threatened intestinal obstruction, faecal impaction, difficulties in swallowing, colonic atony, infective bowel disease and severe dehydration. As undesirable effects, gastrointestinal (GI) troubles, including flatulence and abdominal distension, are mentioned [Celevac<sup>17</sup>].

Microcrystalline cellulose (E 460(i)) may also be used in combination with other polymers such as sodium carboxy methyl cellulose (E 466) and guar gum. Incompatibilities of sodium carboxy methyl cellulose (E 466) have been reported with strongly acidic solutions, soluble salts of iron and some other metals, and with xanthan gum (Martindale, 2016).

Upon oral intake of cellulose-containing medicines, reactions noted were abdominal discomfort, abdominal distension/pain, constipation, dysphagia, dyspepsia, weight decrease or increase and flatulence. In addition, in some cases, a choking sensation was also reported associated to the property of cellulose ethers to swell, forming hydro gels in contact with water (Kamel et al., 2008; FDA, 2015).

## 4. Biological and toxicological data

### 4.1. Absorption, distribution, metabolism and excretion

#### 4.1.1. Microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii))

##### 4.1.1.1. Studies on absorption and metabolism of cellulose

JECFA (1998a,b, 1999a,b) and the SCF (1999) referred to several studies on persorption of microcrystalline cellulose particles in animal models. Persorption corresponds to the enteral translocation of particles into the gut-associated lymph system and is one part of the mechanism of absorption of xenobiotics. However, due to the low surface area available for persorption compared to that available for passive diffusion, persorption is generally a minor quantitative contributor to the absorptive capacity of the GI tract. Thus, in cases where persorption is the major, or only, absorption mechanism, only a small amount (a few percent) of the dose can be absorbed.

### Animal studies

#### Microcrystalline cellulose

Four rats received <sup>14</sup>C-labelled microcrystalline cellulose at 10% or 20% of the diet (Baker, 1966; cited in JECFA, 1998a,b, 1999a,b). The recovery of radioactivity in faeces ranged from 96% to 104% and was complete for all labelled material. There was no evidence of degradation or digestion and no radioactivity appeared in the urine (only limited information available).

Pahlke and Friedrich (1974) used 29 Wistar rats (14 males and 15 females), 8 mini pigs and 9 Beagle dogs to study the absorption of microcrystalline cellulose (Avicel<sup>®</sup> PH-101 or Elcema<sup>®</sup> P 050). The animals were fasted for 12 h prior to oral administration of the test compound in suspension. Rats, dogs and pigs were given 0.5, 140 and 200 g, respectively, of microcrystalline cellulose using whipped cream for preparation of the suspension. In rats, five other vehicles were also used. No details were given about the number of animals used for each experimental trial. The microcrystalline cellulose was stained with Schiff's reagent before application. Venous blood was taken from the animals 1–2 h after administration, haemolysed with saponin and the sediment was examined for particles via light microscopy. The authors reported that small numbers of particles could be detected in venous blood samples by light microscopy, but there were insufficient details to further interpret these findings.

A gavage study in Sprague–Dawley rats used doses of 1,000, 2,000, 3,000, 4,000 and 5,000 mg/kg bw per day microcrystalline cellulose (median particle size 6 µm, 28% of particles < 5 µm) in groups of 5 rats/sex (presumably including control). No absorbed particles were detected in the gut or in the Peyer's patches at 5,000 mg/kg bw per day (no further details; FMC, 1994; cited in SCF, 1999).

In a subchronic gavage study according to current standards, Kotkoskie et al. (1996) examined the possible absorption and translocation of microcrystalline cellulose particles. Groups of 20 male and 20 female Sprague–Dawley rats received once daily via gavage microcrystalline cellulose (median particle size 6 µm, 35% of particles < 5 µm) as a 25% suspension in tap water at dose levels of 0, 500, 2,500 or 5,000 mg/kg bw per day for 90 consecutive days (data on subchronic toxicity are presented in

<sup>17</sup> Celevac, 2016. Product information. <https://www.medicines.org.uk/emc/medicine/20700> (accessed January 2016).

Section 4.2.2.1). Necropsy was conducted on study days 91–94 under conditions that reduced the contamination of tissues with particulates, and tissues/organs were processed for histopathology. After conventional histopathology, sections were also examined by polarised light microscopy for the detection of cellulose particles. The limit of detection of birefringent particles was  $< 1 \mu\text{m}$ . This specific examination revealed a lack of birefringent cellulose particles in all organs and tissues, including gut-associated lymphoid tissue (GALT), liver, lung and spleen. These results indicated no detectable absorption of particles.

### **Powdered cellulose**

In a feeding study using  $\alpha$ -cellulose with Elcema<sup>®</sup>, a mixture of four types of  $\alpha$ -cellulose in the ratio of 1/1/1/1 (particle size 1–50  $\mu\text{m}$  (powder); 1–100  $\mu\text{m}$  (powder); 1–150  $\mu\text{m}$  (fibrillar); 90–250  $\mu\text{m}$  (granulate)) was fed to groups of male and female Wistar rats (number of rats not clearly stated but presumably  $n = 5/\text{dose per sex}$ ) for 30 days at a dietary level of 0% or 50% (equivalent to 45,000 mg/kg bw per day) (Ferch, 1973a,b). Statistical analysis of results was not performed. All rats were subjected to a 'persorption test': on the last day of the treatment period a cellulose-staining dye (substitution of 5% of Elcema<sup>®</sup> by Remalbordo<sup>®</sup>) was added to the diet. The animals were sacrificed 24 h after addition of the dye, followed by histological examination of the GI tract, spleen, liver, kidney and heart for stained particles. Even at this high dose level, no detectable absorption of cellulose particles occurred.

### **Human studies**

#### **Microcrystalline cellulose**

The absorption of ingested radiolabelled microcrystalline cellulose was examined in one male volunteer (no further details reported) (Baker, 1968; referred to by JECFA, 1998a,b, 1999a,b). In a 15-day adaptation period, the subject received 150 g/day of unlabelled microcrystalline cellulose in two portions. He then received 47.6  $\mu\text{Ci}$  of  $^{14}\text{C}$ -labelled microcrystalline cellulose in two portions on one day. The following 10 days the supplementation of the diet with unlabelled microcrystalline cellulose was continued. After oral exposure to the labelled test substance, samples of faeces and urine collected over 24 h were examined for radioactivity. All administered radioactivity ( $98.9 \pm 3.0\%$ ) was recovered from the faeces within 2 days and no radioactivity appeared in the urine or in the expired  $\text{CO}_2$ , suggesting no absorption of microcrystalline cellulose from the GI tract.

In another publication (Pahlke and Friedrich, 1975), the authors reported data on absorption of microcrystalline cellulose (no further details about the test item) in humans. The study was performed in one male volunteer. Due to the insufficient reporting, the Panel considered this study as not relevant for risk assessment.

#### **4.1.1.2. Studies on fermentation of cellulose**

There is evidence that certain high molecular weight dietary polysaccharides can be partially broken down by fermentation in the large intestine of animals and man. However, celluloses are known to be less fermentable than other polysaccharides such as gums, starches or pectins. In addition to intermediate metabolites such as lactic, acrylic or fumaric acids, the main end products of this colonic anaerobic digestive process are short-chain fatty acids (SCFA) such as acetic, propionic and butyric acids, which are absorbed from the colon (Cummings and Englyst, 1987).

### **In vitro studies**

#### **Microcrystalline cellulose**

The fibrolytic microbiota of the human large intestine was examined to determine the numbers and types of cellulolytic and hemicellulolytic bacteria present (Wedekind et al., 1988). Faecal samples from each of five individuals contained bacteria capable of degrading the hydrated cellulose in spinach and in wheat straw pretreated with alkaline hydrogen peroxide (AHP-WS), whereas degradation of the relatively crystalline cellulose in Whatman no. 1 filter paper (PMC) was detected for only one of the five samples. The mean concentration of cellulolytic bacteria, estimated with AHP-WS as a substrate, was  $1.2 \times 10^8/\text{mL}$  of faeces. Pure cultures of bacteria isolated on AHP-WS were able to degrade PMC, indicating that interactions with other microbes were primarily responsible for previous low success rates in detecting faecal cellulolytic bacteria with PMC as a substrate. The cellulolytic bacteria included *Ruminococcus* spp., *Clostridium* sp. and two unidentified strains. According to the authors, this work

demonstrated that many humans harbour intestinal cellulolytic bacteria and that a hydrated cellulose source such as AHP-WS is necessary for their consistent detection and isolation.

Microorganisms involved in the breakdown of cellulose were isolated, quantified and identified from human faecal samples (n = 34) (Robert and Bernalier-Donadille, 2003). The cellulolytic isolates corresponded to new *Ruminococcus* species and to *Enterococcus* species. These isolated species were incubated for 10 days at 37°C with 100 mg of paper cellulose as sole energy source. The end products of cellulose fermentation were mainly succinic acid, acetic acid and ethanol. The authors stated that microcrystalline cellulose degraders could only be enumerated in faecal samples from methane excretors, indicating that these species seem to be linked to methanogenic archaea in the gut.

In further investigations, the composition and activity of the cellulose-degrading bacterial population were shown to vary depending on the presence or absence of methanogens in the human gut (Chassard et al., 2010). The main microcrystalline cellulose-degrading bacteria belonged essentially to *Bacteroidetes* in non-methane-excreting subjects, while they were predominantly represented by *Firmicutes* in methane-excreting individuals. The degradation of cellulose has been shown *in vitro*.

In additional experiments, an anaerobic cellulolytic bacterial strain (*Ruminococcus champanellensis* sp. nov., strain designated 18P13(T)) was isolated from a human faecal sample (Chassard et al., 2012). In *in vitro* experiments, this strain was able to degrade microcrystalline cellulose (Avicel® PH-101), but the utilisation of soluble sugars was restricted to cellobiose. Acetic and succinic acid were the major end products of cellulose and cellobiose fermentation.

### **Powdered cellulose**

Sunvold et al. (1995) evaluated the influence of GI tract microflora from several species on fibre fermentation characteristics *in vitro*. Among other fibrous substrates (beet pulp, citrus pulp and citrus pectin), powdered cellulose (Solka Floc) was incubated for 6, 12, 24, and 48 h with ruminal fluid from cattle or faeces from dogs, cats, pigs, horses or humans. In the case of human faeces, after pooling data across all substrates and fermentation times, organic matter (OM) disappearance was 41.2% and acetic, propionic, butyric acid and total SCFA production were, respectively, 1.70, 0.60, 0.39, and 2.70 mmol/g of OM. Lactic acid production was 0.06 mmol/g of OM. When data were pooled across species, substrate OM disappearance and SCFA production ranked from least to greatest in the following order: cellulose < beet pulp < citrus pulp < citrus pectin. According to the authors, the fermentation of different fibrous substrates by faecal or ruminal microflora from various species seems to be dependent not only on the fermentative activity of the microbial population but on other factors such as lag time and rate of digesta passage.

### **In vivo study**

#### **Microcrystalline cellulose**

In rat studies, evidence of fermentation of microcrystalline cellulose by the microflora of the large intestine was shown. Male Wistar rats (n = 6 per group) were exposed via the diet (adapted from the AIN-76A purified diet; fibre-free) at a level of 5% microcrystalline cellulose (Avicel® PH-101, unlabelled test substance) for 14 days (Hsu and Penner, 1989). Food consumption was analysed, as well as cellulose content in faeces (validated methods). The mean total ingestion of microcrystalline cellulose was 13.1 g/rat and the mean faecal output 11.9 g/rat, resulting in a mean digestibility of 9%. The individual digestibility ranged between 6.4% and 10.8%. The authors stated that this variation may reflect differences in the population of cellulolytic bacteria in the intestine of individual rats.

The fermentation of cellulose was also studied in a comparative study conducted in conventional or germ-free (absence of intestinal microflora) male Wistar rats (Jühr and Franke, 1992). In this study, <sup>14</sup>C-labelled starch-free cellulose (synthesised by fermentation of [U-<sup>14</sup>C]glucose with *Acetobacter xylinum*, was used. No data were given about the form of the test item or further impurities. Groups of 5 rats received a single oral application of 145 kBq of the labelled cellulose in 0.5 mL of physiological saline by gavage (no further details about the dose). Excretion via faeces, urine and exhaled air was measured for 30 h in metabolism cages. The total recovery after 30 h was 101% in both groups, illustrating the validity of the methods. In germ-free rats, 94.6% of the applied radioactivity was detected in the faeces and intestinal content, 4.6% in the carcass, 1.1% in exhaled air (CO<sub>2</sub> measured) and 0.7% in urine. In conventional rats, however, 65.4% of the applied radioactivity was found in the faeces and intestinal content, but 30.0% in exhaled air, 3.9% in the carcass and 1.6% in urine. The authors stated that the portion of the dose remaining within the intestine at 30 h was resistant to fermentation. In germ-free rats, in the absence of fermentation of cellulose via the

intestinal microflora, nearly complete excretion via faeces was demonstrated (94.6%), suggesting no or little absorption from the GI tract. However, the absorption rate of 6.4% (4.6% in the carcass, 1.1% in exhaled air and 0.7% in urine) was clearly above background and may represent persorption and/or metabolism of radiolabelled impurities of the test item. Fermentation of cellulose is unlikely because the germ-free status of the rats was verified before and after the treatment. There is evidence from experiments with conventional rats that fermentation of cellulose occurs and the fermentation products (not identified or specified) were absorbed and mainly used as energy source, since 30% of the applied radioactivity was exhaled via  $^{14}\text{CO}_2$ .

#### 4.1.1.3. Studies on excretion of cellulose in humans

Most of the excretion studies performed in humans used celluloses prepared from botanicals which were, generally, not specified.

##### Unspecified cellulose

Southgate and Durnin (1970) compared the intake and the faecal excretion of proteins, fat, pentosan and cellulose (origin unspecified) in young ( $n = 26$ ) and elderly ( $n = 23$ ) volunteers of both sexes. The authors reported that the apparent digestibility of orally administered cellulose in the human gut ranged from 15% to 26% and from 26% to 55% in young and elderly subjects, respectively.

Kelleher et al. (1984) used  $^{14}\text{C}$ -labelled cellulose prepared from *Cana indica* leaves, which were allowed to photosynthesise in an atmosphere of  $^{14}\text{CO}_2$  for 24 h. The test item contained also labelled starch. The purification of the commercial product resulted in a  $^{14}\text{C}$ -cellulose in which  $> 90\%$  of the starch had been removed. Less than 1% of the test substance was soluble in water and 98% was retained by a  $0.45\ \mu\text{m}$  Millipore filter. The experiment was performed in 10 volunteers (6 elderly and 4 younger subjects without GI disease). All fasted subjects received orally 500 mg  $^{14}\text{C}$ -labelled cellulose mixed with 15 g mashed potatoes, followed by a cup of tea. Thereafter, the volunteers consumed mixed normal diets. Excretion of the applied radioactivity was determined by liquid scintillation measurements of faeces ( $n = 9$ ) and exhaled air ( $n = 8$ ; 4 elderly and 4 younger volunteers). The faecal collection was nearly complete and reached a mean of 92% in nine subjects (one subject excluded from faecal results). A mean of 57% of the applied radioactivity was excreted in the faeces (no data about sampling period) and 16% of applied radioactivity was expired as  $^{14}\text{CO}_2$  (collection time 72 h); an average of 7.5% of the faecal radioactivity was water-soluble. About 4% of the administered radioactivity appeared in the exhaled air 0–10 h after ingestion. In the four elderly subjects, a mean of 18% of the applied dose occurred in expired air 10–72 h after application, but only 7% in the four younger volunteers. The early exhalation of  $^{14}\text{CO}_2$  was discussed by the authors as due to possible metabolic products of impurities (non-cellulose polysaccharides). Metabolites in faeces were not analysed. The authors concluded that a significant quantity of ingested cellulose was metabolised within the human GI tract, absorbed and appeared in the expired air as  $^{14}\text{CO}_2$ .

Walters et al. (1989) used the same purified test item in additional *in vitro* experiments for specifying that the purified  $^{14}\text{C}$ -labelled cellulose was completely hydrolysed by cellulase. In a first trial, six healthy volunteers (40–50 years of age, no further details) received 500 mg of the radiolabelled cellulose after an overnight fast. The exhaled radioactivity was measured 0–24 h after ingestion (no data about other excretion routes); 1.6% of the applied dose was exhaled during the first 4 h, whereas the cumulative amount of exhaled  $^{14}\text{CO}_2$  reached 11% of the applied radioactivity. In a second trial, the degradation of the purified  $^{14}\text{C}$ -labelled cellulose was compared in six subjects with an ileostomy, following total colectomy and rectal resection. Using the same experimental protocol, 1.1% of the applied radioactivity was exhaled within the first 4 h after ingestion, but the cumulative amount in expired air (0–24 h) was only 3% in the ileostomy subjects; negligible  $^{14}\text{CO}_2$  excretion was detected from 15 h onwards. Analysis of ileostomy contents showed that 81% of the administered cellulose was excreted by this route. According to the authors, this comparison of normal and ileostomy subjects revealed the important role of the colonic microflora in the degradation of cellulose in the human GI tract. In a third trial, increase (by 54%) in exhaled  $^{14}\text{CO}_2$  was observed in four healthy volunteers, pretreated with increased dietary fibre for 3 months.

##### Powdered cellulose

The fate of orally ingested  $^{131}\text{I}$ -labelled  $\alpha$ -cellulose was quantified in eight healthy volunteers (four men, aged 27–75 years; four women, aged 21–76 years) by monitoring samples of faeces, urine and blood (Carrier et al., 1982). Details about the test substance were not available, except for the statement that strands  $< 1\ \text{mm}$  were eliminated by sieving before use. The volunteers ingested



100  $\mu\text{Ci}$  of the radiolabelled cellulose with a meal after an overnight fast. Complete urine and stool collections were obtained over a 5-day period after ingestion; in two subjects, faeces were also collected at days 6 and 7 and analysed. Venous blood samples were taken once daily. Aliquot samples of faeces and urine were filtered (0.45  $\mu\text{m}$  Millipore filter) to assess also the unbound radiolabel. No radioactivity was detected in blood, but 87% of the applied dose was excreted via faeces (58% at days 1 and 2) and 2% via urine (1.6% at days 1 and 2). Less than 2% of radioactivity in faeces, but total radioiodine in urine, was unbound. The total recovery of the applied radioactivity was 89%; the authors did not discuss the fate of the remaining amount, but concluded that  $^{131}\text{I}$ -labelled  $\alpha$ -cellulose was resistant to degradation by colonic microflora due to the large fibre size and/or chemical transformation of the test item produced by the radioactive labelling.

#### 4.1.2. Methyl cellulose (MC; E 461)

##### Animal data

Groups of adult male Hooded Wistar rats were fed sucrose-based diets containing 80 g/kg of MC of low (25 cP), medium (400 cP) and high (1,500 cP) viscosity (Topping et al., 1988). After 10 days of adaptation to the three diets, blood and liver were sampled. The whole caeca were also removed and the contents extruded for measurement of weight, viscosity, pH, volatile fatty acids (VFA) and bile acids. The viscosity of stomach and caecal contents was increased in proportion to that of the dietary fibre. Plasma cholesterol and VFA concentrations were unaffected by the viscosity of the dietary fibre. The concentrations of acetic and propionic acid in caecal contents decreased with increasing viscosity of the diet; according to the authors, this effect could be related to the availability of dietary starch and sucrose trapped in the methyl cellulose matrix. Finally, this study gave no indication of fermentation of MC by gut microflora.

Male and female Sprague–Dawley rats (3/sex per group, 185–250 g) were dosed once or once daily over 5 days via gavage with  $\sim 500$  mg/kg bw per day of  $^{14}\text{C}$ -MC labelled in the methoxyl group and with a viscosity of 3,300 cP (Braun et al., 1974). In the single-dose study, the animals were kept in metabolism cages for separate collection of urine, faeces and  $\text{CO}_2$  in expired air. Urine and faecal samples were collected at 6-h intervals for the first 24 h and at 12-h intervals thereafter. The rats were killed 4 days after dosing, the carcasses were skinned and the hearts, livers, lungs, kidneys and GI tracts were removed. In the repeated-dose study, rats were housed in metabolism cages designed for a separate collection of urine and faeces. Samples of excreta were collected at 24-h intervals, the rats were killed 24 h after the final dose, and the carcasses and tissue samples were assayed for radioactivity contents. In the single-dose study, 96–105% of the total dose of  $^{14}\text{C}$  activity was eliminated via the faeces within 48 h. No radioactivity was detected in the expired air and less than 0.1% of the dose was found in the urine, selected tissues and remaining carcass. In the repeated-dose study, there was also no increase in the  $^{14}\text{C}$  activity in the heart, kidney, liver, lung, carcass and skin, as the total amount found in these tissues was less than 0.1% of the total dose. The Panel noted that all the administered MC was eliminated in the faeces of the rats and was neither absorbed intact, nor fermented, in the GI tract.

##### Human data

In an older study (Machle et al., 1944) with two male adults and one 10-year-old girl, it was shown that nearly the entire MC (up to > 95%) was excreted via faeces, practically unaltered, within 3 days. In each experiment, 10 g of MC were given orally in a single dose, except in one experiment, where 5 g were given. The product was taken as a 5% solution and in one experiment as a gel. Irrespective of the accompanying diet (e.g. milk diet, customary diet, bran daily), practically all of the methoxyl groups of ingested MC were recovered from the faeces.

#### 4.1.3. Ethyl cellulose (EC; E 462)

No data available.

#### 4.1.4. Hydroxypropyl cellulose (HPC; E 463)

##### Animal data

Male and female Wistar-Imamichi strain rats (3/sex per group) were dosed once via gavage with 1,300 mg/kg bw of  $^{14}\text{C}$ -HPC (labelling in the methylenic carbon of the hydroxypropyl group),



containing 12% of the hydroxypropyl group (2.74  $\mu\text{Ci}/\text{mg}$ ) (Kitagawa et al., 1976a). After dosing, the rats were placed in metabolism cages and urine and faeces were collected for 96 h. For the detection of possible metabolites in urine, gel filtration was used. Bile duct was cannulated and samples were taken for 24 h and at different time points (6, 12, 24, 48 and 72 h after dosing). Radioactivity was determined in tissues and GI contents (blood, liver, kidney, lung, heart, spleen, cerebrum, cerebellum, thymus, thyroid, fat, stomach and intestinal contents from the upper part of the duodenum to the caecum, testes, prostate, uterus, ovaries). Up to 69% and 97% of the radioactivity were excreted in the faeces within 24 and 96 h, respectively. Only minor amounts (up to 2.6%) were excreted in urine within 96 h. Less than 0.015% was excreted in bile within 24 h after dosing. Due to the very minor amounts of  $^{14}\text{C}$ -HPC excreted via urine, no specific metabolites could be identified. Their molecular weight was slightly higher than that of glycerol or glucose and the elution position was different from propylene glycol, which was present at a level of  $< 2\%$  in the test material. Apart from liver and kidneys, radioactivity was not detectable in other tissues examined. Peak amounts in livers were given with 1.02% of the dose in male rats 12 h after dosing, and with 0.026% of the dose in females, 24 h after dosing.

