SCIENTIFIC OPINION

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Safety of a change in specifications for the food additive hydroxypropyl methyl cellulose (E 464)

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)

Abstract

Following a request from the European Commission, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion regarding the safety of an amendment to specifications for the food additive hydroxypropyl methyl cellulose (HPMC, E 464). It is requested that the limit of the sum of the two isomers (1-chloro-2-propanol and 2-chloro-1-propanol) of propylene chlorohydrin (PCH) in HPMC be raised from 0.1 mg/kg to 1.0 mg/kg, in order to align it with the limit defined by the Joint FAO/WHO Expert Committee on Food Additives in 2011. The ANS Panel received a dossier from the applicant and subsequently requested additional data. The ANS Panel noted that not all information was made available and that exposure to PCH is likely to be underestimated, since data were provided only for a limited number of authorised uses and that other sources of PCH were not included. The Panel also noted that the available data, for both PCH isomers, were indicative of a genotoxic hazard and that the results available from preliminary studies were deemed insufficient to make any conclusion regarding developmental toxicity. In addition, because no carcinogenic effects were observed in relevant chronic/carcinogenicity studies at the same time, it was impossible to derive a lower benchmark dose level. A margin of exposure approach to impurities, as suggested by EFSA guidance in 2012, was also not possible. Moreover, when considering a threshold of toxicological concern approach to genotoxic effects, the Panel was unable to make any conclusion regarding the absence of risk. Finally, analytical data provided for a total of 12 different batches of HPMC showed that PCH levels were in compliance with current specifications. The Panel concluded that the data available are insufficient to support a change in specification from 0.1 to 1 mg PCH/kg HPMC.

Keywords: food additive, hydroxypropyl methyl cellulose, HPMC, E 464, propylene chlorohydrin, PCH, change in specifications

Requestor: European Commission

Question number: EFSA-Q-2013-00283

Correspondence: fip@efsa.europa.eu

Panel Members: Fernando Aguilar, Riccardo Crebelli, Alessandro Di Domenico, Birgit Dusemund, Maria Jose Frutos, Pierre Galtier, David Gott, Ursula Gundert-Remy, Claude Lambré, Jean-Charles Leblanc, Oliver Lindtner, Peter Moldeus, Alicja Mortensen, Pasquale Mosesso, Dominique Parent-Massin, Agneta Oskarsson, Ivan Stankovic, Ine Waalkens-Berendsen, Rudolf Antonius Woutersen, Matthew Wright and Maged Younes.

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Summary

The European Commission requests that the European Food Safety Authority (EFSA) provides a scientific opinion regarding the safety of an amendment to the specifications for the food additive hydroxypropyl methyl cellulose (E 464), in accordance with Regulation (EC) No 1331/2008, which establishes a common authorisation procedure for food additives, food enzymes and food flavourings.

The Organisation des Fabricants de produits Cellulosiques Alimentaires (OFCA) requested that the limit of propylene chlorohydrin (PCH) in hydroxypropyl methyl cellulose (HPMC) be raised from 0.1 mg/kg to 1.0 mg/kg, in order to align it with the limit defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 74th meeting in 2011. Thus, the limit defined for PCH in Regulation (EU) No 231/2012 would be changed from "not more than 0.1 mg/kg" to "not more than 1 mg/kg". Furthermore, this limit of PCH would apply to the sum of the two isomers (1-chloro-2-propanol and 2-chloro-1-propanol) by using the following wording "1.0 mg/kg for the sum of both isomers".

The Panel received a dossier from the applicant and subsequently requested additional data. The original dossier stated that substantial toxicological data were available for PCH and that the safety data from JECFA (1969–1973) should be taken into account. In its initial discussions, the Panel was made aware of published studies which appeared to contradict the statements of the applicant. Therefore, in agreement with EFSA's 2012 guidance for submission for food additives evaluation the Panel requested data regarding the levels of PCH in at least five representative batches of HPMC that were independently produced.

The applicant cited ambiguities related to the PCH limit stated by the EC and to inaccuracies in the analytical method used for measuring PCH levels as reasons for their proposal to raise the PCH specification limits. According to the applicant, high background noise produced by the halogen-specific detector used for analysis made the results unreliable. Also, the wax column permitted co-elution of the PCH with unknown compounds leading to inaccuracies and higher apparent PCH concentrations. The Panel noted that the proposal to raise the PCH specification limits did not appear to be supported by the results obtained with methods 2 and 3.