When 250 or 1,000 mg/kg bw of  $^{14}\text{C}$ -HPC were administered to rats in a 5% aqueous solution, radioactivity no greater than 0.01% of the administered dose was detected in organs, urine and expired air (Industrial Bio-Test Lab, 1964; unpublished report, cited in JECFA, 1990). Recovery of activity in the faeces varied from 98.3% to 102.7%. Hence, orally ingested material was not absorbed from the GI tract of the rat and was excreted quantitatively in the faeces, principally in the first 48 h. To check on enterohepatic circulation, two additional rats with cannulated bile ducts were administered 1,000 mg/kg bw of radiolabelled material. Bile was collected for 72 h, but no significant activity was found.

#### 4.1.5. Hydroxypropyl methyl cellulose (HPMC; E 464)

##### Experimental data

###### In vitro

Wyatt et al. (1988) compared the effect of a fibre-free diet and of diets containing non-digestible polysaccharides, including HPMC, on rat caecal and colonic physiology and microflora. All polysaccharide-containing diets led to enlargement of the caecum and colon, associated with increased weight of contents and tissue. *In vitro*, HPMC remained almost completely unfermented, with only 5% of the substrate utilised after 7 days of incubation. According to the authors, caecal and colonic enlargement would be due to tissue hypertrophy in response to increased bulk of contents, irrespective of the nature of that bulk, which varies with diet. It would be unlikely that SCFA or other microbial metabolites are the stimulus for the trophic response seen when non-digestible dietary polysaccharides are fed to rats.

###### In vivo

Groups of three young male and three young female Sprague–Dawley rats were dosed with 500 mg/kg bw per day of  $^{14}\text{C}$ -HPMC (viscosity of 2.25 cP) once or once daily over five consecutive days via gavage (Gorzinski et al., 1986). After single dosing, about 73–101% of the applied dose was excreted within 24 h via faeces (100–105% within 72 h), while within 72 h only up to 1.5% was found in urine, 0.2% in carcass and tissues and 0.07% in expired air. In bile, a collection over 24 h in two male rats gave 0.05% of the applied dose. In plasma, the elimination of radioactivity was monophasic, with a half-life of about 2 h. Most of the residual radioactivity was found in the GI tract (no details given). In urine, methyl ethers of glucose and oligomers were identified, determined by thin layer chromatography in 6-h urine from one animal of each sex. Also, after dosing once daily over 5 days, most of the applied dose was excreted via faeces (97–104%), while only 1% was recovered in urine. There was no evidence of accumulation in the tissues examined (adrenals, brain, heart, liver, kidneys and spleen). A determination in exhaled air was not performed.

##### Human data

In a study with 25 young and healthy adults (23 males and 2 females), each person was given 3 graduated doses of HPMC ranging from 0.6 to 8.9 g (Knight et al., 1952). The time interval between the doses was at least 1 week. Following each dose, stool specimens were collected at approximately 24-h intervals for 72 or 96 h. For a determination of the quantitative amounts of HPMC in the samples of dried faeces, an analytical procedure, consisting of a methoxyl determination made directly on the

faeces samples, was used. Nearly all of the ingested substance (average of total recovery 97%, with a range of 89–110%) was excreted via faeces within 96 h following administration.

#### 4.1.6. Ethyl methyl cellulose (EMC; E 465)

##### Animal data

After feeding a single dose of 600 mg of EMC in the diet of rats, about 90% of the dose was recovered from the faeces by the end of the fourth day (Gage, 1962; unpublished report, as cited in JECFA, 1990). Nearly all alkoxy groups remained attached to the cellulose chain during passage through the gut.

#### 4.1.7. Sodium carboxy methyl cellulose (NaCMC; E 466)

##### Experimental data

Wyatt et al. (1988) compared the effect of a fibre-free diet and of diets containing non-digestible polysaccharides, including CMC, on rat caecal and colonic physiology and microflora. All polysaccharide-containing diets led to enlargement of the caecum and colon, associated with increased weight of contents and tissue. CMC had the most marked effect and treated animals had watery faeces. *In vitro*, CMC was poorly fermented even after prolonged incubation. According to the authors, caecal and colonic enlargement would be due to tissue hypertrophy in response to increased bulk of contents, irrespective of the nature of that bulk, which varies with diet. It would be unlikely that SCFA or other microbial metabolites are the stimulus for the trophic response seen when non-digestible dietary polysaccharides are fed to rats.

Adiotomre et al. (1990) investigated the effects of dietary fibres, including NaCMC, on caecal fermentations by using fresh human microflora. Evolution of SCFA and water-holding capacity after fermentation were also measured. Among other polysaccharides, NaCMC (E 466 grade) yielded low amount of total SCFA (25.8 vs 15.5 mmol/L for controls). The major SCFA produced were acetic and propionic acids, with smaller amounts of butyric, isobutyric, valeric and isovaleric acids. By contrast, the amount of water held by 1 g of the fermented residue was the highest in the case of NaCMC (11.9 vs 0.91 g/g for controls).

The absorption and excretion of NaCMC was examined in rats (Bär et al., 1995b). The  $^{14}\text{C}$  label was in the two carbon atoms of the carboxymethyl group and the specific radioactivity was 0.27  $\mu\text{Ci}/\text{mg}$ . One group of four male and four female Wistar rats were fed diets with 5% unlabelled CMC for a 2-week adaption period, followed by a single oral dose of 500 mg/kg bw of a  $^{14}\text{C}$ -CMC solution via gavage. Immediately after dosing, the rats were housed for 48 h in metabolism cages and then for 72 h in open metabolism cages. Expired  $\text{CO}_2$  was collected every 2 h during the first 24 h and then at 12-h intervals until 48 h. Urine and faeces were collected. At study termination on day 5, the animals were killed and plasma and blood cells were collected. The content of the GI segments, testes, prostate, uterus, ovaries, adrenals, urinary bladder, as well as samples of perirenal fat, abdominal skin and skeletal muscle, together with the remaining carcass were collected and weighed. The  $^{14}\text{C}$  administered and recovered in excreta and different organ and tissue samples was determined by liquid scintillation counting. At the end of the 5-day sampling period, the total mean recovery rate for  $^{14}\text{C}$ -CMC was 98.07% and the applied dose was mainly excreted via faeces (94.39%). Most of the radioactivity was excreted within 48 h after dosing. In the contents of the GI tract, only about 0.01% of the dose was recovered. Only small amounts were excreted via urine (less than 2% of the dose). Via expired  $\text{CO}_2$ , only < 1% of the applied radioactivity was expired within 48 h. The highest expiration rate was seen during the first 2 h after dosing (0.19%). Only a small incorporation of the  $^{14}\text{C}$  label in organs and tissues was seen (0.58%), with highest amounts found in fat, skin, muscles and liver. An examination of the pooled faeces showed that about 51% of the label from  $^{14}\text{C}$ -CMC remained with the sediment obtained by centrifugation of the faecal suspensions. As most of the soluble  $^{14}\text{C}$ -labelled excretion products were extracted in a first extraction step (the second step provided only about 10% of the radioactivity of the first step), the label was apparently tightly bound to the faecal matrix or was incorporated in the bacterial cells. Gel permeation chromatography (GPC) of the dosing solutions and the faecal extracts revealed that CMC was depolymerised during intestinal passage.

In an unpublished study (Wiebe, 1962; cited in JECFA, 1990),  $^{14}\text{C}$ -labelled CMC containing up to 0.34% radioactive sodium glycolate was given orally to two groups of five male rats each, in a dose of 400 mg. No detectable activity (less than 0.02% of the dose) was found in the livers and kidneys and

about 0.14% of the administered radioactivity was found in the 48-h urine samples. This amount, however, could be accounted for the free radioactive glycolate present in the test compound.

Five rats (strain and sex not specified) were fed 5 g of CMC collectively and faeces were collected at 24-h intervals. Approximately 90% of the applied dose was recovered in the faeces (Shelanski and Clark, 1948; as cited in JECFA, 1990).

In a study with six young albino rats, CMC was given via diet at levels of 5, 10 or 14% during four periods of 10 days each. About 96.3–100% of the applied doses could be recovered in faeces (Ziegelmeier et al., 1951).

In a study with three rabbits, CMC was given via diet at levels of 4.76% and 9.09% during two periods of 10 days each. About 48–57% of the applied doses could be recovered in faeces (Ziegelmeier et al., 1951).

## Human data

In three volunteers given daily oral doses of 20 or 30 g of CMC over 4 days, about 90% of the applied doses could be recovered in faeces. The dosing was well tolerated (Ziegelmeier et al., 1951).

### 4.1.8. Cross-linked sodium carboxy methyl cellulose (E 468)

As reported above, a comparative disposition study was carried out with  $^{14}\text{C}$ -labelled sodium CMC and enzymatically hydrolysed sodium CMC in adult Wistar rats (8 animals/sex; age not stated) (Bär et al., 1995b). The enzymatically hydrolysed sodium CMC was recovered from the GI tract with an unchanged molecular weight, whereas the sodium CMC was reduced in molecular size. This implies that the passage of CMC through the rat GI tract leads to the breakdown of some of the monomers of CMC to units similar to the ones of its enzymatically hydrolysed counterpart. JECFA (2003) noted that the cross-linked form will be more insoluble in water and therefore less likely to be absorbed and degraded than the parent compound itself.

### 4.1.9. Enzymatically hydrolysed carboxy methyl cellulose (E 469)

The absorption and excretion of partially enzyme-hydrolysed  $^{14}\text{C}$ -labelled enzymatically hydrolysed carboxy methyl cellulose was compared in rats to those of CMC in the above described study (Bär et al., 1995b). Following the same experimental protocol, most of the radioactivity (89–99% of the dose) was excreted within 48 h after dosing. In the contents of the GI tract, at study termination, only about 0.01% of the dose could be recovered. Only small amounts (about 2%) were excreted via urine. Via expired  $\text{CO}_2$ , only < 1% was expired within 48 h. Only a small incorporation of the  $^{14}\text{C}$  label in organs and tissues was seen (0.75%), with highest amounts in fat, skin, muscles and liver. An examination of the pooled faeces showed that about 35% of the label from  $^{14}\text{C}$ -enzymatically hydrolysed carboxy methyl cellulose remained with the sediment obtained by centrifugation of the faecal suspensions. The label was apparently tightly bound to the faecal matrix or was incorporated in the bacterial cells. GPC of the dosing solutions and the faecal extracts revealed that enzymatically hydrolysed carboxy methyl cellulose was excreted nearly intact.

### 4.1.10. Summary

Animal and human data clearly demonstrated that microcrystalline and powdered cellulose are not absorbed intact in the GI tract and could be fermented during their passage through the large intestine by strains of bacteria found in the human colon. Microcrystalline, powdered and modified celluloses would be less fermented than other polysaccharides such as gums, starches or pectins. A comparative human study in normal and ileostomy subjects confirmed the role of the intestinal microbiota in the degradation of  $^{14}\text{C}$ -labelled cellulose in the human GI tract. Data on methyl cellulose (E 461), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) demonstrated that these modified celluloses would not be absorbed intact and not fermented in the GI tract of animals or humans. They are excreted intact mainly via faeces (more than 90% of the administered doses), while only minor amounts of radiolabelled material are excreted via urine or via expired air (as  $^{14}\text{CO}_2$ ) and there is no indication for accumulation in the body. Overall, by comparing their respective databases, the Panel considered that microcrystalline, powdered and modified celluloses would not be absorbed intact and would be less fermented than other polysaccharides such as gums, starches or pectins.

## 4.2. Toxicological data

Specific toxicity data were not available for all the celluloses for all endpoints. Where studies were conducted, the specifications of the celluloses tested were not always clearly stated. However, given their structural, physicochemical and biological similarities, the Panel considered it possible to read-across between all the celluloses.

### 4.2.1. Acute oral toxicity (E 460(i), E 460(ii), E 462–464, E 466)

Data on acute oral toxicity were available for microcrystalline cellulose, powdered cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose and sodium carboxy methyl cellulose. Apart from one unpublished study with hydroxypropyl methyl cellulose, where an oral LD<sub>50</sub> value of > 1,000 mg/kg bw was given for rats (CTFA, 1978), the oral LD<sub>50</sub> values for the different other celluloses were consistently  $\geq$  3,000 mg/kg bw (Documentation provided to EFSA n. 20, 25, Freeman, 1996a; referred to by JECFA, 1998a,b, 1999a,b, Palotta, 1959; referred to by JECFA, 1998a,b, 1999a, b, Rowe et al., 1943; Shelanski and Clark, 1948; Industrial Bio-Test Lab, 1962; Kitagawa et al., 1970, 1976b; Moreno, 1977). Therefore, the Panel considered the oral acute toxicity of these compounds to be low.

### 4.2.2. Short-term and subchronic toxicity

#### 4.2.2.1. Microcrystalline cellulose (E 460(i))

Male Wistar rats (n = 6/group) fed a diet containing 5% microcrystalline cellulose (Avicel<sup>®</sup> PH-101; equivalent to 6,000 mg/kg bw per day) for 14 days did not show any effects on body weight gain, final body weight or food consumption in comparison to the controls receiving fibre-free diet (Hsu and Penner, 1989). The treatment induced a significant increase in wet and dry weight of faeces; the cellulose content of faeces reached 70%.

A subacute 28-day gavage study in Sprague–Dawley rats used doses of 1,000, 2,000, 3,000, 4,000 or 5,000 mg Avicel<sup>®</sup> FD-006/kg bw per day microcrystalline cellulose (median particle size of 6  $\mu$ m; 28% of particles with size < 5  $\mu$ m) in groups of five rats/sex (presumably including control) (FMC 1994, referred to by SCF, 1999). No adverse toxicological effects occurred at any dose level. No persorbed particles were detected in the gut or in the Peyers' patches at 5,000 mg/kg bw per day. The Panel noted that the test substance was not compliant with the specifications of the food additive.

Male Wistar rats (n = 12 per group) were fed for 4 weeks a diet (26% casein) supplemented with 0%, 5%, 10% or 20% microcrystalline cellulose (Avicel<sup>®</sup>) (equivalent to 0, 2,500, 5,000 or 10,000 mg/kg bw per day) (Sundaravalli et al., 1971). Rats were sacrificed and the body composition was analysed (moisture, fat and protein). No effects were detected. The body weight gain was not significantly influenced. In additional experiments, groups of 12 male Wistar rats received for 5 weeks a cholesterol-rich diet (1.5% cholesterol) supplemented with 0% or 20% microcrystalline cellulose. Cellulose treatment reduced significantly the total cholesterol level in plasma and liver and increased the excretion of bile acids in the faeces.

Groups of five male rats (no data about strain) received 0.5% or 10% microcrystalline cellulose (equivalent to 450 or 9,000 mg/kg bw per day) in their diet for 8 weeks (Asahi Chemical Industry Co., 1966; referred to by JECFA, 1998a,b, 1999a,b). The high-dose group showed slightly lower body weights than control rats. Haematology, serum chemistry and vitamin B1 levels in blood and faeces revealed no differences from control (no further details available).

In a study in Russian (Dorkina et al., 2007), groups of six male and six female Wistar rats received 0, 200, 1,000 or 2,000 mg microcrystalline cellulose/kg bw per day (dosing not clearly stated, presumably by gavage and in mg/kg bw per day) for 2 months. The treatment reduced significantly the body weight gain in males and females at  $\geq$  1,000 mg/kg bw per day, and in females also at the low dose level. However, the initial weight at the start of the experiment differed between groups (difference > 10%), limiting the evaluation of this result. Haematology revealed statistically significant effects even at the low dose level for some parameters (e.g. thrombocytopenia, erythrocytopenia, decreased haematocrit, reticulocytosis, reduced number of monocytes but increased number of lymphocytes and segmented neutrophils, decreased coagulation time). However, no data were given on the historical range of these parameters. The toxicological relevance of these effects is questionable and not in accordance with other studies.



Sprague–Dawley rats (20/sex per group) received a diet containing 0%, 5% or 10% microcrystalline cellulose (Avicel<sup>®</sup> PH-101; median particle size 70 µm) (equivalent to 0, 4,500 or 9,000 mg/kg bw per day) for 13 weeks (Schmitt et al., 1991). Animals were observed daily for overt signs of toxicity. Body weights and food consumption were determined weekly for all animals. Haematology and clinical chemistry analyses were performed at termination (n = 10/group). Liver, adrenals, testes with epididymides, and kidney weights were recorded for all animals. All animals were necropsied and tissues from control and high-dose animals, as well as all gross lesions from all animals, were examined microscopically (no further data). No treatment-related clinical signs or effects on mean body weight or mean body weight gain were noted. The food consumption was increased in treated groups due to the reduced caloric intake. In haematology and clinical chemistry, no treatment-related effects were detected. There were no notable gross pathologic findings at necropsy and no effects on organ weights. Microscopic evaluation of tissues revealed no treatment-related lesions, including in the GI tract. The Panel considered 10% microcrystalline cellulose in the diet (9,000 mg/kg bw per day, the highest dose tested) as the no observed adverse effect level (NOAEL). Similar results were obtained with cellulose fibre (Cellulon<sup>™</sup>; cellulose produced by a bacterial fermentation process) using the same experimental design and dose levels (Schmitt et al., 1991).

Groups of 20 male and 20 female CrI:CD(R) BR/VA/Plus rats received a diet containing 0 (control), 25,000 or 50,000 mg Avicel<sup>®</sup> RCN-15/kg diet (a mixture of 85% microcrystalline cellulose with 15% guar gum) (equal to 1,794 or 3,769 mg/kg bw per day for males and 2,131 or 4,446 mg/kg bw per day for females) for 90 days (Freeman, 1992a referred to by JECFA 1998). The study was performed according to the current guidelines. During the study, no treatment-related clinical signs were noted. In the high-dose group, increased food consumption was observed during several weeks, probably due to the increased dietary fibre content. This effect was also observed during the first week in the 25,000 mg/kg group. However, body weight gain was not affected. No treatment-related effects were detected in clinical chemistry and haematology. Necropsy revealed no evidence of treatment-related changes. The organ weights were unaffected by the treatment. Histopathology was performed in 34 organs or tissues, including the GI tract and GALT of the ileum; there was no evidence for toxicity of microcrystalline cellulose (data from secondary source). The author noted that the NOAEL was 50,000 mg/kg diet, the highest dose tested, (equal to 3,769 mg/kg bw per day for males and 4,446 mg/kg bw per day for females). The Panel agreed with this NOAEL.

In a 90-day study performed according to the current guidelines, Sprague–Dawley rats (20/sex per group) received a diet containing 0 (control), 25,000 or 50,000 mg Avicel<sup>®</sup>CL-611/kg diet (microcrystalline cellulose and carboxy methyl cellulose) (equivalent to 0, 2,250 or 4,500 mg/kg bw per day), Documentation provided to EFSA n. 32). Clinical signs were recorded daily. Body weights and food consumption were measured weekly. Blood samples were collected at termination for haematology and clinical chemistry. Necropsy was conducted on study days 91–94 and organ weights (brain, liver, kidneys, adrenals and testes) measured. Thirty-three organs/tissues of high-dose and control rats were examined histopathologically. No treatment-related clinical signs and no mortality occurred. In males, no effect on body weight and body weight gain was observed. Males of both treated groups showed increased food consumption. This effect was considered to be a response to the decreased caloric content of the test diets. Females of both treated groups showed a decreased body weight and body weight gain, without any significant increase in food consumption. The effect on body weight gain was considered to be caused by the decreased caloric intake. There were no other toxicologically relevant effects in treated animals with respect to clinical chemistry, haematology and absolute and relative organ weights. No treatment-related lesions were detected in histopathology. The authors considered the high-dose group (4,500 mg/kg bw per day) as the NOAEL of this study. The Panel agreed with this NOAEL.

A subchronic gavage study was conducted according to current standards to evaluate the potential toxicological effects associated with intestinal translocation of microcrystalline cellulose (Kotkoskie et al., 1996). Sprague–Dawley rats (20/sex per group) received once daily, via gavage, microcrystalline cellulose (Avicel<sup>®</sup> FD-006, median particle size 6 µm, 35% particles < 5 µm) for 90 consecutive days as a 25% (weight/volume preparation) suspension in tap water at dose levels of 0, 500, 2,500 or 5,000 mg/kg bw per day. The suspension was prepared fresh daily. The control group received the same dosage volume of tap water as the high-dose group. Clinical signs were recorded daily after dosing. Body weights and food consumption were measured weekly. Blood samples were collected at termination for haematology and clinical chemistry. Necropsy was conducted on study days 91–94 and the organ weights (brain, liver, kidneys, adrenals and testes) measured. Thirty three organs/tissues of high-dose and control rats were examined histopathologically. After conventional histopathology,



sections were also examined by polarised light microscopy for the detection of cellulose particles (limit of detection of birefringent particles  $< 1 \mu\text{m}$ ). No treatment-related mortality occurred. The only treatment-related clinical sign noted during the study was pale faeces at  $\geq 2,500 \text{ mg/kg bw per day}$ . This effect was attributed to the presence of the test material in the faeces and was not considered by the authors to be of toxicological relevance. Body weights or body weight gains were not significantly altered. There were no toxicologically relevant effects in treated animals with respect to food consumption, clinical chemistry, haematology, absolute and relative organ weights. No treatment-related lesions were detected in histopathology. Particularly, there were no macroscopic or microscopic findings of microemboli or inflammation in any tissue, including the spleen, intestinal wall and GALT. Polarised light examination revealed a lack of birefringent cellulose particles in all organs and tissues from all high-dose rats, indicating no intestinal uptake. According to the authors, the NOAEL of this subchronic gavage study was  $5,000 \text{ mg/kg bw per day}$ , the highest dose tested. The Panel agreed with this conclusion. The Panel noted that the test substance was not compliant with the specifications of microcrystalline cellulose (E 460(i)) used as food additive.

#### 4.2.2.2. Powdered cellulose (E 460(ii))

##### Rats

In a subacute feeding study performed according to current standards, fermentation-derived cellulose (60% purity, mixed with 20% sodium carboxy methyl cellulose and 20% sucrose; white powder) was administered to groups of six male and six female F344 rats at dietary levels of 0, 1.25, 2.5 or 5.0% for 28 days (equal to 1,340, 2,644 or 5,331 mg/kg bw per day for males and 1,280, 2,611 and 5,230 mg/kg bw per day for females) (Hagiwara et al., 2010). The treatment had no effects on clinical signs, mortality, body weight, food and water consumption. No adverse effects were detected in urinalysis, ophthalmology, haematology, blood biochemistry and histopathology. Slightly increased absolute and relative caecum weights were measured at necropsy in females ingesting  $\geq 2.5\%$  dietary level. This effect was considered by the authors to be a physiological adaptation to the poorly absorbed cellulose. The authors concluded that the NOAEL was 5.0% of the test item in the diet, equal to 5,331 mg/kg bw per day for males and 5,230 mg/kg bw per day for females. The Panel agreed with this conclusion.

The addition of 15% cellulose to the diet of six male rats for 5 weeks did not produce clinical signs of toxicity or statistically significant changes on body weight gain when compared with animals that received a fibre-free diet (Shiau and Ong, 1992). Food intake was significantly increased in cellulose-treated animals, probably due to the high concentration of non-nutritive material in the diet.

In a feeding study with Elcema<sup>®</sup> (a mixture of four types of  $\alpha$ -cellulose in the ratio of 1/1/1/1 (particle size 1–50  $\mu\text{m}$  (powder), 1–100  $\mu\text{m}$  (powder), 1–150  $\mu\text{m}$  (fibrillar), 90–250  $\mu\text{m}$  (granulate)), groups of male and female Wistar rats ( $n = 20/\text{sex per group}$ ) were fed for 90 days at a dietary level of 0% or 10% (equivalent to 0 or 9,000 mg/kg bw per day) (Ferch, 1973a,b, 1974). Statistical analysis of results was not performed. Treated rats gained less weight than those in the control group ( $> 10\%$  reduction in males and maximal 8% in females). Food consumption was not affected. No treatment-related mortality and behavioural abnormalities were observed. Urinalysis, haematology and clinical chemistry at week 0, 6 and 13 revealed similar results for test and control groups. At necropsy, some of the treated rats had distended stomachs, which often contained considerable amounts of the test diet. The absolute liver and kidney weights of male rats and the ratio of the weight of these organs to brain weight were decreased in test animals (no data about statistical significance). No treatment-related histopathological effects were reported (totally 31 organs examined, no further data). The effects on liver and kidney weight were considered by the authors to be without toxicological relevance, since no effects were detected in histopathology and clinical chemistry. Overall, no toxic effects were induced in this subchronic feeding study in rats at a dose of 10%  $\alpha$ -cellulose in the diet (equivalent to 9,000 mg/kg bw per day), the highest dose tested. The Panel agreed with the NOAEL of 9,000 mg/kg bw per day proposed by the authors. The Panel noted that the test substance was not compliant with the specifications of powdered cellulose (E 460(ii)) used as food additive.

Rats (15/sex per group) received 0% or 10% cellulose powder in the diet (equal to 8,200 mg/kg bw per day for male rats and 9,800 mg/kg per day for female rats) for 3 months (Rowland et al., 1982). Rats given the cellulose powder exhibited higher food consumption than control animals, presumably due to the high concentration of non-nutritive material in their diet. No effects were observed on the following parameters: body weight, water intake, haematology, clinical chemistry, urinalysis, organ weights or histopathology.

A group of 20 male and 20 female Crl:CDBR rats received for 13 weeks a basal diet containing a mixture of 10% powdered cellulose (Solka-Floc<sup>®</sup>, no further data) (equivalent to 9,000 mg/kg bw per day) and 10% fructose; concurrent controls were fed the basal diet (Kruger et al., 1999). All rats were checked once daily for clinical signs. Body weight and food consumption were measured once weekly. Haematological and clinical chemistry parameters were determined in blood collected just prior to necropsy. Macroscopic and microscopic pathology was performed. Histopathology of 34 organs and all gross lesions was performed. No clinical signs were detected and all rats survived to study termination. No effects were reported on body weight. Treated rats apparently consumed more food to compensate for the reduction in caloric intake. The treatment induced no significant haematological or clinical chemistry effects and no changes in organ weights (determined in liver, kidney, adrenals, testes, brain, heart, thymus, spleen, thyroid, epididymal fat pads, mesenteric lymph nodes). No treatment-related effects were reported in the histopathology. No adverse effects were induced in rats receiving a diet supplemented with 10% powdered cellulose (equivalent to 9,000 mg/kg bw per day) for 13 weeks.

The effects of a synthetic diet (AIN-76<sup>TM</sup>) containing 5% cellulose (equivalent to 2,500 mg/kg bw per day; no further details) were compared with a cereal-based diet (4.5% crude fibre, no further details) in female rats (n = 6 per group) (Bieri et al., 1977). After an exposure period of 24 weeks, no effects were noted on terminal body weight, absolute and relative organ weight of liver and kidney or haemoglobin and haematocrit values. No further parameters were measured.

Groups of 10–15 male Sprague–Dawley rats were fed diets containing 0%, 10%, 20% or 30% cellulose fibre (equivalent to 0, 5,000, 10,000 or 15,000 mg/kg bw per day; no further details), for 23 weeks (Nigro et al., 1979). A slight increase in body weight (measured every fourth week) was found at the low dose, but a dose-dependent decrease was observed at  $\geq 20\%$  in the diet, significant at the high dose level (30%). Food consumption was increased at  $\geq 20\%$  and faecal wet weight increased in all treatment groups. No further parameters measured.

#### 4.2.2.3. Methyl cellulose (MC; E 461)

##### Rats

Groups of five male and five female rats (not further specified) were dosed via diet with 0 or 10% of MC (4,000 cP) (equivalent to 0 or 9,000 mg/kg bw per day) for 95 days. In females, the feed intake was decreased, resulting in decreased body weight gains (107 vs 125 g in controls). At study termination on day 95, the rats were autopsied and tissues were examined microscopically. The dosing had no adverse effects on relative organ weights of heart, liver, spleen and kidneys. However, the stomachs of the dosed rats were 15% heavier compared with controls. There were no significant microscopic changes indicative of toxic effects in the examined visceral organs (not further specified) (Tainter, 1943).

In two 90-day studies, groups of 10 male and 10 female Sprague–Dawley rats were dosed via diet with 0%, 1%, 3% and 10% low viscosity (10 cP) MC (equivalent to 0, 900, 2,700 or 9,000 mg/kg bw per day) or with 0%, 3% and 10% high viscosity (4,000 cP) MC (equivalent to 0, 2,700 or 9,000 mg/kg bw per day). In both studies, weekly body weight and food consumption records were kept. Haematological evaluations (packed cell volume (PCV), haemoglobin (Hb) determinations and erythrocyte and total and differential leucocyte counts) were conducted during week 12 on five male and five female rats from controls and high-dose rats. Urine analyses were performed in five rats of each sex fed 0% and 10% of the test material. Blood urea nitrogen (BUN), serum alkaline phosphatase (AP) and serum glutamic-pyruvic transaminase were determined terminally. At termination, weights of brain, heart, liver, kidneys and testes were recorded and gross examinations were carried out on all rats. Histopathology was conducted on haematoxylin-eosin-stained sections of selected tissues from rats fed the control and 10% diets. Most, or all, of the following tissues were examined: thyroid, pituitary, trachea, lung, aorta, heart, liver, kidneys, adrenals, spleen, pancreas, stomach, small and large intestine, reproductive organs, urinary bladder, brain, spinal cord, peripheral nerve, skeletal muscle, bone marrow, mesenteric and mediastinal lymph nodes and any lesions suggesting a possible pathological change. Male rats receiving diets containing 10% low viscosity MC (10 cP) showed a significant decrease in their mean starved body weight at autopsy ( $p < 0.05$ ). The average weights, in grams, of few of the organs from these rats were also significantly lower than controls. Especially in male rats dosed with  $\geq 3\%$  of low and high viscosity MC, a dose-dependent and significantly increased feed consumption was noted. For all other investigated parameters, no adverse effects were reported (McCollister et al., 1973). In both trials, there were no significant adverse effects at a dose level of 3% (equivalent to 2,700 mg/kw bw per day).

#### 4.2.2.4. Ethyl cellulose (EC; E 462)

##### Rats

In an unpublished study with rats, no adverse effects were reported in 80 animals dosed via diet with 1.2% of EC (equivalent to 1,080 mg/kg bw per day) (Hake and Rowe, 1963; cited in JECFA, 1990).

#### 4.2.2.5. Hydroxypropyl cellulose (HPC; E 463)

##### Rats

Groups of 10 young adult male and 10 female Wistar rats were administered daily doses of 0, 1,500, 3,000 or 6,000 mg/kg bw of HPC via gavage for 30 days. The rats were weighed every day and food consumption was measured twice a week during the experiment. After 30 days, blood samples were collected for the following tests: aspartate aminotransferase (serum glutamic-oxaloacetic transaminase (S-GOT)), alanine aminotransferase (serum glutamate-pyruvate transaminase (S-GPT)), total cholesterol, AP, blood sugar, total protein, haematocrit, Hb, white blood cell (WBC) and red blood cell (RBC) counts and specific gravity of whole blood and blood plasma. Furthermore, urine of the individual rats was collected for the qualitative detection of glucose, protein and pH. Then the rats were autopsied and the wet weight of liver, kidneys, heart, spleen, brain (including cerebellum), lungs, adrenals and testes or ovaries were recorded. For histopathological examination, besides these organs, stomach, small intestine and bone were also prepared. For all examined parameters, no adverse effects were reported (Kitagawa et al., 1976b).

In an unpublished study, groups of five male and five female rats were dosed via diet with 0, 0.2, 1 or 5% of HPC (equivalent to 0, 180, 900 or 4,500 mg/kg bw per day) over 90 days. The dosing caused no adverse effects on mortality, growth, food utilisation, urinalysis, haematological indices, organ weight, gross pathology and histopathology. Higher dietary levels (not further specified) resulted in increased food consumption and decreased food utilisation due to the inertness of the material (Industrial Bio-Test Lab, 1963; cited in JECFA, 1969).

#### 4.2.2.6. Hydroxypropyl methyl cellulose (HPMC; E 464)

##### Rats

Groups of five male Wistar rats were dosed via diet with 0 or 10% of HPMC over 12 days and at termination, caecum and ascending colon were removed (Wyatt et al., 1988). The dosing caused an up to fivefold increase in the wet weights of the full and empty caecum and ascending colon and a decrease in the density of bacteria in the caecum and colon. HPMC was poorly fermented by caecal and colonic bacteria *in vitro*, with only 5% of the substrate being utilised after 7 days of incubation (no significant change in the level of reducing-ends in the culture fluid). The authors concluded that caecal and colonic enlargement was due to tissue hypertrophy in response to increased bulk of contents, and that it is unlikely that SCFA or other microbial metabolites are the stimulus for the trophic response.

In a study with weanling rats (no further data), groups of 10 male and 10 female rats were dosed via diet with 0%, 2%, 10% or 25% of HPMC (equivalent to 0, 2,400, 12,000 or 30,000 mg/kg bw per day) over 30 days (Hodge et al., 1950). The highest dose level caused diarrhoea and a decrease in body weights; three of 10 males and six of 10 females died. At this dose level, there was also a minor decrease in red blood cell counts, while other haematological and also urine parameters, as well as organ weights, were unchanged. A histopathological examination revealed no adverse effects.

In an additional study, groups of 10 male and 10 female young Wistar-derived rats were dosed via diet with 0%, 1%, 3%, 10% or 30% (equivalent to 0, 900, 2,700, 9,000 or 27,000 mg/kg bw per day) of HPMC over 121 days (McCollister and Oyen, 1954). All rats were weighed twice weekly during the study and examinations included general appearance, mortality and average daily food consumption. Whenever possible, failing animals were sacrificed when moribund. Terminal haematological values were obtained from a group of five female control rats and five female rats dosed with 10% HPMC. At termination, all surviving rats were killed and examined. The livers and kidneys from each rat were weighed. Haematoxylin-eosin-stained sections from these organs, as well as from the pancreas, lung, heart, spleen, testes and adrenals were prepared for histological examination. At a dose level of 30%, body weight gain was markedly decreased and four male and six female rats died during the study due to undernourishment. In male rats dosed with 10%, there also was a slight body weight gain retardation. No other adverse effects were reported and the histological examination revealed no abnormalities in any of the different groups.

In two follow-up studies, groups of 10 male and 10 female young Wistar-derived rats were dosed via diet with 0%, 0.3%, 1%, 3%, 10% or 20% of HPMC containing 24–27% methoxyl groups and 3–5.5% hydroxypropoxyl groups (equivalent to 0, 270, 900, 2,700, 9,000 or 27,000 mg/kg bw per day) for 90 days or with HPMC containing 19–24% methoxyl groups and 4–12% hydroxypropoxyl groups for 84 days (McCollister et al., 1961). During the studies, the rats were observed frequently for changes in gross appearance and behaviour. Body weights were determined twice weekly and average food consumption per rat was recorded. At termination, gross pathological examinations were performed, and the lungs, heart, liver, kidneys, testes and spleen were removed and weighed. Portions of these tissues, as well as adrenals and pancreas, were prepared for a microscopic examination. In addition, blood haematocrit values were determined. In the first study, three rats of each sex died after dosing with 20% and there was a marked reduction in body weight gain in both sexes. In male rats, a significant retardation in body weight gain was seen at 10%. In none of the dosed animals, were adverse histopathologic effects noted. Also, in the second study, male rats showed a significant retardation of body weight gain at the 20% level and a slight retardation at 10%. As a result of undernourishment, there were also corresponding changes in liver, kidney and testes weights. The gross and microscopic examination gave no indications for adverse effects and there were no changes in haematocrit values. In both trials, there were no significant adverse effects at a dose level of 3% (equivalent to 2,700 mg/kg bw per day).

In an additional, unpublished study, groups of 10 male and 10 female young rats (unknown strain) were dosed via diet with 0%, 1%, 3% and 10% of HPMC (higher viscosity; 31,800 cP) and with 0%, 1%, 3% and 10% of HPMC (lower viscosity; 8,480 cP) (equivalent to 0, 900, 2,700 or 9,000 mg/kg bw per day) over 92 days (McCollister and Copeland, 1967; cited in JECFA, 1990). The treatment gave no adverse effects concerning mortality, growth, general appearance and behaviour, body weights, food consumption, haematological and clinical chemistry analysis, organ weights and at gross and histological examination.

In two other 90-day studies, groups of 10 male and 10 female Dow-Wistar rats were dosed via diet with 0%, 1%, 3% and 10% low viscosity (10 cP) HPMC or with 0%, 3% and 10% high viscosity (4,000 cP) HPMC (equivalent to 0, 900, 2,700 or 9,000 mg/kg bw per day) (McCollister et al., 1973). No adverse effects concerning mortality, body weight gain, food consumption, urine and haematological analyses and serum chemistry were noted. Terminal absolute and relative organ weights showed no treatment-related effects. The gross and histopathological examination of the tissues revealed similar findings for control and treated rats. At all dose levels (i.e. up to 9,000 mg/kg bw per day), no adverse effects were reported in both studies.

Groups of 15 male and 15 female young Sprague–Dawley rats were dosed via diet with 0%, 1% or 5% low viscosity (4.22 cP) HPMC (equivalent to 0, 900 or 4,500 mg/kg bw per day) for 90–91 days (Schwetz, 1976). No adverse effects concerning mortality, body weight gain, food consumption, urine and haematological analyses and serum chemistry were noted. Terminal absolute and relative organ weights showed no treatment-related effect. The gross and histopathological examination of the tissues revealed similar findings for control and treated rats. For none of the examined parameters were adverse effects reported at a dose level up to 4,500 mg/kg bw per day.

Groups of five male and five female Crj:CD (SD) IGS rats (SPF) with an age of about 5 weeks and body weights of 125–176 g were dosed via gavage with 0, 505, 1,020 or 2,100 mg/kg bw per day of HPMC (viscosity of 2.83 mPa.s) for 91 days (Obara et al., 1999). For both high-dose male and female rats, lower body weights were observed from day 28 onwards; in high-dose males there was also a decrease in food consumption and urine volume. However, these differences were not statistically significant. For all other examined endpoints, no treatment-related effects were noted. Based on the decrease in body weights, the authors of this study identified a NOAEL value of 1,020 mg/kg bw. However, the Panel considered the level of 2,100 mg/kg bw per day as the NOAEL, because the effects observed were all not statistically significant.

## Rabbits

In groups of six rabbits (approximately 6 months old and with a body weight of 1.8 kg) dosed via diet with 0%, 10% or 25% of HPMC (equivalent to 0, 3,000 or 7,500 mg/kg bw per day) over 30 days, no adverse effects concerning body weights, urine analysis, haematology, organ weights and histological examination were reported (Hodge et al., 1950).



## Dogs

Two dogs (body weights of 11 and 13 kg) were dosed with 25 or 50 g/day, respectively, of HPMC over 30 days (Hodge et al., 1950). In the higher dose animal, effects such as diarrhoea, slight weight loss and slight depression in RBC count were noted, while the lower dose in the other animal was well tolerated. An urine analysis and a histological examination revealed no adverse effects at both dose levels.

In another study, groups of two male and two female beagle dogs were dosed via diet with 0, 2 and 6% low viscosity (10 cP) HPMC for 90 days (equivalent to 0, 500 or 1,500 mg bw per day) (McCollister et al., 1973). Body weights and food consumption were recorded weekly. Before starting the study and at termination on day 90, standard haematological and clinical chemistry parameters (PCV, Hb, RBC, total and differential WBC, BUN, AP, S-GOT, S-GPT and bromosulfothalein retention) were determined and also tissues from all dogs were examined. No adverse effects concerning mortality, body weight gain, food consumption, urine and haematological analyses and serum chemistry were noted. Terminal absolute and relative organ weights showed no treatment-related effect. The gross and histopathological examination of the tissues revealed similar findings for control and treated dogs. There was no evidence of storage of the test material within the cells of the reticuloendothelial system of the dosed animals.

Groups of four male and four female adult beagle dogs were dosed via diet with 0, 1 or 5% low viscosity (4.22 cP) HPMC (equivalent to 0, 250 or 1,250 mg/kg bw per day) for 90–91 days (Schwetz, 1976). No adverse effects concerning mortality, body weight gain, food consumption, urine and haematological analyses and serum chemistry were noted. Terminal absolute and relative organ weights showed no treatment-related effects. The gross and histopathological examination of the tissues revealed similar findings in control and treated dogs. For none of the examined parameters, were adverse effects reported.

### 4.2.2.7. Ethyl methyl cellulose (EMC; E 465)

No data available.

### 4.2.2.8. Sodium carboxy methyl cellulose (NaCMC; E 466)

## Rats

Groups of five male Wistar rats were dosed via diet with 0% or 10% of CMC (equivalent to 0 or 12,000 mg/kg bw per day) over 12 days, and at termination caecum and ascending colon were removed (Wyatt et al., 1988). Dosed rats showed diarrhoea from the second day of dosing and a significantly increased output of faecal material. There was a visible enlargement of the caecum and increased wet weights of contents and tissues (almost eightfold increase in weight of caecal contents coupled with a doubling of tissue weight). The dosing had no significant effect on the density of bacteria in the caecum and colon. The aerobic microflora of the control group was composed primarily of *Streptococcus* spp. (95–100% of isolates), while both in caecal and colonic contents of CMC-dosed rats, almost exclusively *E. coli* was found (93% of isolates). No enterotoxin activity was detected in the *E. coli* strains isolated from CMC-fed rats with diarrhoea, and serotyping showed that the strains (O54 and O24) belonged to groups that are not commonly associated with diarrhoea in man. A substantial amount of unfermented CMC was present in the intestinal contents of dosed rats and the proportion of CMC increased distally. CMC appeared not to have been hydrolysed to shorter chain lengths, as no increase in reducing ends could be demonstrated compared with animals maintained on the control diet. CMC also was poorly fermented by caecal and colonic bacteria *in vitro*. By day 7 of incubation, there was only a very slight increase in the level of reducing-ends in the culture fluid and dosed rats had significantly lower SCFA concentrations in the caecum. The authors concluded that caecal and colonic enlargement was due to tissue hypertrophy in response to increased bulk of contents and that it was unlikely that SCFA or other microbial metabolites were the stimulus for the trophic response.

In an unpublished study, 12 rats/per group (not further specified) were dosed over 21 days with a high-protein diet containing 0% or 15% sodium CMC of 10 viscosity grades (35–4,500 cP) or four other vegetable gums (Anderson, 1986; cited in JECFA, 1990). Animals were weighed on alternate days. Body weight gain in animals dosed with one sample of CMC exceeded that of controls and body weight gains in animals dosed with two other CMC samples were less than that of controls. Average faecal water content (measured as %) was increased in all CMC-fed animals from 1.9- to 3-fold, and average filled caecal weight (g/kg bw) was increased 1.5- to 3.3-fold, compared to controls. It was noted that there was a tendency for CMC samples of low molecular weight to produce high faecal wet



weights. Measurement of the viscosities of completely hydrated samples of CMC indicated that CMC can have large or narrow molecular weight distributions. It was suggested that different molecular weight distributions in samples of CMC may produce different physiologic or dietary responses.

In a study with young male Sprague–Dawley rats (3-weeks old) with conventional gut microflora, six animals per group were dosed via diet with 0% or 5% of CMC (equivalent to 0 or 6,000 mg/kg bw per day) over 4 weeks (Mallett et al., 1984). At termination, the caecal contents from each animal were collected and used for different enzyme assays. In dosed rats, the body weights were decreased (about 9%) compared to the control group, and the weights of both the caecal wall and caecal contents were significantly increased. The treatment significantly increased the total bacterial population of the caecum and the mean caecal ammonia concentration was increased fourfold. All measured enzyme activities (azoreductase,  $\beta$ -glucosidase,  $\beta$ -glucuronidase, nitrate reductase, nitro reductase and urease) were also significantly increased compared to controls. The authors assumed that the  $\beta$ -1,4-glucose backbone of CMC was susceptible to attack by gut bacteria.

In groups of three young rats (no further data), the dosing with 0% or 14% of CMC (equivalent to 0 or 16,800 mg/kg bw per day) via diet over 5 weeks caused no adverse effects (Ziegelmeier et al., 1951).

In a study with limited documentation, the daily dosing of 10 rats (no further data) with 300–500 mg of CMC over 2 months caused no adverse effects (Werle, 1941).