In response to the Panel's request for data on PCH levels in at least five batches, results were provided for two HPMC products (seven batches for one product and five for the other). All of the reported PCH values were below the current specification of 0.1 mg/kg. The Panel noted that these results appear to contradict the stated technical need for the change in specification.

The Panel noted that the requested literature search had not identified the ADME (absorption, distribution, metabolism and excretion) references cited in the National Toxicology Program (NTP) report. The data on ADME were mainly on 1-chloro-2-propanol and limited data on 2-chloro-1-propanol or the mixture of both isomers were available.

The Panel noted that the ADME data indicated that an active intermediate might be formed, which appeared to be detoxified by glutathione conjugation leading to cysteine conjugate excretion.

The Panel does not agree with the applicant's statement in the dossier that "propylene chlorohydrins are not genotoxic". The Panel noted that 1-chloro-2-propanol and the mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) showed mutagenic properties in S. typhimurium strains TA 1535 and TA 100 in the absence and presence of an S9 fraction. The mixture of 1-chloro-2-propanol isomers was mutagenic and clastogenic in cultured mammalian cells, with and without metabolic activation with S9, and induced micronuclei and morphological transformation in hamster embryo cells. The same mixture was also weakly positive in a mutation test using *Drosophila* germ cells and clastogenic in rat bone marrow *in vivo.* Based on the available results, the Panel concluded that data on PCH (both 1-chloro-2-propanol and the mixture of 1-chloro-2-propanol and 2-chloro-1-propanol) were indicative of a genotoxic hazard. The Panel also noted that there is some additional uncertainty concerning the no observed adverse effect level (NOAEL) of 58 mg 1-chloro-2-propanol (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol)/kg bw (body weight) per day proposed by the applicant, as some parameters in the reproductive toxicity study were tested only in the high-dose group and not in the mid- and low-dose groups.

The Panel concluded that the NOAELs for sub-chronic toxicity and from the NTP carcinogenicity study were similar (around 35 mg/kg bw in rats). The Panel considered that the lack of neoplastic effects in this study at all three dose levels tested in mice and rats demonstrated a lack of carcinogenic potential. However, it was not possible to derive a lower benchmark dose level ($BMDL_{10}$) as there were no relevant lesions at any dose level. Furthermore, the Panel considered that using a margin of exposure (MOE) approach was not applicable because PCHs do not fulfil the basic criteria, of being both genotoxic and carcinogenic, that are required for this method to be applicable.

It should be noted that, in the toxicology attachment provided by the applicant, it is stated that "No malformations were observed upon gross examination", whilst the authors of the study concluded that chloropropanol may be a weak teratogen. The Panel also noted that, although this is only a preliminary study with a low number of animals, the author conclusion is valid. Therefore, in the absence of a full prenatal developmental study by gavage, performed in accordance with current Organisation for Economic Cooperation and Development guidelines, the Panel cannot make any conclusions on developmental toxicity. The EFSA Scientific Committee considered that clear evidence of genotoxicity in somatic cells *in vivo* should be regarded as an adverse event per se, since genotoxicity is also implicated in other degenerative diseases. The Panel noted that when evidence of genotoxicity is less clear, using a weight of evidence evaluation, negative carcinogenicity data can be considered more conclusive on a case-by-case basis. However, since *in vivo* genotoxicity has occasionally been associated with teratogenic potential and given the results of the Philips study, the Panel considered that the carcinogenicity data were not sufficient to make the conclusion that there was no genotoxic risk in this case.

The Panel noted that the exposure estimates provided by the applicant to support the change in specifications were based on the normal use levels of HPMC for a limited number of its authorised uses (around 20 out of 70). The Panel would be able to compare the exposure from these selected uses only with the available toxicological database. The Panel considered this assumption to be driven by huge uncertainties, knowing that there are other uses of HPMC and that there are also other sources of PCH exposure from other cellulosic or hydroxypropyl food additives. If these uncertainties are confirmed, then the anticipated exposure to PCH would be higher than estimated and, therefore, any conclusions based on the comparison of the exposure with a toxicological reference point ($BMDL_{10}$ or NOAEL) are likely to result in a significant underestimation of the risk.