In another study, 10 female Wistar rats with an age of 82–98 days were fed a diet containing 20% CMC (equivalent to 18,000 mg/kg bw per day) for 63 days (Rowe et al., 1943). All animals were weighed twice weekly and individual weight records were kept throughout the experimental period. The treatment caused slight growth retardation and a laxative effect was observed. Organ weights and gross examination revealed no abnormalities. Also, the results of a histopathological examination of the liver, kidney, spleen, pancreas and adrenal gland revealed no significant differences between the treated and the control groups.

Groups of albino Wistar outbred rats [CrI:WI(WU)BR] (20/sex, 8 weeks of age) were dosed via diet with 0, 2.5, 5 and 10% of CMC (equivalent to 0, 2,250, 4,500 or 9,000 mg/kg bw per day) for 91–95 days in male rats and for 98–102 days in female rats (Bär et al., 1995a). To adapt the animals of the mid- and high-dose groups, the dose of CMC was increased gradually from 2.5% on days 1–3 to 5% on days 4–7 and to 10% from day 7 onwards. The mean intakes were 0, 1,360–1,570, 2,810–3,230 or 6,150–6,800 mg/kg bw per day (higher values are for female rats) and the dietary sodium content was 0.31%, 0.57%, 0.81% and 1.27%. In general, the ingestion of CMC was well tolerated, except that all high-dosed rats had some diarrhoea during the entire study period. In both sexes, there was a dose-dependent increase in food intake, although in high-dosed females the body weights were slightly below control values. Also, the water intake was dose dependently increased in both sexes and was statistically significant in low-dosed males and in mid- and high-dosed rats of both sexes. In high-dosed female rats, the activity of plasma AP was significantly increased, while in high-dosed male rats, the alanine aminotransferase activity was significantly increased. As a result of the higher water intake, urine volumes in both sexes also increased dose dependently. In addition, a dose-dependent increase of sodium, calcium and citrate in urine was seen at  $\geq 5\%$  CMC (equivalent to 4,500 mg/kg bw per day). Terminal body weights did not differ significantly between treated groups and controls. The dosing caused some changes in absolute or relative organ weights of liver and kidneys, but especially for the caecum weights, there was a clear dose-dependent increase both in male and female rats. A gross examination at autopsy revealed the presence of caecal enlargement and an increased liquid content in the intestinal tract, particularly in high-dosed male and female rats. On microscopic examination, an increased incidence of dilatation of the colon was observed in high-dosed females. The renal observations included an increased occurrence of pelvic urothelial hyperplasia (5 out of 20 rats) and pelvic nephrocalcinosis (6 out of 20 rats) in low-dosed male animals. Corticomedullary nephrocalcinosis was seen in five of out 20 high-dosed female rats. In the bladder of male rats, an increased incidence of diffuse epithelial hyperplasia was found in 8 out of 20 high-dosed rats. For all the reported findings, a toxicological concern was not concluded, as the findings in the GI tract were considered to be a consequence of the accumulation of poorly absorbed water-soluble material in the caecum and colon, while the findings in kidneys and urinary bladder were attributed to the four times higher concentration of sodium in the CMC diet compared with the basal diet.

## Dogs

In a study with dogs, aged about 16 weeks (not further specified), three groups of 10 animals each were dosed with 0, 500 or 1,000 mg/kg bw per day of CMC via diet for 6 months (Shelanski and Clark, 1948). None of the animals died during the course of the study. The dosing was well tolerated and

caused no changes in body weights. At study termination, all dogs were autopsied; the histological examination of stomach, intestines, spleen, liver, kidney, heart, lung and pancreas revealed no changes.

#### 4.2.2.9. Cross-linked sodium carboxy methyl cellulose (E 468)

##### Rats

Cross-linked sodium carboxy methyl cellulose was administered to Sprague–Dawley CD rats (20 animals/sex, 8 weeks old) at 0, 10,000 or 50,000 mg/kg diet for 90 days (equal to 757 and 893 or 3,922 and 4,721 mg/kg bw per day for males and females, respectively) (Freeman et al., 2003). The study was conducted according to a current OECD guideline and in compliance with Good Laboratory Practice (GLP). A control group of 20 animals were given a basal diet. No treatment-related deaths took place and no clinical signs were reported in response to the test article administration. The body weights were significantly reduced in males receiving 50,000 mg/kg during the last 3 weeks of the study (*p* value not stated). The food consumption of females receiving the 50,000 mg/kg dose was significantly increased (*p* value not stated) compared to controls during most of the study (data not shown). No effects were seen in the clinical chemistry or haematology investigations, no treatment-related findings were noted in the necropsy and there were no effects on organ weights or organ-to-brain ratios. The study authors concluded that the daily administration of 50,000 mg/kg cross-linked sodium carboxy methyl cellulose did not result in adverse effects in rats following 90-day exposure, and the NOAEL was concluded to be 3,922 mg/kg bw per day for males and 4,721 mg/kg bw per day for females.

##### Dogs

In a 1-year study, groups of two dogs (strain and sex not further specified) were dosed with 0, 100, 300, 1,000 or 3,000 mg cross-linked sodium carboxy methyl cellulose/kg bw per day. The treatment had no adverse effects on body weights and none of the animals died. Urine analyses (performed twice monthly) and haematological examinations performed prior to starting, four times during the experiment and terminally, showed no changes. At necropsy, organ weights were recorded and a number of tissue sections (heart, lung, spleen, stomach, large and small intestine, pancreas, liver, gall bladder, adrenal, kidney, bladder, gonads, thyroid, bone marrow and brain) were examined microscopically. No adverse effects were reported (Hodge et al., 1950).

#### 4.2.2.10. Enzymatically hydrolysed carboxy methyl cellulose (E 469)

##### Rats

Groups of albino Wistar outbred rats [CrI:WI(WU)BR] (20 per sex, 8 weeks of age) were dosed via diet with 0%, 2.5%, 5% and 10% of enzymatically hydrolysed carboxy methyl cellulose for 91–95 days for male rats and for 98–102 days for female rats (Bär et al., 1995a). To adapt the animals of the mid- and high-dose groups, the dose of enzymatically hydrolysed carboxy methyl cellulose was increased gradually from 2.5% on days 1–3, to 5% on days 4–7, and to 10% from day 7 onwards. The mean intakes were equal to 0, 1,340–1,560, 2,830–3,170 or 5,920–6,610 mg/kg bw per day (higher values are for female rats) and the dietary sodium content was 0.31%, 0.51%, 0.72% and 1.16%. In general, the ingestion of enzymatically hydrolysed carboxy methyl cellulose was well tolerated, except that all high-dosed rats had some diarrhoea during the entire study period. In both sexes, there was a dose-dependent increase in food intake, although in high-dosed females the body weights were slightly below control values. Also, the water intake was dose-dependently increased and the differences were statistically significant in mid- and high-dosed rats of both sexes. In high-dosed females, Hb concentrations and PCVs were slightly decreased (details were not provided). Plasma AP activity was significantly increased in high-dosed male rats and in mid-dosed female rats. As a result of the higher water intake, urine volumes also increased with increasing doses of enzymatically hydrolysed carboxy methyl cellulose in male and female rats. In addition, a dose-dependent increase in sodium, calcium and citrate was seen at  $\geq 2.5\%$  enzymatically hydrolysed carboxy methyl cellulose. Terminal body weights did not differ significantly between treated groups and controls. The dosing caused some slight changes in absolute or relative organ weights of liver and kidneys, but especially for the caecum weights, there was a clear dose-dependent increase both for male and female rats. Gross examination at autopsy revealed the presence of caecal enlargement and an increased liquid content in the intestinal tract, particularly in high-dosed male and female rats. On microscopic examination, an increased incidence of dilatation of the colon was observed in high-dosed females. The renal observations included an increased occurrence of pelvic urothelial hyperplasia in males

dosed with 10% (6 out of 20 rats) and pelvic nephrocalcinosis at  $\geq 5\%$  in males (five or nine of 20 rats). In the bladder of male rats, an increased incidence of diffuse epithelial hyperplasia was found in five of 20 high-dosed rats. For all the reported findings, a toxicological concern was not concluded, as the findings in the GI tract were considered to be a consequence of the accumulation of poorly absorbed water-soluble material in the caecum and colon, and the findings in kidneys and urinary bladder were attributed to the four times higher concentration of sodium in the enzymatically hydrolysed carboxy methyl cellulose diet compared with the basal diet.

#### 4.2.2.11. Summary

Data are available for microcrystalline cellulose, powdered cellulose, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, sodium carboxy methyl cellulose and enzymatically hydrolysed carboxy methyl cellulose.

- The subacute and subchronic toxicity of microcrystalline and powdered cellulose was low. The NOAELs found in oral studies (gavage, drinking water or diet) in rats ranged from 3,769 to 9,000 mg microcrystalline cellulose/kg bw per day (5–10% in the diet) and 9,000 mg powdered cellulose/kg bw per day (the highest dose tested in each case). No effects were detected when an increased percentage of particles below the recommended particle size in the EU specifications was present in the test substance.
- In two 90-day feeding studies, groups of 10 male and 10 female Sprague–Dawley rats were dosed via diet with 0%, 1%, 3% and 10% low viscosity (10 cP) methyl cellulose or with 0%, 3% and 10% high viscosity (4,000 cP) methyl cellulose. In high-dosed male rats, a significant decrease in body weights was noted, while for all other investigated parameters no adverse effects were reported (McCollister et al., 1973). Therefore, a NOAEL value of 3% (equivalent to 2,700 mg/kg bw per day) was derived from this study.
- In a 30-day gavage study, groups of 10 young adult male and female Wistar rats were dosed with 0, 1,500, 3,000 or 6,000 mg/kg bw of hydroxypropyl cellulose. Even at the highest dose level, no adverse effects were reported (Kitagawa et al., 1976b).
- In two 90-day feeding studies, groups of 10 male and 10 female Dow–Wistar or Sprague–Dawley rats were dosed via diet with 0%, 1%, 3% and 10% low viscosity (10 cP) hydroxypropyl methyl cellulose or with 0%, 3% and 10% high viscosity (4,000 cP) hydroxypropyl methyl cellulose. In both trials, even at the highest dose level of 10% (equivalent to 9,000 mg/kg bw per day), no adverse effects were reported (McCollister et al., 1973).
- In another study, groups of five male and five female Crj:CD (SD) IGS rats (SPF) were dosed via gavage with 0, 505, 1,020 or 2,100 mg/kg bw per day of hydroxypropyl methyl cellulose (viscosity of 2.83 mPa.s) for 91 days. Apart from a decrease in body weights in high-dosed male and female rats, which was not statistically significant, no treatment-related effects were noted for all the other endpoints examined. The food consumption data were limited, but suggested decreased food consumption. The authors of this study gave a NOAEL value of 1,020 mg/kg bw (Obara et al., 1999). The Panel considered 2,100 mg/kg bw per day of hydroxypropyl methyl cellulose as the NOAEL in this study.
- In two parallel studies, groups of 20 Albino Wistar outbred rats [CrI:WI(WU)BR] per sex were dosed with carboxy methyl cellulose or enzymatically hydrolysed carboxy methyl cellulose via diet with 0%, 2.5%, 5% and 10% (equal to 0, 1,500, 3,000 and 6,000 mg/kg bw per day) for up to 102 days (Til, 1992; Bär et al., 1995a). Although, in both studies, some effects (increased caecum weights, urothelial hyperplasia, pelvic nephrocalcinosis, corticomedullary nephrocalcinosis, increased incidence of diffuse epithelial hyperplasia in the urinary bladder) were observed, for all the reported findings, a toxicological concern was not concluded for carboxy methyl cellulose or enzymatically hydrolysed carboxy methyl cellulose itself, as the findings in the GI tract were considered to be a consequence of the accumulation of poorly absorbed water-soluble material in the caecum and colon, and the findings in kidneys and urinary bladder were attributed to the up to fourfold higher concentration of sodium in the fed diet compared with the basal diet.

Several additional studies, which do not meet current criteria for toxicological testing or which were not available (unpublished data) for evaluation, supported the low toxicity of this group of modified celluloses.

Although there were some inconsistencies in the data, the main effects seen were decreases in body weight gain at the highest dose, which are likely to be due to the amount/bulk of cellulose in the

diet decreasing the nutrient intake. The NOAEL values reported ranged from 2,000 up to 9,000 mg/kg bw per day.

### 4.2.3. Genotoxicity

#### 4.2.3.1. Microcrystalline cellulose (E 460(i))

##### *In vitro*

Avicel<sup>®</sup> RCN-15 (a mixture of 85% microcrystalline cellulose with 15% guar gum, no further data) was not mutagenic in a bacterial reverse mutation assay in the *Salmonella* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 when tested as suspension in dimethylsulfoxide (DMSO) at five dose levels between 50 and 5,000 µg/plate, with and without metabolic activation by rat liver S9-mix in an unpublished study performed in compliance with GLP (Batt, 1992).

The same test system resulted also in no mutagenic activity with the test substance Avicel<sup>®</sup> AC-815 (composed of 85% microcrystalline cellulose and 15% calcium alginate). In this study, *E. coli* WP2uvrA or *S. Typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to the test substance suspended in DMSO at doses from 10 to 5,000 µg/plate in the presence or absence of metabolic activation with rat liver S9-mix (Documentation provided to EFSA n. 37).

In a third bacterial reverse mutation assay, using *S. Typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, microcrystalline cellulose suspended in DMSO produced no increase in revertants at doses up to 5,000 µg/plate (no further information available) (FMC, 1991; referred to by SCF, 1999).

Avicel<sup>®</sup> RCN-15 was tested in a mammalian gene mutation assay in L5178Y mouse lymphoma cells in an unpublished study performed in compliance with GLP (Cifone, 1992). The test material was dissolved in DMSO at 100 mg/mL and tested in replicated experiments at six dose levels, ranging from 100 to 1,000 µg/mL (top concentration based on the 1% final concentration of DMSO in culture medium) both with and without metabolic activation. In both cases, a 4-h treatment was applied. No toxicity and no increase in forward mutation at the thymidine kinase locus which was dose-related or exceeded twice the solvent control was observed. Precipitation in culture medium was observed above 100 µg/mL. Positive control substances, methyl methanesulfonate and 3-methylcholanthrene, elicited a distinct mutagenic response. The Panel noted that an extended treatment without metabolic activation was not performed, but this was not recommended in the relevant OECD Guideline at the time the study was performed. In another study, also Avicel<sup>®</sup> CL-611 was tested with negative results in the same test system (Documentation provided to EFSA no. 34).

Avicel<sup>®</sup> RCN-15 was tested in an unscheduled DNA synthesis assay in rat liver primary cells in an unpublished study performed in compliance with GLP (McKeon, 1992). The test substance was formulated in DMSO and applied at seven concentrations in the range 5–1,000 µg/mL in a first trial and at 10 concentrations in the range 10–1,000 µg/mL in a second trial. Precipitation was observed from 5 µg/mL onwards in the first trial and from 10 g/mL onwards in the second trial. No overt toxicity (i.e. < 78 of survival) and no induction of unscheduled DNA synthesis was observed in both experiments. The positive control 2-acetylaminofluorene elicited the expected positive response. The Panel noted that in this study a lower solubility of Avicel<sup>®</sup> RCN-15 was reported, compared to the previous study by Cifone (1992).

##### *In vivo*

Avicel<sup>®</sup> RCN-15 was tested in a mouse bone marrow micronucleus assay in ICR mice (Murli, 1992). The test material was mixed in a commercial diet at a concentration corresponding to an exposure level of 5,000 mg/kg bw. The feed (1 g) was administered during an exposure time of 10 h, and animals (groups of five males and five females) sacrificed 24, 48, and 72 h after the end of the exposure period. No increase of micronucleated polychromatic erythrocytes and no evidence of toxicity to bone marrow was observed in treated animals. A significant positive response was induced by the positive control cyclophosphamide, administered by gavage 24 h before sacrifice. This study was performed in compliance with GLP.

Negative results were also reported with other microcrystalline cellulose preparations in other unpublished studies (Documentation provided to EFSA n. 35–36).

#### 4.2.3.2. Methyl cellulose (MC; E 461)

In a screening of cosmetic ingredients, MC was tested for mutagenicity in a spot test with *S. Typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic



activation. Negative results were reported by the study authors at the single dose level tested (50 µg/plate) (Blevins and Taylor, 1982).

In another published study, MC was not mutagenic in bacterial reverse mutation assays in *S. Typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98, with and without metabolic activation, up to the maximum dose of 70 mg/plate (Ishidate et al., 1984). In the same study, MC was not clastogenic in an *in vitro* chromosomal aberration assay in Chinese hamster fibroblasts (CHL), only carried out without metabolic activation, up to the maximum tested concentration of 4 mg/mL. The Panel noted that only a qualitative indication of the outcome of tests, with no presentation of experimental results, was available from the above studies.

Further results of genotoxicity tests on MC are reported in an unpublished study (Litton Bionetics Inc., 1974) on the test material Methocel (FDA 71-264). Negative results were obtained in the following assays:

- two host-mediated assays in rats, dosed once or five times either with 4.75, 47.5 or 475 mg/kg bw by gavage or with 5,000 mg/kg via feed, using *S. Typhimurium* strains TA1530 and G46 and yeast strain D3 as indicator organisms for reverse mutation and mitotic recombination induction, respectively;
- a chromosome aberration assay with human embryonic lung cells (WI-38), only performed without metabolic activation, at doses of 80, 800 and 8,000 µg/mL;
- an *in vivo* chromosome aberration assay in rat bone marrow, following single or repeated (five times) oral administration of the test article, either at 4.75, 47.5 or 475 mg/kg bw by gavage, or at 5,000 mg/kg bw per day via feed;
- a dominant lethal assay with male rats at identical dosage regimen as above.

The Panel noted that these studies, although acceptable at the time they were performed, are based on limited and/or obsolete experimental protocol/system.

#### 4.2.3.3. Ethyl cellulose (EC; E 462)

No data available.

#### 4.2.3.4. Hydroxypropyl cellulose (HPC; E 463)

No data available.

#### 4.2.3.5. Hydroxypropyl methyl cellulose (HPMC; E 464)

No data available.

#### 4.2.3.6. Ethyl methyl cellulose (EMC; E 465)

No data available.

#### 4.2.3.7. Sodium carboxy methyl cellulose (CMC; E 466)

Sodium CMC was not mutagenic in bacterial reverse mutation assays in the *S. Typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98, with and without metabolic activation up to the maximum dose of 2.5 mg/plate (Ishidate et al., 1984). In the same study, sodium CMC was not clastogenic in an *in vitro* chromosomal aberration assay in CHL, only carried out without metabolic activation, up to the maximum tested concentration of 1 mg/mL. The Panel noted that no raw data were presented for these tests, which were carried out in the framework of a large screening of food additives.

Further results on sodium CMC were available from an unpublished study (Litton Bionetics Inc., 1975, 1980). In this study, sodium CMC was not mutagenic in the following tests:

- bacterial reverse mutation tests with *S. Typhimurium* TA1535, TA1537 and TA1538, both in the presence and the absence of metabolic activation, at concentrations of 2.5–10% (suspension test) and 5 mg/plate (spot test);
- a mitotic recombination assay with *Saccharomyces cerevisiae* D4 at concentrations of 0.25%, 0.5% and 1%, only performed without metabolic activation;

The Panel noted that these studies, although acceptable at the time they were performed, are based on limited and/or obsolete experimental protocol/system.

Negative results in an Ames test with *S. Typhimurium* TA98, TA100, TA1535, TA1537 and TA1538, both in the presence and absence of metabolic activation at doses from 0.5 to 5,000 µg/plate, were



also reported in another unpublished study (Litton Bionetics Inc., 1980), not available to the Panel for evaluation.

#### 4.2.3.8. Cross-linked sodium carboxy methyl cellulose (E 468)

The literature search and the EFSA call for data did not produce any genotoxicity data for cross-linked sodium carboxy methyl cellulose (E 468). The previous evaluation by the SCF (1996) refers to available genotoxicity data, but no further information is available. The most recent evaluation by JECFA (2003) does not discuss the genotoxicity of cross-linked sodium carboxy methyl cellulose (E 468).

#### 4.2.3.9. Enzymatically hydrolysed carboxy methyl cellulose (E 469)

No data available.

#### 4.2.3.10. Summary

Avicel<sup>®</sup> RCN-15 (a mixture of 85% microcrystalline cellulose with 15% guar gum) did not induce mutagenic effects in the presence or absence of a metabolic activation system in bacterial reverse mutation assays (Batt, 1992), in the mammalian cell gene mutation assays (Cifone, 1992) and in an *in vitro* test for unscheduled DNA synthesis (McKeon, 1992). Avicel<sup>®</sup> RCN-15 was also tested negative in mouse bone marrow micronucleus assays (Murli, 1992). Negative results were also reported in the following unpublished studies: bacterial reversion assay with Avicel<sup>®</sup> AC-815 (85% microcrystalline cellulose and 15% calcium alginate) (Documentation provided to EFSA no. 34), *in vivo* micronucleus assays with Avicel<sup>®</sup> PH-101 (pharmaceutical microcrystalline cellulose, nominal mean particle size of 50 µm) (Documentation provided to EFSA no. 36) and Avicel<sup>®</sup> CL-611 (Documentation provided to EFSA no. 35). The Panel noted that, even though the majority of these studies were carried out with mixtures containing microcrystalline cellulose and guar gum rather than with microcrystalline cellulose alone, the latter was present as main component (85% by weight) in such mixtures. Therefore, the results of the studies can be considered directly relevant for the evaluation of the genotoxicity of E 460 (i). Based on the negative results reported, the Panel concluded that microcrystalline cellulose (E 460 (i)) is not genotoxic. The Panel also considered that this conclusion can be extended to powdered cellulose (E 460(ii)), which is structurally similar to E 460(i) but with a higher polymerisation degree.

Concerning modified celluloses, experimental data on genotoxicity were only available for methyl cellulose (E 461) and sodium carboxy methyl cellulose (E 466).

Both substances were negative in Ames tests with different *S. Typhimurium* strains, both with and without metabolic activation (Litton Bionetics Inc., 1975, 1980, Blevins and Taylor, 1982; Ishidate et al., 1984). Negative results were also obtained in a chromosomal aberration assay in CHL (Ishidate et al., 1984), only performed without metabolic activation, and in host-mediated assays with yeast and bacteria (Litton Bionetics Inc., 1974, 1975).

MC was also tested with negative results in an *in vitro* chromosomal aberration assay in human embryonic lung cells (WI-38), and *in vivo* in a chromosome aberration assay in rat bone marrow and in the dominant lethal assay in male rats (Litton Bionetics Inc., 1974).

The Panel noted that most genotoxicity test results on MC and sodium CMC were from limited and/or briefly reported studies, not available for direct evaluation to the Panel. However, the Panel also noted that there is a long history of use of MC and CMC as vehicles for non-water-soluble substances in *in vitro* and *in vivo* genotoxicity assays (as recommended in OECD TG 478, 2015 and TG 483, 2015). Therefore, the Panel concluded that MC and CMC do not raise concern for genotoxicity.

The Panel also considered that read-across from MC (E 461) to the other modified celluloses bearing similar simple substituents (E 462, E 463, E 464, E 465) was justified, as the SAR analysis of E 462, E 463, E 464, E 465 using the OECD QSAR Toolbox did not highlight any structural alert for genotoxicity in their structure. Thus, the Panel concluded that E 462, E 463, E 464, E 465 did not raise genotoxic concern either.

Similarly, the Panel considered scientifically justified the read-across from sodium CMC (E 466) to its products of enzymatic hydrolysis (E 469) or cross-linking (E 468), which in a similar *in silico* analysis were shown not to bear additional structural determinants of genotoxicity.

Overall, the Panel concluded that microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)) and modified celluloses (E 461–466 and E 468–469) do not raise concern for genotoxicity.

#### 4.2.4. Chronic toxicity and carcinogenicity

##### 4.2.4.1. Microcrystalline cellulose (E 460(i))

###### Rats

Groups of male and female rats (no further details) received the control diet or a diet containing 330 mg/kg microcrystalline cellulose (equivalent to 16.5 mg/kg bw per day), for a period of 6 months (Yartsev et al., 1989; cited in JECFA, 1990). Six rats per group were then sacrificed, necropsy performed, and tissues processed for histopathology (no further data available). No effects of the treatment were reported.

An unpublished chronic feeding study is reported in the secondary literature (limited information; no further details available). Three groups of 50 male and 50 female rats received in their diet either 30% 'ordinary cellulose' or microcrystalline cellulose or microcrystalline cellulose gel for 72 weeks (equivalent to 15,000 mg/kg bw per day). The type of control was not specified. No clinical signs were reported. The body weights of males treated with microcrystalline cellulose gel were higher than those of the controls. No such effects were detected with the other cellulose formulations. Food efficiency, survival and haematology were comparable in all groups. The liver and kidney weights of males receiving microcrystalline cellulose gel were increased. Histopathology showed some dystrophic calcification of renal tubules in females on microcrystalline cellulose. All other organs appeared unremarkable. The tumour incidences did not differ between treated and control groups (Documentation provided to EFSA no. 40).

A combined one-generation reproductive/chronic toxicity study was performed in rats (Lewerenz et al., 1979; referred to by SCF, 1999) (Lewerenz et al., 1981a; referred to by SCF, 1999), in which males and females of the F1 generation were fed microcrystalline cellulose (90% of particles < 20 µm) at 0 (control), 30, 100 or 200 g/kg diet (equivalent to 0, 1,500, 5,000 or 10,000 mg/kg bw per day), for up to 2 years (data from secondary sources, no further details available). The treatment induced some growth depression of the F1 rats at the top dose during the early growth phase only. Food consumption was increased in all treated groups. After 12 months, particles were detected in some organs but no microemboli were identified. Some impairment of the renal function without any associated histopathological changes and of some haematological changes in the highest dose group could not be confirmed in the surviving rats treated for 2 years. This study was also reported in the JECFA evaluation (Lewerenz et al., 1981b; referred to by JECFA, 1999a,b). The Committee stated that the available data are not sufficient for evaluation due to the high mortality during the course of the study, the evidence of confounding infection, the limited number of animals for histopathology and the absence of details of the first year of treatment. Limited information on this study was published in a review (Steege et al., 1980) and did not permit a firm conclusion. The Panel agreed with this conclusion.

###### **Initiation–promotion studies**

The Panel noted a series of studies which had investigated potential beneficial effects of microcrystalline cellulose administration on the development of cancer in laboratory animals treated with an initiating agent (Freeman et al., 1978, 1980; Nigro et al., 1979; Yamamoto, 1994; Cohen et al., 1996; Chang et al., 1997; Wijnands et al., 1999; Iwane et al., 2002) and a clinical study in humans diagnosed with colon cancer (Hardman et al., 1997). The Panel did not consider these studies relevant to the risk assessment of microcrystalline cellulose as a food additive (E 460(i)).

##### 4.2.4.2. Powdered cellulose (E 460(ii))

###### Monkeys

Intestinal structure of male adult African Green monkeys (n = 4 per group) was studied after feeding diets containing 10% psyllium husk (fibre; highly branched arabino-xylan-polysaccharide) or 10% cellulose (Cellufil®; no further data) (equivalent to 7,500 mg/kg bw per day), for 3.5 years (Paulini et al., 1987). The cellulose-treated animals did not show any abnormality in the GI tract.

##### 4.2.4.3. Methyl cellulose (MC; E 461)

In a feeding study, groups of five female rats (strain not specified) were dosed via diet with 1.66 or 5% of MC (equivalent to 833 or 2,500 mg/kg bw per day) for 6 months (Bauer, 1945). Adverse effects caused by the treatment were not reported.

In another feeding study, 80 young male and female albino rats were dosed with 0.8% MC in the diet (equivalent to 400 mg/kg bw per day) and with 1% MC via drinking water (equivalent to 900 mg/kg bw per day) for about 9 months (Deichmann and Witherup, 1943). As controls, also 80 young male and female albino rats were used. The combined average total daily intake was calculated to be 436 mg of MC per animal. The dosing was well tolerated, there were no effects on growth rate and also water and food intake were unchanged. At necropsy, no gross or microscopic pathological changes were found.

Groups of five male and five female young Sprague–Dawley rats were dosed with diets containing 0%, 0.17% (changed after 6 weeks to 0.5%) or 5% of MC (average intake equal to 690–775 mg/kg bw per day) for 8 months (Bauer and Lehmann, 1951). Group body weights and food and water consumption were recorded weekly. This treatment had no adverse effect on growth, but caused a significantly increased feed intake. Through 3 generations, reproduction was not impaired and the second and third generation rats dosed with 5% MC showed no adverse effects. A macroscopic and microscopic examination of representative animals revealed essentially normal tissues (not further specified). There also was no deposition of abnormal material in the tissues, indicating that no methyl cellulose was absorbed from the intestinal tract. After about 8 months on the experimental diet, the rats were mated. The young of the F1 and F2 generations were kept in groups of five and placed on 5% MC diet, and later mated in the same manner as the F0 generation. Representative animals from each group of the first generation were sacrificed at 8 months of age for macroscopic and microscopic examination. In the second experiment, nine young Sprague–Dawley rats (litter mates of the F1 generation, divided according to sex into three males and six females and weighing between 55 and 116 g), were placed on a modified paired-feeding regimen. This system was employed in order to determine the effect of massive dosage and the 'bulk' producing effects of the questionably inert substance, in contrast to one (cellulose flour) shown to be inert and to possess only a 'bulk' effect when included in the diet. The nine animals were divided into three groups. Rat 1 in each of the three groups received a diet of equal parts of the nutrient and of MC (50% MC diet) *ad libitum*. Rat 2 received a diet of equal parts of the nutrient and cellulose flour (forming the 'bulk' control) *ad libitum*, while rat 3 was given the nutrient alone (nutritive control). Each animal was housed in an individual metabolism cage, and daily records were kept of the urine volume, faeces weight and food and water consumption. Pooled 7-day urine samples from each rat were subjected to quantitative formic acid determinations. This study was terminated after 90 days of dosing. In dosed rats, growth depression was seen, but a subsequent replacement of MC or cellulose diet by the basal diet resulted in marked weight gain. There was no indication that significant amounts of MC were hydrolysed to cellulose and methanol in the intestinal tract.

In a long-term study, groups of 20 male and 20 female Wistar rats were dosed via diet containing 1 or 5% MC (15, 400 or 4,000 cP) (equivalent to 500 or 2,500 mg/kg bw per day) for 2 years (McCollister et al., 1973). Duplicate control groups consisted of 20 males and 20 females each. Additional groups of 10 rats of each sex for each treatment were set up for necropsy examination at 12 and 18 months. Haematological studies (PCV, Hb and total and differential WBC) were conducted at 12, 18 and 24 months on five male and five female rats from the groups fed the control ration and each of the 5% diets. All rats appeared normal and exhibited no unusual behaviour during the test period. Body weights were comparable between all groups and also feed consumption gave no compound-related differences. Mortality was unaffected by treatment and was associated with a variety of commonly observed spontaneous lesions. Haematological studies and determinations of BUN and AP gave normal findings, with no differences between control and treated rats indicative of a compound-related effect. Final mean body and organ weights of rats necropsied after 12, 18 and 24 months revealed a few random statistically significant differences, none of which was associated with treatment. Gross and histopathological examinations of the tissues showed no compound-related changes, nor any evidence to indicate storage of the test material within the cells of the reticulo-endothelial system of treated rats. Also the observed tumours were similar in type and number in treated and control groups.

For obtaining data on tumour incidence from larger numbers of rats, diets identical to those fed in the above cited study as to sample and concentration were administered to groups of 30 male and 30 female Wistar rats for 2 years (McCollister et al., 1973). A control group of 30 rats of each sex received untreated diets. The rats were examined frequently for evidence of tumour development. Body weight records were maintained. Gross pathological examination was conducted on rats dying during the study or sacrificed in extremis, and sections of grossly visible nodules or masses were preserved for histopathological examination. Gross examination was conducted on all rats surviving at

the end of the 2 years. Weights of liver and kidneys were recorded and grossly evident lesions suggestive of tumour formation were preserved for histopathological examination. Also in this study, no adverse effects were seen concerning mortality, body weights, terminal liver and kidney weights or any increase in tumour incidences.

#### 4.2.4.4. Ethyl cellulose (EC; E 462)

No data available.

#### 4.2.4.5. Hydroxypropyl cellulose (HPC; E 463)

##### Rats

Groups of 10 young adult male and 10 female Wistar rats were administered daily doses of 0, 1,500, 3,000 or 6,000 mg/kg bw of HPC via gavage for 6 months (Kitagawa et al., 1976b). Rats were weighed every day and food consumption was measured twice a week during the study. The dosing caused a decrease in body weights of high-dosed male and female rats for 7–8 weeks (statistically significant for females). For the other investigated parameters (haematology, clinical chemistry and histopathology), no significant adverse findings were reported.

#### 4.2.4.6. Hydroxypropyl methyl cellulose (HPMC; E 464)

##### Rats

In groups of 10 male and 10 female rats (strain not specified) dosed via diet with 0%, 20% or 25% of HPMC (equivalent to 0, 10,000 or 12,500 mg/kg bw per day) over 1 year, some retardation in growth was seen at a level of 20% (more pronounced at 25%). The dosing caused no increase in mortality and no adverse effects concerning urine analysis, haematology and histological examination were reported (Hodge et al., 1950).

In a 2-year study, four groups of 50 male and 50 female rats (strain not specified) were dosed via diet with 0%, 1%, 5% or 20% of HPMC (equivalent to 0, 500, 2,500 or 10,000 mg/kg bw per day) (Hodge et al., 1950). In males dosed with 20%, a slight retardation of body weight gain (about 30 g less compared to the other dose groups) was seen during the first year (weights taken weekly). During the second year (weights taken twice weekly), this trend was continued, although there were considerable fluctuations, especially near study termination. The mortality rates between all groups were comparable (60–84%). Pooled urine samples examined periodically for sugar and protein concentrations gave no changes. Also, a haematological examination (total erythrocytes, haemoglobin and total leucocytes) prior to beginning the study, eight times during the study, and at termination, gave no adverse effects. However, there was small decrease in RBC counts and Hb values in both sexes at a dose level of 20% (equivalent to 10,000 mg/kg bw per day), which was attributed to a nutritional origin. Organ weights recorded at autopsy were comparable between all groups and also a histological examination of stomach, small and large bowel, liver, kidneys, lungs, heart, brain, bladder, spleen, testes and bone marrow gave no indications for an adverse or a carcinogenic effect.

#### 4.2.4.7. Ethyl methyl cellulose (EMC; E 465)

##### Mice

In an unpublished study, groups of 50 male and 50 female mice (strain not specified) were dosed via diet with 0%, 0.1% or 1% (equivalent to 0, 150 or 1,500 mg/kg bw per day) of EMC for 2 years. Apart from slightly decreased body weights in the latter part of the study seen in both sexes dosed with 1%, no adverse effects on survival rate, tumour incidence, blood picture and gross and microscopic appearance of internal organs were reported (ICI, 1966; cited in JECFA, 1990).

##### Rats

In a parallel study, groups of 50 male and 50 female rats (strain not specified) were dosed via diet with 0%, 0.1% or 1% (equivalent to 0, 50 or 500 mg/kg bw per day) of EMC for 2 years. Apart from decreased body weights in the latter part of the study seen in male rats dosed with 1%, no adverse effects on survival rate, tumour incidence, blood picture and gross and microscopic appearance of internal organs were reported (ICI, 1966; cited in JECFA, 1990).



#### 4.2.4.8. Sodium carboxy methyl cellulose (NaCMC; E 466)

##### Mice

Groups of 50 male and 50 female young adult Alderley Park SPF mice were dosed via diet with 0, 10,000 or 100,000 mg/kg diet (equivalent to 0, 1,500 or 15,000 mg/kg bw per day) of sodium CMC for 100 weeks (McElligott and Hurst, 1968). In general, the dosing was well tolerated and caused no increased mortality in dosed mice. During the first 30 weeks, body weights and body weight gain were not affected, while thereafter, a retarded growth was noted in dosed mice. The final body weights at 0, 1,500 or 15,000 mg/kg bw per day were 45, 38 and 39 g for male mice and 39, 34 and 36 g for female mice. At necropsy, there was no histological evidence of changes in the intestinal wall indicating absorption of sodium CMC and also no evidence of storage in the regional lymph nodes or elsewhere. Also the tumour incidences were comparable among all groups.

CMC has been used as vehicle in a bioassay of selenium sulfide for possible carcinogenicity. Groups of 50 B6C3F1 mice of each sex were dosed via gavage with 10 mL/kg bw per day of 0.5% aqueous CMC (50 mg/kg bw per day) on 7 days per week for 103 weeks (NCI, 1980). Similar groups of untreated controls were also used. Observations made on the test animals were recorded twice daily. Examinations of animals for clinical signs and the presence of palpable masses were performed and recorded weekly. Mean body weights were recorded every 2 weeks for the first 12 weeks and then monthly for the remaining 93 weeks, with few exceptions. Animals that were moribund and those that survived to the termination of the study were killed and necropsied. Gross and microscopic examinations were performed on major tissues, major organs and all gross lesions from killed animals and from animals found dead. Compared with untreated controls, for all investigated parameters, no adverse effects were reported.

##### Rats

Ten male and 15 female young Wistar rats were fed a diet containing 5% CMC (equivalent to 2,500 mg/kg bw per day) for 201–250 days (Rowe et al., 1943). All animals were weighed twice weekly and individual weight records were kept throughout the experimental period. All the animals that were killed, as well as most of those that died during the study, were examined for gross pathology and lesions. The dosing had no adverse effects on growth rate, mortality and organ weights. Also, the results of histopathological examination of the liver, kidney, spleen, pancreas, adrenal gland, testis and GI tract showed no significant differences between the treated and the control groups.

Two groups of 50 male and 50 female white rats each were dosed with 500 or 1,000 mg/kg bw per day of CMC via diet for 6 months (Shelanski and Clark, 1948). Forty rats were used as controls. The animals were weighed once monthly; other investigated parameters were fertility (not further specified), urine analysis (sugar, albumin and phosphates as well as microscopic examination for casts and blood cells), haematology at the beginning of the experiments, at the end of 3 months and at the end of 6 months (Hb percentage, leucocyte and erythrocyte counts as well as differential counts) and a histopathological examination in five rats from each dose group, as well as in three rats from the control group each month (liver, kidney, spleen, stomach, intestine and heart). For none of the investigated parameters were adverse effects reported.

CMC has been used as vehicle in a bioassay of selenium sulfide for possible carcinogenicity (NCI, 1980). Groups of 50 F344 rats of each sex were dosed via gavage with 1 mL/kg bw per day 0.5% aqueous CMC (5 mg/kg bw per day) on 7 days per week for 103 weeks. Similar groups of untreated controls were also used. Observations made on the test animals were recorded twice daily. Examinations of animals for clinical signs and the presence of palpable masses were performed and recorded weekly. Mean body weights were recorded every 2 weeks for the first 12 weeks and then monthly for the remaining 93 weeks, with few exceptions. Animals that were moribund and those that survived to the termination of the study were killed and necropsied. Gross and microscopic examinations were performed on major tissues, major organs and all gross lesions from killed animals and from animals found dead. Compared with untreated controls, for all investigated parameters, no adverse effects were reported.

Groups of 50 male and 50 female young adult Alderley Park SPF rats were dosed via diet with 0, 10,000 or 100,000 mg/kg diet (equivalent to 0, 500 or 5,000 mg/kg bw per day) of sodium CMC for 104 weeks (McElligott and Hurst, 1968). In general, the dosing was well tolerated and caused no increased mortality in dosed rats. During the first 30 weeks, body weights and body weight gain was not affected, while thereafter a retarded growth was noted in dosed rats. Although the feed intake was increased in dosed rats, the final body weights were dose dependently decreased (511, 498 and



451 g for male rats and 344, 337 and 287 g for female rats at 0, 10,000 or 100,000 mg/kg diet, respectively). Haematological examinations revealed no adverse effects. At necropsy, there was no histological evidence of changes in the intestinal wall indicating an absorption of sodium CMC and also no evidence of storage in the regional lymph nodes or elsewhere. Also the tumour incidences were comparable between all groups.

Four groups of 25 male and female white rats each were dosed via diet for over 2 years with 0, 100, 500 or 1,000 mg/kg bw per day of sodium CMC (Shelanski and Clark, 1948). The weight curves of the four groups were normal except that the groups receiving sodium CMC showed a slight increase in weight compared with control rats. Monthly urine (albumin, casts and sugar) and haematology examinations (Hb, RBC and WBC counts, differential counts) showed no deviations. Litters produced within all groups were kept in their respective groups and placed on the same diet as the parents. This was carried out into the third generation and these animals also showed no deviation from the animals in the control group. At the end of 25 months, 12 of the animals in each group were autopsied; no adverse findings were observed. Also a histological examination (heart, liver, kidney, stomach, intestine, spleen and adrenals) revealed no adverse findings.

#### Guinea pigs

Two groups of 50 male and 50 female guinea pigs each were dosed with 500 or 1,000 mg/kg bw per day of CMC via diet for 6 months (Shelanski and Clark, 1948). Forty guinea pigs were used as controls. The animals were weighted once monthly. Other investigated parameters were fertility (not further specified), urine analysis (sugar, albumin and phosphates, as well as microscopic examination for casts and blood cells), haematology at the beginning of the experiments, at the end of 3 months and at the end of 6 months (Hb percentage, leucocyte and erythrocyte counts, as well as differential counts) and a histopathological examination in five guinea pigs from each dosage group, as well as in three guinea pigs from the control group each month (liver, kidney, spleen, stomach, intestine and heart). For none of the investigated parameters were adverse effects reported.

In a one-year study, groups of 20 guinea pigs each were dosed with 500 or 1,000 mg/kg bw per day of CMC via diet (Shelanski and Clark, 1948). A control group consisted of 15 guinea pigs. Monthly weight records were kept and showed a similar body weight gain in all groups. All animals were autopsied at the end of the first year and none of the animals showed gross pathological changes. Also sections of liver, kidney, spleen, heart, stomach and intestines prepared from each animal and examined histologically showed no changes.

#### **4.2.4.9. Cross-linked sodium carboxy methyl cellulose (E 468)**

No data available.

#### **4.2.4.10. Enzymatically hydrolysed carboxy methyl cellulose (E 469)**

No data available.

#### **4.2.4.11. Summary**

Overall, in a chronic feeding study, no consistent effects on the body weight were reported even at very high doses of microcrystalline cellulose (30% in the diet) (Documentation provided to EFSA n. 40). Food efficiency, survival and haematology were comparable among all groups. The liver and kidney weights of males receiving microcrystalline cellulose gel were increased. Histopathology showed some dystrophic calcification of renal tubules in females of the high dose group. No further treatment-related changes were observed. Other available studies were less suitable for evaluation of this endpoint due to methodological shortcomings (Lewerenz et al., 1981a,b; Paulini et al., 1987) or limited reported information (Bieri et al., 1977; Nigro et al., 1979; Yartsev et al., 1989; cited in JECFA, 1990; Anastasia et al., 1990; Maurer et al., 1990). The Panel concluded that microcrystalline cellulose has no carcinogenic properties.

Data are available for methyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, ethyl methyl cellulose and sodium carboxy methyl cellulose.

Groups of 20 male and 20 female Wistar rats were dosed via diet containing 1% or 5% (up to 5,000 mg/kg bw per day) methyl cellulose (15, 400 or 4,000 cP) for 2 years. For all examined parameters, no adverse effects were reported and also the observed tumours were similar in type and number in treated and control groups (McCollister et al., 1973). For obtaining data on tumour incidence from a larger number of rats, diets identical to those fed in the above cited study as to sample and concentration were administered to groups of 30 male and 30 female Wistar rats for 2

years. Also in this study, no adverse effects were seen concerning mortality, body weights, terminal liver and kidney weights or any increase in tumour incidences (McCollister et al., 1973).

In groups of 10 young adult male and female Wistar rats dosed daily via gavage with 0, 1,500, 3,000 or 6,000 mg/kg bw of hydroxypropyl cellulose for 6 months, apart from a decrease in body weights in high-dosed male and female rats, no other adverse effects were noted (Kitagawa et al., 1976b).

In an older study, groups of 50 male and 50 female rats (strain not specified) were dosed via diet with 0%, 1%, 5% or 20% of hydroxypropyl methyl cellulose (equivalent to 0, 500, 2,500 or 10,000 mg/kg bw per day). Also in this study, apart from a decrease in body weights of high-dosed males, no other significant adverse findings were reported and there was no indication of a carcinogenic effect (Hodge et al., 1950).

For ethyl methyl cellulose, data are available from two unpublished studies with rats and mice. In both trials, groups of 50 male and 50 female animals (strains not specified) were dosed via diet with 0%, 0.1% or 1% (equivalent to 0, 150 or 1,500 mg/kg bw per day for mice; equivalent to 0, 50 or 500 mg/kg bw per day for rats) over 2 years. Apart from decreased body weights in high-dosed animals, no adverse effects on survival rate, tumour incidence, blood picture and gross and microscopic appearance of internal organs were reported (ICI, 1966; cited in JECFA, 1990).