The Panel used an MOE approach only for residuals or contaminants in a food additive that are genotoxic and carcinogenic, which is not the case for PCH; in addition, there are currently no accepted MOE approaches for compounds which are only genotoxic. Instead, it would be possible to compare exposure to a residual with the threshold of toxicological concern (TTC) for genotoxic effects of 0.15 µg/person per day. The Panel noted that the exposure estimated using the current specification of 0.1 mg PCH/kg HPMC would be around this value. Consequently, at the requested new specification of 1 mg/kg, and without considering the fact that only some of the authorised uses (around 20 out of 70 from the legislation) were proposed, the TTC would be exceeded. Therefore, the Panel was unable to conclude that there would not be a genotoxic risk associated with the requested new specification level of 1 mg PCH/kg HPMC.

On the basis of the above considerations, the Panel concluded that the available data are insufficient to support a change in the specification from 0.1 to 1 mg PCH/kg HPMC.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

The Organisation des Fabricants de produits Cellulosiques Alimentaires (OFCA), representing the major manufacturers of cellulose derivatives, has requested from an amendment to the specifications regarding the food additive hydroxypropyl methylcellulose.

Hydroxypropyl methylcellulose is cellulose obtained directly from strains of fibrous plant material and partially etherified with methyl groups and containing a small degree of hydroxypropyl substitution.

One of the purity's criteria is the amount of 'Propylene chlorohydrin' (PCH) identified as the residue formed in starches modified by hydroxypropylation. Currently, the limit of PCH as set by Regulation (EU) No. 231/2012[1] laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No. 1333/2008 of the European Council, reads "Not more than 0.1 mg/kg".

OFCA asks for the limit on PCH to be raised from 0.1 mg/kg to 1.0 mg/kg, in order to align it with the one defined by the joint FAO/WHO Expert Committee on Food Additives (JECFA) at the 74th meeting of JECFA (2011). Thus, the limit defined for PCH in Regulation (EU) No. 231/2012 would be changed from 'Not more than 0.1 mg/kg' to "Not more than 1 mg/kg". Besides, the limit would apply to the sum of the two isomers (1-chloro-2-propanol and 2-chloro-1-propanol) by using the following wording "1.0 mg/kg for the sum of both isomers".

OFCA claims that a relatively substantial toxicology data base is available for the isomers of PCH, 1-chloro-2-propanol and 2-chloro-1-propanol, and that the safety data from JECFA (1969, 1973) should be taken into account. OFCA has provided additional supporting toxicity data for PCH summarised in 'Attachment 5 (Propylene chlorohydrin Residues in hydroxypropyl methylcellulose).

1.1.2. Terms of Reference

The European Commission asks the European Food Safety Authority to provide an opinion on the safety regarding the amendment of specifications on the food additive hydroxypropyl methyl cellulose in accordance with Regulation (EC) No 1331/2008[2] establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.2. Additional information

1.2.1. Existing authorisations and evaluations

In 1989, HPMC (E 464) was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) during its 35th meeting and an acceptable daily intake (ADI) was not allocated. Specifications for the identity and purity were also defined.

In 1992, the Scientific Committee for Food (SCF) issued an opinion on HPMC (E 464) and other modified celluloses and this committee also did not consider it appropriate to set a numerical ADI. The current specifications for HPMC are laid down in the 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. The reported limit for PCH is "not more than 0.1 mg/kg".

Commission Regulation (EU) No 1129/2011[3] reports the maximum permitted levels for use of HPMC in the authorised food categories.

[1] 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, 22.03.2012, p. 1-295

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and flavourings. OJ L 354, 31.12.2008, p. 1–6.

[3] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives, OJ L 295, 12.11.2011, p. 1–177

In 2011, during its 74th meeting, JECFA considered possible changes to the specifications for HPMC. Because of the development of a revised analytical method, JECFA increased the specified levels of PCH to the new limit of not more than 1.0 mg/kg for the sum of both isomers in HPMC. According to JECFA (2011): "In consideration of the proposed revision of the limit for propylene chlorohydrins, the Committee took into account the extensive available toxicological database, most notably studies conducted by the United States National Toxicology Program. These data, together with the Committee's previous estimate of dietary intake of HPMC, indicated that levels of propylene chlorohydrins up to 1 mg/kg in HPMC were not of toxicological concern".

The present opinion specifically refers to the proposed change in specifications and it is focused on the levels of PCH. Full re-evaluation of HPMC (E 464), in accordance with Regulation (EU) No 257/2010,[4] will be carried out in agreement with the re-evaluation programme defined by EFSA and the EFSA Panel on Food Additives and Nutrient Sources added to Food ANS by the deadline of 31 December 2016, as laid down in Annex II of the relevant regulation.