Sodium carboxy methyl cellulose was tested in two studies with groups of 50 male and 50 female Alderley Park SPF mice and Alderley Park SPF rats, respectively (McElligott and Hurst, 1968). The animals were dosed via diet with 0, 10,000 or 100,000 mg/kg diet (equivalent to 0, 1,500 or 15,000 mg/kg bw per day for mice; equivalent to 0, 500 or 5,000 mg/kg bw per day for rats) for up to 104 weeks. In the study with mice, during the first 30 weeks, body weights and body weight gain were not affected, while thereafter, a retarded growth was noted. The final body weights at 0, 10,000 or 100,000 mg/kg diet group were 45, 38 and 39 g for male mice and 39, 34 and 36 g for female mice. At necropsy, there was no histological evidence of changes in the intestinal wall indicating an absorption of the substance and there was also no evidence of storage in the regional lymph nodes or elsewhere. Also, the tumour incidences were comparable between all groups. In rats, during the first 30 weeks, body weights and body weight gain also were not affected, while thereafter a retarded growth was noted. Although the feed intake was increased in dosed rats, the final body weights were dose dependently decreased (511, 498 and 451 g for male rats and 344, 337 and 287 g for female rats at 0, 10,000 or 100,000 mg/kg diet, respectively). Also in this study, there was no evidence of histological changes in the intestinal wall indicating absorption of sodium carboxy methyl cellulose and also no evidence of storage in the regional lymph nodes or elsewhere. The tumour incidences were comparable among all groups.

#### 4.2.5. Reproductive and developmental toxicity

##### 4.2.5.1. Microcrystalline cellulose (E 460(i))

###### Reproductive toxicity studies

###### Rats

In a report available from secondary source (Documentation provided to EFSA n. 41), groups of eight male and 16 female rats were used to produce P, F1a, F1b, F2 and F3 generations after having been fed on diets containing 30% microcrystalline cellulose flour or gel (equivalent to 15,000 mg/kg bw per day). The control received 30% 'ordinary cellulose' (no further details). The authors stated that no adverse effects on reproduction and no developmental effects were seen. The evaluation of this study is hampered by limited and inconsistent documentation.

An unpublished, combined, one-generation reproduction/chronic toxicity study in rats with microcrystalline cellulose (90% of particles < 20 µm) in the diet at levels of 0 (control), 30, 100 or 200 g/kg diet (equivalent to 0, 1,500, 5,000 or 10,000 mg/kg bw per day) (Lewerenz et al., 1979, 1981a; referred to by SCF, 1999) revealed no effects on reproductive parameters (no details reported). Due to the limited reporting and low number of animals involved, the Panel considered this study inadequate for risk assessment.

###### Developmental toxicity studies

###### Rats

Groups of 25 pregnant Sprague–Dawley rats received Avicel® RCN-15 (a mixture of 85% microcrystalline cellulose with 15% guar gum; median particle size of 21 µm, only 1% of particles < 5 µm) in the diet at dose levels of 0%, 2.5% or 5.0% (equal to 0, 2,091 or 4,490 mg/kg bw per day)

*ad libitum* on gestation day (GD) 6–15 (Freeman, 1992b). Animals were fed basal diet at all other times. The treatment in the high-dose group resulted in a significant increase in food consumption from GD 6 to 15, presumably due to the increased fibre content. On GD 20, a caesarean section was performed. Number and distribution of corpora lutea, implantation sites, early and late resorptions, live and dead fetuses and fetal sex were determined. External, visceral and skeletal examinations of the fetuses were performed. There was no evidence for maternal toxicity or any developmental effects of microcrystalline cellulose. The NOAEL for maternal and developmental toxicity was 4,500 mg/kg bw per day.

In a second study, the same experimental design was used, but pregnant rats were exposed to Avicel® CL-611, a mixture of 85% microcrystalline cellulose and 15% sodium carboxy methyl cellulose (Documentation provided to EFSA n. 33). The mean particle size was 32 µm (1% of particles < 5 µm). The doses were of 0%, 2.5% or 5.0% (equal to 0, 2,207 and 4,584 mg/kg bw per day), respectively. In this study, the food consumption was significantly increased at the 2.5% and 5% level. There was no evidence for maternal toxicity or any developmental effects of microcrystalline cellulose. The NOAEL for maternal and developmental toxicity was 4,600 mg/kg bw per day.

#### 4.2.5.2. Powdered cellulose (E 460(ii))

##### Reproductive toxicity studies

###### Mice and rats

The effects of a synthetic diet (AIN-76™) containing 5% cellulose (no further details) (equivalent to 2,500 mg/kg bw per day) for 6 weeks prior to mating and during pregnancy and lactation were compared with a control group receiving a cereal-based diet containing 4.5% crude fibre (no further details) in rats and mice (strains not specified) (Bieri et al., 1977). In each group, 7–8 pregnant rats were used (no further details). Litter size, birth weight, number of weaned F1 rats and body weight of weaned rats were measured. In a first trial, no differences were found between controls and treated rats. In an independent second experiment, birth weight and body weight at weaning were significantly increased in treated rats. No effects on reproduction and lactation were detected in similar experiments with mice. The reporting and the parameters measured were very limited.

##### Developmental toxicity studies

###### Rats

Four groups of 9–12 pregnant Sprague–Dawley rats were fed at GD 6–15 a mixture of four types of α-cellulose (Elcema®; four types of α-cellulose in the ratio of 1/1/1/1: particle size 1–50 µm (powder), 1–100 µm (powder), 1–150 µm (fibrillar), 90–250 µm (granulate)) at dose levels of 0%, 2.5%, 5% or 10% in the diet (equivalent to 0, 1,250, 2,500 or 5,000 mg/kg bw per day) (Ferch, 1973a,b, 1974). Maternal weight was measured every third day and dams were killed on day 21 of pregnancy for caesarean section. Number, sex and weight of fetuses, corpora lutea, early and late resorption, litter size and average backbone length were determined. Fetuses were examined for external malformations, soft tissue or skeletal defects. Additionally, two groups of pregnant rats received at GD 6–15 the basal diet (n = 12) or 10% cellulose in the diet (n = 10) and dams were allowed to deliver the pups, which were maintained to weaning (day 21 postnatal). In these additional groups, the following parameters were measured: duration of pregnancy, number of pups born and surviving postnatal period, and weight of pups at days 7 and 21; pups were killed at day 21 postnatal and histopathology was performed. Presumably basal diet was fed at all other times in both trials. No statistical analysis was performed. The treatment did not induce effects on maternal weight gain. There were no altered parameters after the caesarean section in comparison to the concurrent control, except for a slight, but not dose dependent, increase in early resorptions (limited reporting prevents the evaluation of this effect). However, the authors stated that no effects on early resorptions were noted in comparison to historical controls. The treatment with 10% cellulose did not affect postnatal development. The Panel noted that the test substance was not compliant with the specifications of the food additive.

#### 4.2.5.3. Methyl cellulose (MC; E 461)

##### Reproductive toxicity studies

No studies available.

## Developmental toxicity studies

### Mice

Groups of 20–22 pregnant albino CD-1 outbred mice were dosed once daily via gavage with 0, 16, 74, 345 or 1,600 mg/kg bw per day of a MC (FDA 71-51) suspension in corn oil (dose volume 10 mL/kg bw) from GD 6 to 15. The control group received daily doses of corn oil. A caesarean section was performed on GD 17. In dams dosed with 1,600 mg/kg bw per day, a significant increase in mortality was observed, with a reduced rate of pregnancy in survivors. At term, resorption sites were markedly increased in number, live fetuses were significantly reduced in number and fetal weight decreased. At external and visceral examination of the fetuses, no dose-related abnormalities were observed. At fetal skeletal examination, the ossification of the fetuses was retarded in the 1,600 mg/kg bw group. In fetuses, there was no evidence for developmental effects at any dose level (FDLI, 1973; cited in JECFA, 1990).

Groups of 12–17 pregnant mice (CD/1 strain) were dosed once daily via gavage with 0, 70, 153, 330 or 700 mg/kg bw per day of a MC (FDA 71-51) suspension in corn oil (dose volume 5.8, 12.7, 27.5 or 58.3 mL/kg bw) from GD 6 to 15. The control group received corn oil at the same dose as the highest dose. A caesarean section was performed on GD 17. There were no dose-related effects on growth, mortality or incidences of gross lesions in dams and in the number of implantations, resorptions, live and dead fetuses and fetal weight. No increase in incidences of external, visceral and skeletal abnormalities, reduced weight, or mortality was observed in fetuses from treated dams (Cannon Labs, 1975; cited in JECFA, 1990).

### Rats

Groups of 20–25 pregnant rats (not further specified) were dosed once daily via gavage with 0, 13, 51, 285 or 1,320 mg/kg bw per day of a MC suspension in corn oil (dose volume 6, 1, 1, 2 or 6 mL/kg bw) from GD 6 to 15. The control group received daily doses of corn oil. A Caesarean section was performed on GD 20. In dams dosed with 1,320 mg/kg bw per day, a reduced pregnancy rate was observed. The dosing gave no adverse effects on growth, mortality or incidence of gross lesions in dams. Also, the incidences of implantations, live or dead fetuses and resorptions were not affected. Apart from an increased incidence of extra centres of ossification in vertebrae of fetuses from high dose dams, no increase in incidences of external, visceral and skeletal abnormalities was observed in fetuses. Also, fetal weights were not affected (FDLI, 1973).

Groups of 13–19 pregnant Sprague–Dawley rats were dosed once daily via gavage with 0, 120, 260, 550 or 1,200 mg/kg bw per day of a MC suspension in corn oil (dose volume 12, 1.2, 2.6, 5.6 or 12 mL/kg bw) from GD 6 to 15. The control group received daily doses of corn oil. A Caesarean section was performed on GD 20. There were no dose-related effects on growth, mortality or incidence of gross lesions in dams. The incidences of implantations, live fetuses, corpora lutea, dead fetuses and resorptions in treated dams were within the normal range. Apart from a slight increased incidence of extra centres of ossification in vertebrae of fetuses from the high-dose group, no increase in incidences of external, visceral and skeletal abnormalities was observed in fetuses. Also, fetal weights were not affected (Cannon Labs, 1977).

### Hamsters

Groups of 22–24 Golden hamsters were dosed once daily via gavage with 0, 46, 216 or 1,000 mg/kg bw per day of a MC suspension in corn oil (dose volume 4, 1, 1 or 4 mL/kg bw) from GD 6 to 10. The control group received daily doses of corn oil (negative control). A caesarean section was performed on GD 14. There were no dose-related effects on growth, mortality or incidences of gross lesions in dams. Also, the incidences of implantations, live and dead fetuses and resorptions in dams were within the normal range. In fetuses, there were no increased incidences of external, visceral and skeletal abnormalities and also fetal weights were not affected (FDLI, 1973).

### Rabbits

Groups of 10–17 Dutch belted rabbits were dosed once daily via gavage with 0, 7, 32, 148 or 685 mg/kg bw per day of a MC suspension in corn oil (dose volume 3, 1, 1, 1 or 3 mL/kg bw) from GD 6 to 18. The control group received daily doses of corn oil (negative control). A Caesarean section was performed on GD 29. Dams of the highest dose group showed an increased mortality and a decrease in pregnancy rate in survivors, but no dose-related effects on growth or incidence of gross lesions. The incidences of corpora lutea, implantations, live and dead fetuses and resorptions in dams were within



the normal range. In fetuses, there were no increased incidences of external, visceral and skeletal abnormalities and also fetal weights were not affected (FDLI, 1973). Due to the high mortality in the high-dose group, the Panel considered this study not relevant for risk assessment.

#### **4.2.5.4. Ethyl cellulose (EC; E 462)**

##### **Reproductive and developmental toxicity studies**

No studies available.

#### **4.2.5.5. Hydroxypropyl cellulose (HPC; E 463)**

##### **Reproductive toxicity studies**

No studies available.

##### **Developmental toxicity studies**

###### Rats

Pregnant Wistar rats (JCL) were dosed via gavage from GD 7 to 17 with 0, 200, 1,000 or 5,000 mg/kg bw hydroxypropyl cellulose of low substitution (L-HPC) (Kitagawa et al., 1978a). Since L-HPC is insoluble in water, 8 g were suspended in 100 mL of 1% gum arabic solution. The amounts of suspension administered were 2.5, 12.5 or 62.5 mL/kg bw in the low-, mid- and high-dose group. The control group received 62.5 mL/kg bw of 1% gum arabic solution. The group sizes were 21–24 animals for caesarean section and 12–15 animals for spontaneous delivery. During the gestation period, general symptoms, mortality and abortion rates of dams were examined. Body weight, and food and water intake were recorded every 3 days. On GD 21, 21–24 pregnant females in each group were necropsied by caesarean section. The number of corpora lutea, implantations, viable and dead fetuses, and resorbed embryos were counted, and positions of implantations were observed. Body weight of all viable fetuses were weighed individually and examined for external abnormalities and gender. Two to three of the viable fetuses per litter (random) were stained with alizarin Red S and examined for skeletal anomalies and development. The other fetuses were examined for visceral abnormalities. In the control, low-, mid- and high-dose groups, litter weight (36.6, 34.4, 33.8 and 28.4 g) and the percentage of pre-implantation loss (14.9%, 11.1%, 14.75 and 24.4%) and post-implantation loss (6.2%, 6.5%, 10.2%, and 13.5%) were significantly increased in the high-dose group. The number of live fetuses in the control, low-, mid- and high-dose groups was 9.0, 9.0, 8.8 and 7.5. The authors considered the effect in the high-dose group not as treatment-related, as this effect (pre-implantation loss) occurred before administration of the test substance. Furthermore, no decrease in the number of liveborn pups was observed in the dams which were allowed to litter. Furthermore, these effects were not observed in the litters of dams which were allowed to deliver. The Panel agreed with the authors.

Twelve to 15 dams in each group of the study described above were allowed to deliver spontaneously (Kitagawa et al., 1978a). Condition of each delivery was observed and the number of viable and stillborn pups was counted. Body weight, sex and external anomalies were examined. Litters were not adjusted. During the lactation period, general behaviour of the dam and newborn were observed, and at the third day after birth and once a week thereafter, food consumption and body weight gain of pups were recorded. The body weight of each newborn was weighed individually at the time of birth and weaning. Otherwise, the body weight was recorded as 'litter weight' by weighing a whole litter of each dam. Incisors eruption and eye opening were recorded. Pups were weaned at the 28th day of birth and dams were dissected. On the 35th day, each weanling rat was examined for the general behaviour and nervous functions, including anomalies in general behaviour, righting reflex, corneal reflex, pinna reflex, body posture, traction and responsiveness to sounds. All of the weanling rats from each litter were examined for skeletal anomalies taking soft X-ray pictures, except for two males and two females randomly selected. Each was then dissected for the examination of visceral malformations and positioning. One male and one female from each litter were sacrificed and wet weights of brain, heart, lung, liver, spleen, kidney, thymus, adrenal, testis epididymis, prostate gland, ovary, pituitary gland and thyroid gland were weighed. The weaning ratio was calculated conventionally from the data obtained at 3 weeks. Offspring (F1) grown over 5 weeks were examined for weight gains. After maturity, conditioned avoidance test and reproductive ability were examined. For the reproductive ability, F1 offspring (42 from the 200 mg/kg bw group, 56 from the 1,000 mg/kg bw group and 52 from the 5,000 mg/kg bw group) were used. Each male was examined for descent



of testis at an age of 4 weeks, and each female was examined for opening of vagina at the age of 5 weeks. At weeks 10–11 (about 1 week prior to mating), the oestrus cycle of each female was examined, and in week 11–12, each male and female among the same group were housed together and observed for mating. The mating period was 15 days and during this period, oestrus cycle of each female was examined every day until mating was confirmed. Each male and female was separated after the confirmation of mating and the body weight of each female was recorded daily until GD 21. On GD 21, a caesarean section was performed in each female, and implantations, corpora lutea, viable or non-viable fetuses and the weight of each fetus were recorded, as well as external anomalies of each fetus. In this part of the study, no effects on the number of pups, pup survival, growth and physical development and reproduction (parameters) were observed. The authors considered *in utero* exposure to the highest dose of 5,000 mg/kg bw per day as the NOAEL for this study and the Panel agreed with this conclusion.

## Developmental toxicity studies

### Rabbits

In a study with Himalayan rabbits, groups of 11–12 pregnant animals were dosed daily via gavage with 0, 200, 1,000 or 5,000 mg/kg bw of HPC during GD 6–18 (Kitagawa et al., 1978b). The test substance was suspended in 1% arabic gum solution. The dose volume in the control, low-, mid- and high-dose group was 50, 10, 10, or 50 mL/kg bw. Caesarean sections were performed on GD 29 and all fetuses were examined for skeletal and organ malformations. In high-dose dams, a slight body weight loss was seen up to GD 18. In the mid-dose group, the resorption rate was significantly increased (3.4%, 6.1%, 27.4% and 7.4%). The mean number of viable fetuses in the control, low-, mid-, and high-dose group was 7.0, 5.5, 4.8 and 5.7 (historical control 6.37). Mean fetal viable weights were comparable between the different groups. In high-dose dams, the pre-implantation loss was significantly increased (13.6%, 22.4%, 17.0% and 29.2%). The treatment gave no indications for an increased incidence of malformations. The Panel noted that from this study no NOAEL can be derived, as the number of fetuses was low in all dose groups.

#### 4.2.5.6. Hydroxypropyl methyl cellulose (HPMC; E 464)

No studies available.

#### 4.2.5.7. Ethyl methyl cellulose (EMC; E 465)

### Reproductive and developmental toxicity studies

No studies available.

#### 4.2.5.8. Sodium carboxy methyl cellulose (NaCMC; E 466)

### Reproductive toxicity studies

### Rats

In a study with male and female Sprague–Dawley-derived rats, 20 male and 40 female rats were dosed via gavage with 0 or 200 mg/kg bw per day of CMC (aqueous solution, dose volume 10 mL/kg bw) for at least 60 days (males) or for least 14 days (females) prior to mating, and also during the 6-day mating period (Fritz and Becker, 1981). One half of the females were dosed until sacrifice on GD 14, while the other half was dosed until weaning of the progeny (day 28 after birth). The treatment caused no adverse reactions in the parents and also average body weights were comparable throughout the study. In dams, no adverse effects concerning mating efficiency, pregnancy rate, mean numbers of corpora lutea and implantation sites, ratios of corpora lutea to implantation sites or resorption rates were noted. For the offspring, there were also no significant changes in litter sizes, sex ratios, body weight gains, nesting behaviour, eye opening and pinna detachment. In addition, behavioural testing (e.g. righting and direct pupillary reflex) gave no adverse effects. Data were not described very detailed in the publication. The only dose tested (200 mg/kg bw per day) did not induce effects with regards to parental toxicity, reproduction and development and behaviour of the offspring.

## Developmental toxicity studies

### Mice

In a study with pregnant albino CD-1 outbred mice, groups of 19–24 animals were dosed via gavage with 0, 16, 74, 345 or 1,600 mg/kg bw per day of sodium CMC in corn oil (dose volume 10

mL/kg bw per day) from GD 15. The treatment caused no mortality, and no adverse effects were observed on implantation or on fetal survival. There was also no increase in abnormalities of soft or skeletal tissues compared with non-dosed controls (FDLI, 1975).

#### Rats

In another study with pregnant Wistar-derived rats, groups of 19–22 animals were dosed via gavage with 0, 16, 74, 345 or 1,600 mg/kg bw per day of sodium CMC in corn oil (dose volume 5, 1, 1, 2 or 5 mL/kg bw) from GD 6 to 15. The treatment caused no mortality, and no adverse effects were observed on implantation or on fetal survival. There was also no increase in abnormalities of soft or skeletal tissues compared with non-dosed controls (FDLI, 1975).

#### **4.2.5.9. Cross-linked sodium carboxy methyl cellulose (E 468)**

##### **Developmental studies**

#### Rats

Cross-linked sodium carboxy methyl cellulose was administered to pregnant Sprague–Dawley CD rats (25 females; age not stated) at 0, 10,000 or 50,000 mg/kg diet *ad libitum* from GD 6 to 15 (equal to 0, 910 or 4,554 mg/kg bw per day) (Freeman et al., 2003). During GD 0–6 and GD 15–20, the animals received an untreated basal diet, which was also given to the control animals for the duration of the experiment. The animals were observed daily with detailed assessment of test material-related effects. Body weight and food consumption were recorded. Clinical examinations included recording the onset and duration of any toxicological effects. On GD 20, the animals were terminated, and all dams examined for gross lesions, implantation sites, late resorptions and status of fetuses and corpora lutea. The fetuses were examined for external alterations, after which they were euthanised, and visceral and skeletal examinations carried out to detect abnormalities. No treatment-related clinical signs or deaths were reported in the study. There were also no effects on body weight, uterine weights or food consumption in dams compared to controls. No effects were noted in dams during necropsy, and there were no external, skeletal or visceral malformations in the fetuses. The study authors concluded that there were no treatment-related statistically significant findings in the developmental study. The Panel agreed with this conclusion and considered 4,554 mg/kg bw per day as the NOEL for this study.

#### **4.2.5.10. Enzymatically hydrolysed carboxy methyl cellulose (E 469)**

No data available.

#### **4.2.5.11. Summary**

Data from dietary reproductive studies were available for microcrystalline cellulose, powdered cellulose and sodium carboxy methyl cellulose, (Documentation provided to EFSA n. 41, Lewerenz et al., 1979; referred to by SCF, 1999; Bieri et al., 1977; Fritz and Becker, 1981).

Several prenatal developmental studies with oral dosing via diet of microcrystalline cellulose, powdered cellulose and via oral gavage of methyl cellulose, hydroxypropyl cellulose, sodium carboxy methyl cellulose and cross-linked sodium carboxy methyl cellulose have been performed in mice, rats, hamsters and/or rabbits (FDLI, 1973, 1975; Cannon Labs, 1975, 1977; Kitagawa et al., 1978a,b; Fritz and Becker, 1981; Freeman, 1992b).

From these studies, it can be concluded that adverse effects on reproduction and development were unlikely.

- The reproductive toxicity studies with **microcrystalline cellulose** and **powdered cellulose** did not report adverse effects up to 15,000 and 7,500 mg/kg bw per day, respectively. However, the Panel noted that the studies were limited in design and reporting. In prenatal developmental toxicity studies in rats, doses up to 10% powdered cellulose in the diet (equivalent to 5,000 mg/kg bw per day) did not induce maternal or developmental toxicity and did not affect postnatal development (FDLI, 1973; Ferch, 1973a,b, 1974; Cannon Labs, 1975; Freeman, 1992b).
- From prenatal developmental toxicity studies by gavage in mice, rats and golden hamsters, the Panel derived a NOEL of 700, 550 and 1,000 mg **methyl cellulose**/kg bw per day, respectively (vehicle: corn oil, daily dosing from GD 6 to 15) (FDLI, 1973; Cannon Labs, 1977).

- In rats, no treatment-related developmental effects were observed at 5,000 mg **hydroxy propyl cellulose**/kg bw per day, the highest dose tested (Kitagawa et al., 1978a). Himalayan rabbits dosed daily by gavage with 0, 200, 1,000 or 5,000 mg **hydroxypropyl cellulose**/kg bw per day (Kitagawa et al., 1978a) showed a decreased number of viable fetuses in all treated groups. Therefore, the Panel could not derive a NOAEL from this study.
- Besides the reproductive study with a single dose, in which no reproductive effects were observed, a prenatal developmental toxicity study in mice and in rats was performed with **sodium carboxy methyl cellulose**. In mice and rats, no effects on maternal or developmental toxicity were observed after daily dosing up to 1,600 mg **sodium carboxy methyl cellulose**/kg bw per day by gavage (vehicle: corn oil, daily dosing from GD 6 to 15) (FDLI, 1975).
- **Cross-linked sodium carboxy methyl cellulose**, when fed in the diet in a prenatal developmental toxicity study at doses equal to 910 or 4,554 mg/kg bw per day from GD 6 to 15 to rats, gave no effects on maternal or developmental toxicity (Freeman et al., 2003). The NOAEL of this study was 4,554 mg/kg bw per day.

#### 4.2.6. Hypersensitivity, allergenicity and food intolerance

In the JECFA evaluation (1998), the Committee stated that microcrystalline cellulose had no skin sensitising properties in guinea pigs (Freeman, 1991; referred to by JECFA, 1998a,b, 1999a,b) (Freeman, 1996b; referred to by JECFA, 1998a,b, 1999a,b).

In human subjects suffering from seasonal allergic rhinitis, inhalation of inert cellulose powder or oxidised cellulose has been reported to improve hay fever symptoms (Josling and Steadman, 2003; Shani et al., 2011). However, this was not confirmed in other studies where the authors concluded that microcrystalline cellulose did not prove to be significantly better than placebo in treating seasonal allergic rhinitis symptoms (Paz Lanberg et al., 2016).

Anaphylactic reactions to carboxy methyl cellulose have been rarely reported (Dumond et al., 2009) and this was usually when the cellulose was injected. Two patients who presented that reaction, did not react to orally administered CMC (Rival-Tringali et al., 2008).

Overall, the Panel considered that there is no indication for an hypersensitivity potential for celluloses (E 460–466, E 468–469) used as food additives.

#### 4.2.7. Other studies

##### 4.2.7.1. Human studies

###### Microcrystalline cellulose

In patients given 30 g microcrystalline cellulose per day as dry flour (one male) or gel (one female) for 5 weeks (added to the free-choice diet), no significant effects in the GI tract were noted during the administration period (Tusing, 1964).

Microcrystalline cellulose was used in the treatment of constipation in obese patients. After recording control values for body weight, body mass index, total and fractionated cholesterol, triglycerides, A and B lipoproteins, uric acid, glucose and glycosylated haemoglobin, a group of 30 subjects received for 4 weeks 2.4 g microcrystalline cellulose in tablet form in the morning and 3.6 g in the evening (Adamii et al., 1998). In a further trial using the same experimental design, 10 obese subjects were treated for 8 weeks and additionally blood counts and plasma iron levels were determined. Each subject stated in a questionnaire data about evacuations and clinical symptoms. No adverse effects were reported. Improved defecation was observed in 83% of the patients in the first trial and in 9 out of 10 subjects in the second trial. No changes were found in controlled parameters.

In an unpublished clinical study (data from secondary source), eight male and eight female volunteers supplemented for 6 weeks their diet with 30 g microcrystalline cellulose per day as either dry powder or gel (15% aqueous) (Documentation provided to EFSA n. 39. The treatment period followed 2 weeks without supplementation. No adverse findings were reported on body weight. Most subjects complained of fullness and mild constipation. Haematology was normal in all subjects and clinical chemistry showed no differences between treatment and control periods. There was no evidence for effects on liver or kidney function; urinalysis produced normal findings. Analysis of faeces revealed no effects on faecal flora but the amount of cellulose in faeces increased 5–8 times during the test period. Microscopy revealed the presence of microcrystalline cellulose in faeces.

The effects of microcrystalline cellulose were compared in a double-blind, cross-over trial in 20 poorly controlled Type 2 diabetic patients (initially 16 women and six men; ages ranged from 40 to 76 years, mean 63 years) (Niemi et al., 1988). There were 12-week control and treatment periods separated by a 4-week wash-out period. Cellulose was given at 15 g/day for a 2-week period and then at 5 g/day for the remaining 10-week period. Parameters in the control and treatment period were measured after 6 and 12 weeks. Questionnaire survey was performed by physicians. Four patients reported mild flatulence or loose stools during the treatment. There was no significant change in body weight and blood pressure. Serum zinc, ferritin or urinary magnesium excretion were not altered during the treatment period. There was no effect on fasting blood glucose level, glycosylated haemoglobin, serum high density lipoprotein cholesterol and serum triglycerides.

No change was noted in blood chemistry parameters in eight healthy male volunteers after daily ingestion of 30 g microcrystalline cellulose as supplement to their diet for 15 days (limited information, data from secondary source) (Asahi Chemical Industry Co., 1966; referred to by JECFA, 1998a,b, 1999a,b). Furthermore, faecal flora did not show any alterations. The absorption of  $I^{131}$ -triolein was unaffected, and examination of urine, blood and faecal levels of vitamin B1 during treatment showed no difference from control periods. D-xylose absorption was lower during microcrystalline cellulose ingestion.

In 11 healthy female volunteers (19–22 years old), the post-prandial serum vitamin A concentration was measured 3, 5, 7 and 9 h after oral administration of 300,000 IU vitamin A-palmitate given with a formula diet, to which 0 or 40 g microcrystalline cellulose (no further details) were added (Kasper et al., 1979). The areas under the serum vitamin A concentration curves were significantly increased, suggesting an increased amount of vitamin A being absorbed.

A group of 12 female volunteers (non-pregnant, not lactating; no further data) received 5 g microcrystalline cellulose in 200 mL water containing 15 g sucrose and 3 mg radiolabelled Fe as iron sulfate. Controls (n = 12) received the same amount of Fe in a jelly containing 5 g pectin. Microcrystalline cellulose did not appear to inhibit the uptake of iron (Gillooly et al., 1984).

Three men received 10 g of cellulose in addition to a low-fibre diet for 20-day (Ismail-Beigi et al., 1977). The faecal excretion of zinc and calcium was increased. The magnesium balance became negative in two subjects and the phosphorus balance negative in one subject. When cellulose was added to a fibre-rich diet, faecal excretion of calcium and zinc increased in two subjects and magnesium in one.

#### *α-Cellulose and unspecified celluloses*

Bradlow et al. (1994) tested  $\alpha$ -cellulose in a clinical study. Using a randomised clinical trial, groups of 20 female volunteers received daily for 3 months 20 g packets of  $\alpha$ -cellulose (women aged 27–48 years, mean body weight 59 kg) or a placebo (aged 18–53 years, mean body weight 57 kg). The packets were mixed with fruit juice. Urine and blood samples were collected at the end of each month. Haemoglobin, platelets, several clinical chemistry and endocrinological parameters were determined. The urinary 2-OH-oestrone:oestriol oestrogen metabolite ratio was measured monthly at the same time of the menstrual cycle. In most subjects, the cellulose was well tolerated; a few subjects dropped out of treatment group because they found the cellulose suspension very unpleasant to consume (no details about final group number). No other unpleasant symptoms or problems were reported in the questionnaire. No differences between control and treatment group were observed in any parameter examined.

A cross-over design study (two periods of 4 weeks) in a group of 10 healthy volunteers (six women and four men; mean age 24 years) was undertaken to determine the effects of daily dietary supplementation with 15 g/day  $\alpha$ -cellulose fibre (99.5% pure; derived from wood; Sigma Chemical Comp.) (Hillman et al., 1985) on serum lipid levels. There was no significant change in dietary intakes except for the fibre supplement. Each subject acted as his/her own control. At the end of both the control and test periods, fasting blood sample were taken. Cellulose did not alter serum total cholesterol, triglycerides, high-density lipoprotein cholesterol, or the ratio of high-density lipoprotein to total cholesterol.

Seven healthy women consumed a low-fibre diet of constant composition and the same metabolically controlled diet, to which 16 g of refined cellulose (Solca Floc, 85% wood  $\alpha$ -cellulose and 15% non-glucose hemicellulose) was added for 30 days (Slavin and Marlett, 1980). After cellulose consumption, the mean daily wet stool weight, main dry stool weight and the frequency of defecation were increased. Cellulose shortened the transit time. Faecal excretion of calcium and magnesium were increased after cellulose consumption.



The effects of 21 g pure cellulose added to a low-fibre diet were tested in nine non-anaemic adolescent girls (age 16–18 years, average weight and height 48 kg and 155 cm) (Godara et al., 1981). The girls consumed first a low-fibre diet for 21 days and thereafter the high-fibre diet. The cellulose intake increased the faecal excretion of calcium, phosphorus and the serum levels of calcium, inorganic phosphorus and iron were decreased.

King et al. (1982) reported no effects on daily excretion of zinc, copper, calcium and magnesium after feeding cellulose (0.5 g/kg bw per day) to 5 young men for 9 days in an egg white protein formula. Faecal iron was increased in the cellulose group. Another group (n = 5) was fed for 15 days egg white protein formula to which cellulose (0.5 g/kg bw per day) or phytate (3 g/day) was added. The phytate diet did not increase the faecal dry solids but the faecal excretion of calcium, magnesium and zinc in this group was increased. Serum calcium, magnesium and zinc did not change with the phytate feeding. According to the authors, the alteration in absorption of calcium, magnesium and zinc is due to phytate and not to the fibre components.

After intake of 15 g cellulose/day for 4 weeks by 5 males and 5 females (age 21–26 year), the effects on stool pH, transit time and weight were measured (Hillman et al., 1983). In females, nausea, retching (n = 1) and cramps and urgency (n = 1) were reported. The stool pH was decreased, the stool transit time decreased and the stool weight increased.

The effects of 14 g/day of purified cellulose (99% cellulose on a dry basis) for 24 days was studied in 13 healthy male and female adults (age 23–60 years) (Spiller et al., 1980). The subjects were chosen on the basis of slow intestinal transit time and low faecal output when consuming their normal diets. Faecal weight increased and the transit time decreased. During the study, the faecal volatile fatty acids concentration did not change.

#### Methyl cellulose (MC; E 461)

In three healthy adults, 5 g of methyl cellulose (4,000 cP) given twice daily over 8 days both increased the number of stools per day and also the volume of the stools about twofold (Tainter, 1943).

In an older study, it was reported that the intake of 2.5–5.25 g of MC taken orally as gel was mildly constipating (Bauer, 1945).

In 29 and in 8 patients suffering from acute and/or chronic constipation, the daily uptake of 1–3 g of MC over 3–180 days or the daily uptake of 6 g over 4–240 days caused a significant relief and was well tolerated without toxicity (Schweig, 1948).

In six patients suffering from irritable bowel associated with diarrhoea or constipation, the oral uptake of 2 g of MC every 4 h resulted in abdominal comfort and the reduction of number of stools (Bargen, 1949).

In two female patients with an age of 31 and 35 years, after an oral uptake of 60–90 mL of a short-chain MC preparation daily over 5 days, symptoms such as generalised oedema, mental cloudiness, and poor coordination of some skeletal muscle actions were observed, which disappeared within 72 h of cessation of intake. During administration, sodium and water retention, increase in serum osmolality and an up to 75% decrease in urinary aldosterone excretion were seen (Crane et al., 1969).

In an unpublished study, five adult male volunteers were given daily doses of 250 mg/kg bw of MC, divided into three equal portions, over a period of 23 consecutive days. The treatment was well tolerated and gave no allergic responses or alteration in normal elimination patterns. MC administered as a prehydrated gel caused increased faecal weights (wet and dry basis), but had variable effects on intestinal transit time, causing increased transit time in three subjects and decreased transit time in the other two. Haematology, serum biochemistry and urinalysis parameters were within normal limits. Small, but significant, reductions were observed in faecal volatile fatty acids and neutral sterols, but breath hydrogen levels were not affected (Eastwood et al., 1988, 1990).

In a study with 50 healthy adults (44 women and six men with an age of 18–70 years), the subjects were dosed with 19 g of a placebo during the first week (Hamilton et al., 1988). Then each of the subjects was randomised to 1 of 3 arms: a second week of a daily dose of 19 g of placebo, or 19 g of the bulk laxative containing 2 g MC, or 38 g of the laxative containing 4 g of MC. Nine subjects completed the protocol on placebo, 20 subjects on 2 g MC and 21 on 4 g MC. At the end of the study, stools were analysed for faecal weight, faecal solids, water content and percentage of water. In a second phase of the study, during the first week of the protocol, all subjects (135 women and 14 men with an age of 18–70 years) took the 19 g of placebo. The second part of this protocol was a 10-day treatment period during which the subjects were randomised into one of four daily dosage groups: the study bulk laxative containing 1, 2 or 4 g MC in a total weight of 9.5, 19 or 38 g, respectively, or a similar-appearing preparation, 11 g in weight, containing 3.4 g of psyllium as positive control. Only 59



subjects (56 women, three men) entered into the second part of phase 2 (15 on 3.4 g psyllium, 15 on 1 g MC, 15 on 2 g MC and 14 on 4 g MC). The age range of this group was 19–59 years. As in phase 1, stools were analysed for total weight, faecal solids, total water content and percentage water. MC in daily doses of 4 g caused a statistically significant increase in faecal frequency, faecal water and faecal solids. The results of the second phase showed that the dosing with MC statistically significantly increased stool frequency, water content and faecal solids.

#### Ethyl cellulose (EC; E 462)

No data available.

#### Hydroxypropyl cellulose (HPC; E 463)

In a clinical trial, patients (aged 18 years or more) with chronic watery diarrhoea, presumably secondary to idiopathic bile acid malabsorption, were randomly assigned to two groups given either colestyramine (n = 13, 7 females/6 males) or HPC (n = 13, 11 females/2 males) (Fernández-Bañares et al., 2015). Both substances were given in the form of 4 g sachets twice daily for 8 weeks. The Panel noted that the two adverse effects (muscle pain, nasopharyngitis) observed in the HPC group seem not to be associated with the intake of HPC, thus HPC is considered to be well tolerated in doses of two or three 4 g sachets per day.

#### Hydroxypropyl methyl cellulose (HPMC; E 464)

In a study with 25 young and healthy adults (23 males and two females), each person was given three graduated doses of HPMC ranging from 0.6 to 8.9 g. The time interval between the doses was at least 1 week. Following each dose, stool specimens were collected at approximately 24-h intervals for 72 or 96 h. A mild laxative effect was noted in 11 cases and a mild constipating effect in 16 cases (Knight et al., 1952).

#### Ethyl methyl cellulose (EMC; E 465)

No data available.

#### Sodium carboxy methyl cellulose (CMC; E 466)

The daily dosing of five male volunteers (age: 24–58 years; body weights: 73–84 kg) with 3 × 5 g of sodium CMC over 23 days was well tolerated. Also routinely examined parameters such as clinical chemistry, haematology, urine analysis, glucose tolerance, serum cholesterol, triglyceride and phospholipids, and breath hydrogen and methane concentrations were not adversely affected. Other effects such as shortening of intestinal transit times, increased faecal weights or changes in faecal bile acids or faecal fat were attributed to an increased cellulose intake without any toxicological significance (Anderson et al., 1986).

In 11 male and female patients with an age of 27–82 years, daily doses of 10 g of sodium carboxy methyl cellulose over 6 months were well tolerated. A haematological examination (RBC and WBC counts, differential smears of WBCs and haematocrit) showed no significant variations. No patients experienced anaemia or a leukopenia and bone marrow studies of three patients during the early part of the test period, as compared to similar studies at the end of 6 months, revealed no changes (Brick, 1952).

In a study with 12 men with an age of 26–62 years and body weights ranging from 77.5 to 111.5 kg, one basal diet containing relatively low-fibre foods was given to the subjects throughout the 20 weeks of the study (Behall et al., 1984). Four fibre sources, locust bean gum, karaya gum, CMC and cellulose were used as the fibre supplements. The four fibre sources were added singly to the basal diet for 4 weeks at the level of 0.75 g fibre/100 cal. The basal diet alone and with the four fibre sources was given in a randomised rotation pattern during the five 4-week dietary periods so that each diet was followed by each of the other diets an equal number of times. The basal diet provided approximately 50.4% of the calories from carbohydrates, 35% from fat and 14.6% from protein. The diet had a P/S ratio of 0.39, and contained 640 mg cholesterol, 27 g crude fibre, and 6.33 g neutral detergent fibre per 2,550 kcal. Caloric intake of the men ranged from 2,550 to 3,600 kcal/day and 19.1 to 27.0 g/day added fibre source, respectively, when given. At the end of each 4-week dietary period, two fasting blood samples were drawn, one for serum analysis and one in ethylenediaminetetraacetic acid (EDTA) to be used for analysis of plasma lipoprotein cholesterol for high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL). Fasting serum samples were analysed for cholesterol, triglycerides and free fatty acids (FFA). At the end of the 4 weeks, total serum cholesterol had dropped significantly (from 196 to 164 mg/dL) and

plasma LDL decreased significantly (from 131 to 107 mg/dL). No statistically significant changes were noted for triglycerides, FFA, VLDL, or HDL levels. The ratio of HDL/VLDL+LDL cholesterol was significantly higher than the ratios after consumption of the basal diet alone (0.33 versus 0.28).

#### Cross-linked sodium carboxy methyl cellulose (E 468)

No data available.

#### Enzymatically hydrolysed carboxy methyl cellulose (E 469)

No data available.

#### Summary

Overall, there is no evidence that microcrystalline cellulose or other unmodified celluloses in repeated doses up to 35 g/person adversely affect clinical chemistry and haematological parameters, as well as the absorption and/or the metabolism of dietary constituents.

Several celluloses have been used in patients suffering from diarrhoea or constipation. In general, it can be concluded that an oral uptake of up to 5,000 mg/person per day is well tolerated. Even a daily uptake of 6,000 mg over 4–240 days was well tolerated.

#### **4.2.7.2. Case reports**

Cases of oesophageal and GI obstruction have been reported in the literature following intake of methyl cellulose (Belmont, 1952; Hedayaty and Shuman, 1953; Friedman and Alessi, 1954; FDA, 2015), carboxy methyl cellulose (Leger et al., 1952; Monod-Broca, 1952; Soullard et al., 1952; Derobert and Heully, 1956; FDA, 2015) and cellulose fibre diet pills (Jones and Pillsbury, 1990).

## **5. Discussion**

The present opinion deals with the re-evaluation of the safety of microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) as food additives. These celluloses are authorised as food additives in accordance with Annex II and Annex III of Regulation (EC) No 1333/2008.

Cellulose is a linear glucose homopolymer consisting of glucopyranose units linked by  $\beta$ -1,4-glycosidic bonds; its molecular formula is  $(C_6H_{10}O_5)_m$ , with the DP dependent on the origin of the cellulolytic material. Cellulose molecular weight has been calculated to fall approximately in the range 50,000–2,500,000. In modified celluloses, the chemical and physical characteristics of the native substances are modified in order to confer different technological properties for particular food applications. They are obtained from fibrous plant material and the modifications consist mainly in depolymerisation, etherification (with methyl, ethyl or hydroxypropyl groups), or the formation of salt of the carboxymethyl ether of native cellulose. The preparation of modified celluloses can also involve physical (E 460(ii)) or enzymatic treatments (E 469).

Animal and human data clearly demonstrated that microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) are not absorbed intact in the GI tract but could be fermented during their passage through the large intestine by strains of bacteria found in the human colon, although to a lesser degree than other polysaccharides such as gums, starches or pectins. Data for methyl cellulose (E 461), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469) demonstrated that these modified celluloses are not absorbed intact, not fermented and are excreted intact via the faeces.

Data on acute oral toxicity are available for microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464) and sodium carboxy methyl cellulose (E 466). These data indicate low oral acute toxicity, which would also apply to the other celluloses for which specific data were not available.

Short-term and subchronic toxicity studies have been performed with microcrystalline cellulose (E 460(i)), powdered cellulose (E 460(ii)), methyl cellulose (E 461), ethyl cellulose (E 462), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), sodium carboxy methyl cellulose (E 466), cross-linked sodium carboxy methyl cellulose (E 468) and enzymatically hydrolysed carboxy methyl cellulose (E 469). In the majority of studies, animals were dosed via diet at levels up to 10%. Effects on body weight at the highest dose tested (10%) were reported in some, but not all

studies, which may reflect nutritional constraints rather than toxicity. No adverse effects were reported with most of the tested celluloses, except for local effects on caecal size due to the presence of undigested fibre. Groups of 20 Wistar rats per sex were dosed with sodium carboxy methyl cellulose (E 466) or enzymatically hydrolysed carboxy methyl cellulose (E 469) via diet with 0%, 2.5%, 5% and 10% (up to 6,800 mg/kg bw per day) for up to 102 days (Til, 1992; Bär et al., 1995a). Effects on caecal weight, urothelial hyperplasia, pelvic nephrocalcinosis, corticomedullary nephrocalcinosis and increased incidence of diffuse epithelial hyperplasia in the urinary bladder were observed. The findings in kidneys and urinary bladder were attributed to the concentration of sodium, which was up to fourfold higher in the test diet compared with the basal diet. The Panel noted that this was a plausible explanation for the reported findings.

Data concerning genotoxicity are available for microcrystalline cellulose (E 460(i)), methyl cellulose (E 461) and sodium carboxy methyl cellulose (E 466) (Litton Bionetics Inc., 1974, 1975, 1980; Blevins and Taylor, 1982; Ishidate et al., 1984; Batt, 1992; Cifone, 1992; McKeon, 1992; FMC, 1991, 1994, 1995; Murli, 1992). Overall, despite the limitations of some of the studies, the Panel concluded that the available data and the *in silico* analysis conducted by the Panel, did not identify structural determinants indicating a genotoxic concern for microcrystalline cellulose, methyl cellulose and carboxy methyl cellulose. The Panel considered that these results could be read-across to the other modified and unmodified celluloses covered by this opinion, for which the same conclusion can be reached.

Chronic toxicity studies have been performed with microcrystalline cellulose (E 460(i)), methyl cellulose (E 461), hydroxypropyl cellulose (E 463), hydroxypropyl methyl cellulose (E 464), ethyl methyl cellulose (E 465) and sodium carboxy methyl cellulose (E 466). Although there were some inconsistencies in the data, the main effects seen were decreases in body weight gain at the highest dose, which are likely to be due to the amount/bulk of celluloses in the diet leading to nutritional imbalance. Furthermore, in a chronic feeding study with microcrystalline cellulose (E 460(i)), some dystrophic calcification of renal tubules was observed in the high dose group (15,000 mg/kg bw per day). The NOAEL values ranged up to 9,000 mg/kg bw per day. The Panel concluded that microcrystalline cellulose and modified celluloses have no carcinogenic properties and that there was no reason to expect carcinogenic properties with powdered cellulose (E 460(ii)).

Concerning reproductive and developmental toxicity, data are available for microcrystalline cellulose (E 460(i)), methyl cellulose (E 461), hydroxypropyl cellulose (E 463) and sodium carboxy methyl cellulose (E 466). The substances were tested in mice, rats, hamsters and/or rabbits with oral dosing via gavage (FDLI, 1973, 1975; Ferch, 1973a,b; Cannon Labs, 1975, 1977; Kitagawa et al., 1978a,b; Fritz and Becker, 1981; Freeman, 1992b). Adverse effects on reproductive performance or developmental effects were not observed with modified and unmodified celluloses at doses greater than 1,000 mg/kg bw by gavage (often the highest dose tested).

Specific toxicity data were not always available for all the celluloses for all endpoints. In general, the most complete data sets were available for microcrystalline cellulose (E 460(i)) and sodium carboxy methyl cellulose (E 466). Given the similarities in their structure, relevant physicochemical, metabolic and toxicological properties, the Panel considered it possible to read-across between all the celluloses.

In addition, the Panel noted that methyl cellulose (E 461) and sodium carboxy methyl cellulose (E 466) were frequently used in the formulations for administration of xenobiotics by gavage in chronic, reproductive and developmental toxicity and carcinogenicity studies. In these studies, there should be a control group receiving the formulation alone. Although modified cellulose levels were usually only up to 2%, given the number of studies and group sizes in these studies, the overall number of animals tested would be very large. The Panel considered that the absence of reported adverse effects from such vehicle control groups provided additional evidence of the lack of safety concern for modified celluloses at levels up to 2% in the vehicle.

There was evidence that repeated doses up to 35 g/person of microcrystalline cellulose or powdered cellulose did not adversely affect clinical chemistry and haematological parameters and had no effect on the absorption and/or the metabolism of dietary constituents.

Some modified celluloses have been used in patients suffering from diarrhoea or constipation. In general, it can be concluded that an oral ingestion of up to 6,000 mg/person per day for 8 months was well tolerated.

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 (Chassaing et al., 2015). Other emulsifiers have been reported to alter the gut microbiota, promote gut inflammation, promote obesity and to impair glycaemic control in experimental studies with animals (Swidsinski

et al., 2009a,b; Renz et al., 2012; Merga et al., 2014; Cani and Everard, 2015; Chassaing et al., 2015; Romano-Keeler and Weitkamp, 2015; Lecomte et al., 2016; Shah et al., 2017).

The Panel noted that some of the effects associated with emulsifiers are not systematically studied as specific endpoints in toxicity studies performed according to current toxicity testing guidelines, therefore, they would have to be investigated on a case-by-case basis if indicated by the results of the general toxicity testing, as recommended in the Guidance for submission of food additives (EFSA ANS Panel, 2012). However, the Panel noted that the histopathological findings reported in some of the studies are not seen in long-term studies at high doses of celluloses.

The Panel considered that based on the animal data, the toxicity of microcrystalline, powdered and modified celluloses was low and that NOAELs were generally the highest dose tested (up to at least 9,000 mg/kg bw per day). The available data in humans indicate that daily doses of up to 6,000 mg for around 8 months were not associated with adverse effects; however in line with many other dietary fibres, large bolus intakes of celluloses were occasionally associated with laxation, but there was a lack of dose–response data available. Overall, there were no indications that humans would be more sensitive than laboratory animals.

*The Panel considered that in line with the conceptual framework, it would be useful if risk managers had an indicative total exposure (daily consumption value) for microcrystalline, powdered and modified celluloses used as food additives, which would not pose a safety concern and uses up to this value would not require a further risk assessment. The Panel considered this could be based on all the reported NOAELs from subchronic and chronic toxicity studies (ranging from 2100 to more than 9000 mg/kg bw/day either the highest doses tested or the highest doses tested not inducing nutritional imbalance) taking into account human data and allowing for interindividual uncertainty. The Panel considered that an indicative total exposure (daily consumption value) of around 660 to 900 mg/kg bw per day for microcrystalline, powdered and modified celluloses could be identified.*

To assess the dietary exposure to celluloses (E 460–466, E 468 and E 469) from their use as food additives, the combined exposure was calculated based on (1) maximum levels of data provided to EFSA (defined as the *maximum level exposure assessment scenario*) and (2) reported use levels (defined as the *refined exposure assessment scenario brand-loyal and non-brand-loyal consumer scenario*).

Celluloses (E 460–466, E 468 and E 469) are authorised in a wide range of foods. The Panel did not identify brand loyalty to specific food categories in infants and toddlers (e.g. flavoured drinks). Further, the Panel considered that the non-brand-loyal scenario covering other population groups was appropriate and a realistic scenario for risk characterisation because it was assumed that the population would probably be exposed long-term to the food additive present at the mean reported use level in processed food.

A refined estimated exposure assessment scenario taking into account the FSMP for infants and young children (FC 13.1.5.1 dietary foods for infants for special medical purposes and special formulae for infants and 13.1.5.2 dietary foods for babies and young children for special medical purposes as defined by Commission Directive 1999/22/EC in which E 466 is authorised) was also performed to estimate exposure for infants and toddlers who may be on a specific diet. However, no reported use levels were made available by industry for these food categories. Thus, MPLs of E 466 for FSMP were used. The Panel noted that according to Mintel, very few baby foods were on the European market containing E 466. This was in line with the fact that no data were submitted for the food categories 13.1.5.1 and 13.1.5.2.

A refined estimated exposure assessment scenario taking into account the consumption of food supplements for consumers only was also performed to estimate exposure for children, adolescents, adults and the elderly, as exposure via food supplements may deviate largely from that via food, and the number of food supplements consumers may be low depending on populations and surveys.

The refined estimates were based on 26 out of 84 food categories in which celluloses (E 460–466, E 468 and E 469) are authorised. The Panel considered that the uncertainties identified would, in general, result in an overestimation of the exposure to celluloses (E 460–466, E 468 and E 469) as food additives in European countries considered in the EFSA European database for the refined scenario if it is considered that the food additives may not be used in food categories for which no usage data have been provided.

The Panel noted that given the information from the Mintel's GNPD, it may be assumed that celluloses (E 460–466, E 468 and E 469) are used in food categories for which no data have been provided by food industry. The main food categories, in terms of amount consumed, not taken into account were processed fermented milk products, cheeses (unripened, processed), fish and fishery



products and breakfast cereals. However, according to the Mintel GNPD (Appendix B), in the EU market, a small percentage (< 1%) of food products belonging to these food categories are labelled with celluloses (E 460–466, E 468 and E 469). Therefore, the Panel considered that if these uncertainties were confirmed, it would result in a slight underestimation of the exposure.

The Panel further noted that the exposure to celluloses (E 460–466, E 468–469) from their use according to the Annex III to Regulation (EC) No 1333/2008 was not considered in the exposure assessment.

The Panel also noted that the refined exposure estimates were based on information provided on the reported levels of use of celluloses (E 460–466, E 468–469). If actual practice changes, this refined estimates may no longer be representative and should be updated.

## 6. Conclusions

### General population

Following the conceptual framework for the risk assessment of certain food additives re-evaluated under Commission Regulation (EU) No 257/2010 (EFSA ANS Panel, 2014) and given that:

- their structural, physicochemical and biological similarities, allows for read-across between all the celluloses
- animal and human data demonstrate that microcrystalline cellulose (E 460(i)) and powdered cellulose (E 460(ii)) are not absorbed intact in the GI tract but could be fermented by intestinal microbiota. Chemically modified celluloses are not absorbed intact, nor fermented, but are excreted intact via the faeces
- using the read-across approach, adequate data on short- and long-term toxicity and carcinogenicity and reproductive toxicity are available,
- despite the limitations of some of the studies, the available data do not indicate a genotoxic concern for microcrystalline cellulose, methyl cellulose and carboxy methyl cellulose, and by read-across, of the other modified and unmodified celluloses
- no adverse effects were reported after repeated doses up to 35 g/person of microcrystalline cellulose or powdered cellulose; oral ingestion of some modified celluloses up to 6,000 mg/person per day for 8 months in patients suffering from diarrhoea or constipation was well tolerated;
- adequate combined exposure data were available; in the general population, the highest 95th percentile refined exposure assessment estimates calculated based on the reported data from food industry was 506 mg/kg bw per day in toddlers (brand-loyal scenario)
- an indicative high refined exposure assessment of up to 448 mg/kg bw per day for the elderly has been calculated at the 95th percentile among the population classes consuming food supplements

The Panel concluded that there was no need for a numerical ADI and that there would be no safety concern at the reported uses and use levels for the unmodified and modified celluloses (E 460(i); E 460(ii); E 461–466; E 468 and E 469). The Panel further suggested an indicative total exposure (daily consumption value from food additive use) of 660–900 mg/kg bw per day where these conclusions would remain valid.

### Infants and young children consuming foods for special medical purposes and special formulae

Concerning the use of sodium carboxy methyl cellulose (E 466) in 'dietary foods for special medical purposes and special formulae for infants' (FC 13.1.5.1) and in 'dietary foods for babies and young children for special medical purposes as defined in Directive 1999/21/EC' (FC 13.1.5.2), and given that:

- for infants and toddlers consumers only of FSMP, the highest 95th percentile refined exposure estimate was 1,557 mg/kg bw per day in infants
- 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;

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. The Panel noted that E 466 seemed not to be used in these food categories as no use or use levels were submitted by industry and only very few food belonging to these categories appeared to be labelled with E 466.



## 7. Recommendations

The Panel recommended that:

- The European Commission considers lowering the maximum limits for the toxic elements arsenic, lead, mercury and cadmium present as impurities in the EU specifications for unmodified and modified celluloses re-evaluated in the present opinion (E 460(i), E 460(ii), E 461, E 462, E 463, E 464, E 465, E 466, E 468, E 469) should be revised to ensure that these food additives will not be a significant source of exposure to these toxic elements in food.

### Documentation provided to EFSA

- 1) Association of the European Self-Medication Industry (AESGP), 2016. Data on usage levels of E 460i, E 460ii, E 461, E 463, E 464, E 466 and E 468 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 27 May 2016.
- 2) Aviko, 2016. Data on usage levels of E 461 and E 464 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 10 May 2016.
- 3) Dr Loges Naturheilkunde neu entdecken, 2016. Data on usage levels of E 460i, E 460ii and E 464 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 27 April 2016.
- 4) European Dairy Association (EDA), 2016. Data on usage levels of E 460i, 461 and E 466 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 30 May 2016.
- 5) European Federation of Associations of Health Products Manufacturers (EHPM), 2016. Data on usage levels of E 460i, E 463, E 464 and E 466 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 31 May 2016.
- 6) Food Drink Europe (FDE), 2016. Data on usage levels of E 460i, E 460ii, E 461, E 462, E 463, E 464, E 466, E 468 and E 469 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 31 May 2016.
- 7) Food Supplements Europe (FSE), 2016. Data on usage levels of E 460i and E 468 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 15 July 2016.
- 8) International Chewing Gum Association (ICGA), 2016. Data on usage levels of E 460i, E 462, E 464 and E 466 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 31 May 2016.
- 9) Krüger GmbH & Co., 2016. Data on usage levels of E 460i, E 464, E 466 and E 468 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 25 May 2016.
- 10) Organisation des Fabricants de produits Cellulosiques Alimentaires (OFCA), 2016. Data on usage levels of E 460i, E 460ii, E 461, E 462, E 464, E 463, E 464, E 466 and E 468 in foods in response to the EFSA call for food additives usage level and/or concentration data in food and beverages intended for human consumption (2015). Submitted to EFSA on 31 May 2016.
- 11) EMA (European Medicines Agency). Communication to EFSA request of 4 May 2015, for information on a certain group of substances used as food additives.
- 12) Baker EM, 1968. Unpublished report by U.S. Army Medical Research and Nutrition Laboratory, Fitzsimmons General Hospital. Submitted by FMC, 12 October 2016.
- 13) FMC Internal report. Internal report. Subject: Particle Size Distribution Evaluation of Avicel CL611 (EN15828695) and RC591 (HN15828445) by Laser Diffraction (Malvern Mastersizer 3000). Submitted to EFSA on 12 October 2016.

- 14) Itacel Farmoquimica Ltda (formerly Blanver Farmoquimica Ltda), 2016. Data submitted in response to the call for technical data on certain starches and celluloses authorised as food additives in the EU. Submitted to EFSA on 27 June 2016.
- 15) Cutisin, 2010. Data submitted in response to the call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Submitted to EFSA on 29 October 2010.
- 16) Devro, 2010. Data submitted in response to the call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Submitted to EFSA on 2 February 2010.
- 17) FMC Corporation, 1982a. Avicel PH-101. Acute dermal toxicity in rabbits. FMC Study number I1982-620. Submitted to EFSA on 12 October 2016.
- 18) FMC Corporation, 1982b. Avicel PH-101. Primary eye irritation in rabbits. FMC Study number I1982-621. Submitted to EFSA on 12 October 2016.
- 19) FMC Corporation, 1982c. Avicel PH-101. Acute inhalation LC50. FMC Study Number I1982-622. Submitted to EFSA on 12 October 2016.
- 20) FMC Corporation, 1982d. Avicel PH-105 Acute oral toxicity in rats. FMC Study Number I82-623. Submitted to EFSA on 12 October 2016.
- 21) FMC Corporation, 1982e. Avicel PH-105. Acute dermal toxicity in rabbits. FMC Study Number I1982-624. Submitted to EFSA on 12 October 2016.
- 22) FMC Corporation, 1982f. Avicel PH-105. Primary skin irritation. FMC Study Number I1982-625 (Unpublished). Submitted to EFSA on 12 October 2016.
- 23) FMC Corporation, 1982g. Avicel PH-105. Primary eye irritation. FMC Study Number I1982-626 (Unpublished). Submitted to EFSA on 12 October 2016.
- 24) FMC Corporation, 1982h. Avicel PH. Acute inhalation LC50. FMC Study Number I1982-627 (Unpublished). Submitted to EFSA on 12 October 2016.
- 25) FMC Corporation, 1982i. Avicel CL611. Acute oral toxicity in rats. FMC Study Number I82-615 (Unpublished). Submitted to EFSA on 12 October 2016.
- 26) FMC Corporation, 1982j. Avicel CL611. Acute dermal toxicity in rabbits. FMC Study Number I1982-616 (Unpublished). Submitted to EFSA on 12 October 2016.
- 27) FMC Corporation, 1982k. Avicel CL611. Acute inhalation LC50. FMC Study Number I1982-619 (Unpublished). Submitted to EFSA on 12 October 2016. Submitted to EFSA on 12 October 2016.
- 28) FMC Corporation, 1982l. Avicel CL611. Primary skin irritation. FMC Study Number I1982-617 (Unpublished). Submitted to EFSA on 12 October 2016.
- 29) FMC Corporation, 1982m. Avicel CL611. Primary eye irritation. FMC Study Number I1982-618 (Unpublished). Submitted to EFSA on 12 October 2016.
- 30) FMC Corporation, 1991a. Avicel PH. Skin sensitization in guinea pig. FMC Study Number I1991-1184 (Unpublished). Submitted to EFSA on 12 October 2016.
- 31) FMC Corporation, 1991b. Avicel PH101. *Salmonella*/Mammalian-Microsome Plate Incorporation Assay (Ames Test). FMC Study Number I1991-1189 (Unpublished).
- 32) FMC Corporation, 1992a. Avicel CL-611. Ninety-day feeding study in rats. FMC Study Number I92-1711. (Unpublished). Submitted to EFSA on 12 October 2016.
- 33) FMC Corporation, 1992b. Avicel CL-611. Teratology study in rats (dietary). FMC Study Number I92-1712. (Unpublished). Submitted to EFSA on 12 October 2016.
- 34) FMC Corporation, 1994a. Mutagenicity test on Avicel CL-611 in the L5178Y TK+/- mouse lymphoma forward mutation assay with a confirmatory repeat. (Study conducted by Hazleton Washington, Inc.) FMC Study Number I94-1834. (Unpublished). Submitted to EFSA on 12 October 2016.
- 35) FMC Corporation, 1994b. Mutagenicity test on Avicel CL-611 in an *in vivo* mouse micronucleus assay. (Study conducted by Hazleton Washington, Inc.) FMC Study Number I94-1835. (Unpublished). Submitted to EFSA on 12 October 2016.
- 36) FMC Corporation, 1994c. Mutagenicity test on Avicel PH101 Pharmaceutical in an *in vivo* mouse micronucleus assay. (Study conducted by Hazleton Washington, Inc.) FMC Study Number I94-1837. (Unpublished). Submitted to EFSA on 12 October 2016.
- 37) FMC Corporation, 1995. Mutagenicity test with Avicel AC-815 in the *Salmonella-Escherichia coli*/mammalian microsome reverse mutation assay with a confirmatory assay (Study conducted by Coming Hazleton). FMC Study Number I95-2047. (Unpublished). Submitted to EFSA on 12 October 2016.

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- 42) Mars, 2010. Data submitted in response to the call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Submitted to EFSA on 19 May 2010.
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- 44) Organisation des Fabricants de produits Cellulosiques Alimentaires (OFCA), 2009. Data submitted in response to the call for scientific data on food additives permitted in the EU and belonging to the functional classes of emulsifiers, stabilisers and gelling agents. Submitted to EFSA on 1 December 2009.
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- 46) Pre-evaluation document prepared by Fraunhofer on modified celluloses (E 461-466, 469).
- 47) Pre-evaluation document prepared by Peter Fisk Associates Ltd cross-linked carboxy methyl cellulose (E 468).
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## Abbreviations

AAS	atomic absorption spectroscopy
ADI	acceptable daily intake
AESGP	Association of the European Self-Medication Industry
AGU	anhydroglucose unit
AHP-WS	wheat straw pretreated with alkaline hydrogen peroxide
ANS Panel	EFSA Panel on Food Additives and Nutrient Sources added to Food
AOAC	Association of Official Analytical Chemists
AP	alkaline phosphatase
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstract Service
CHL	Chinese hamster fibroblast cell line
CHO	Chinese hamster ovary cells
CMC	carboxy methyl cellulose

DMSO	dimethylsulfoxide
DP	degree of polymerisation
DS	degree of substitution (the average number of substituted hydroxyl groups per glucose)
EDA	European Dairy Association
EDTA	ethylenediaminetetraacetic acid
EHPM	European Federation of Associations of Health Products Manufacturers
EINECS	European Inventory of Existing Commercial Chemical Substances
EMA	European Medicines Agency
EMC	ethyl methyl cellulose
FAO	Food and Agriculture Organization
FC	freezing–cooking
FCS	Food Classification System
FDA	Food and Drug Administration
FDE	FoodDrinkEurope
FFA	free fatty acids
FSE	Food Supplements Europe
FSMP	food for special medical purpose
GALT	gut-associated lymphoid tissue
GC	gas chromatography
GD	gestation day
GI	gastrointestinal
GLP	good laboratory practice
GNPD	Global New Products Database
GPC	gel permeation chromatography
GRAS	Generally Recognized as Safe
Hb	haemoglobin
HDL	high-density lipoprotein
HMWSDF	high-molecular weight soluble dietary fibres
HPC	hydroxypropyl cellulose
HPLC	high-performance liquid chromatography
HPMC	hydroxypropyl methyl cellulose
ICGA	International Chewing Gum Association
ICP-AES	inductively coupled plasma atomic emission spectroscopy
IR	infrared
JECFA	Joint FAO/WHO Expert Committee on Food Additives
L-HPC	hydroxypropyl cellulose of low substitution
LD <sub>50</sub>	lethal Dose, 50% i.e. dose that causes death among 50% of treated animals
LDL	low-density lipoprotein
MA	metabolic activation
MALDI MS	matrix-assisted laser desorption/ionisation mass spectrometry
MC	methyl cellulose
MCC	microcrystalline cellulose
MHEC	methyl hydroxyethyl cellulose
MPL	maximum permitted level
MS	mass spectrometry
NaCMC	sodium carboxy methyl cellulose
NDA Panel	EFSA Panel on Dietetic Products, Nutrition and Allergies
NDO	non-digestible oligosaccharides
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OFCA	Organisation des Fabricants de produits Cellulosiques Alimentaires
OM	organic matter
OPEFB	oil palm empty fruit pulp
PCV	packed cell volume
PCH	propylene chlorohydrin
PMC	propyl methyl cellulose

QS	<i>quantum satis</i>
RBC	red blood cell
SCF	Scientific Committee on Food
SCFA	short-chain fatty acids
S-GOT	serum glutamic-oxaloacetic transaminase
S-GPT	serum glutamate pyruvate transaminase
TAMC	total anaerobic microbial count
TYMC	total combined yeast and mould count
UV/VIS	ultraviolet/visual (spectrometry)
VFA	volatile fatty acids
VLDL	very low-density lipoprotein
WBC	white blood cell
WG	Working Group
WHO	World Health Organization



**Appendix A – Summary of the reported use levels (mg/kg or mg/L as appropriate) of celluloses (E 460–466, E 468 and E 469) provided by industry**

**Appendix B – Number and percentage of food products labelled with celluloses (E 460–466, E 468 and E 469) out of the total number of food products present in the Mintel GNPD per food subcategory between 2012 and 2017**

**Appendix C – Concentration levels of celluloses (E 460–466, E 468 and E 469) used in the refined exposure scenarios (mg/kg or mL/kg as appropriate)**

**Appendix D – Total estimated exposure of celluloses (E 460–466, E 468 and E 469) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day)**

**Appendix E – Main food categories contributing to exposure to celluloses (E 460–466, E 468 and E469) for the maximum and refined (brand-loyal and non-brand-loyal) scenarios**

Appendix A–E can be found in the online version of this output ('Supporting information' section): <https://doi.org/10.2903/j.efsa.2018.5047>