2. Data and Methodologies

2.1. Data

The applicant has provided an exposure estimate for PCH from HPMC according to the Food Additives Intake Model (FAIM) for certain authorised uses and use levels (Table 3). This estimate assumed that all processed foods (around 20 usages proposed by the applicant out of the 70 authorised uses) contain HPMC as a food additive at the maximum reported use levels, and that the PCH residual is at the proposed 1 mg/kg specification limit in all HPMC food additives. In addition, a list of toxicological studies conducted with PCH was also provided.

2.2. Technical data

2.2.1. Identity of the substance

Hydroxypropyl methyl cellulose (HPMC) (E 464) is cellulose, obtained directly from strains of fibrous plant material, partially etherified with methyl groups and containing a small degree of hydroxypropyl substitution (Commission Regulation (EU) No 231/2012).

According to JECFA (JECFA, 2011), HPMC is a methyl cellulose, modified by treatment with alkali and propylene oxide, to which a small number of 2-hydroxypropyl groups are attached through ether links to the anhydroglucose units of the cellulose. The article of commerce may be further specified by viscosity.

The chemical names are hydroxypropyl methyl cellulose and 2-hydroxypropyl ether of methylcellulose.

According to EU specifications (Commission Regulation (EU) No 231/2012), the polymers contain substituted anhydroglucose units with the following general molecular formula:

- $C_6H_7O_2(OR_1)(OR_2)(OR_3)$, where R_1, R_2 and R_3 can be one of the following:
  - H
  - CH_3
  - $CH_2CHOHCH_3$
  - $CH_2CHO(CH_2CHOHCH_3)CH_3$
  - $CH_2CHO[CH_2CHO(CH_2CHOHCH_3)CH_3]CH_3$.

According to the JECFA specifications (JECFA, 2011), the chemical formula for HPMC is:

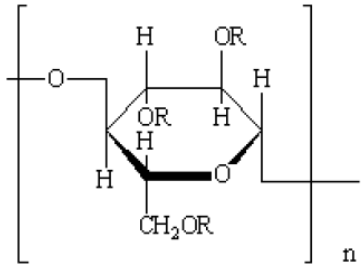
- $[C_6H_7O_2(OH)_x(OCH_3)_y(OCH_2CHOHCH_3)_z]_n$, where:

4 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

- z = 0.07 – 0.34
- y = 1.12 – 2.03
- x = 3 – (z + y), where z + y = degrees of substitution.

The molecular weight of the unsubstituted structural unit is 162.14 g/mol. The structural unit with 1.19 degrees of substitution is approximately 180 g/mol, and with 2.37 degrees of substitution is 210 g/mol. The molecular weights of the macromolecules range from about 13 000 (where "n" is about 70) to about 200 000 (where "n" is about 1 000). The CAS registry number is 9004-65-3.

The structural formula for HPMC is given in Figure 1.



R = H or CH_3 or $CH_2CHOHCH_3$

Figure 1: The structural formula of hydroxypropyl methyl cellulose (JECFA, 2011)

HPMC is a hygroscopic white or off-white powder, or can be found as granules or fine fibres (JECFA, 2011). The additive is used as an emulsifier, a thickening agent and a stabiliser, and it is permitted at *quantum satis* levels.

HPMC swells in water, producing a clear to opalescent, viscous colloidal solution. It is insoluble in ethanol.

2.2.2. Specifications

Specifications have been defined in Commission Regulation (EU) No 231/2012 on specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council.

The content of methoxyl groups (–OCH3) is not less than 19 % and not more than 30 %, and the content of hydroxyl-propoxyl groups (-OCH2CHOHCH3) is not less than 3 % and not more than 12%, for the anhydrous compound.

The purity of HPMC is specified in EU and JECFA specifications (Table 1).

Table 1: Specifications of the purity of hydroxypropyl methyl cellulose (E 464) according to Commission Regulation (EU) No 231/2012 and JECFA (2011)

Purity	Commission Regulation (EU) No 231/2012	JECFA (2011)
Loss on drying	≤ 10 % (105 °C, 3 hours)	≤ 10 % (105 °C to constant weight)
Sulphated ash	≤ 1.5 % (viscosity ≥ 50 mPa.s)	≤ 1.5 % (viscosity ≥ 50 cp)
	≤ 3 % (viscosity < 50 mPa.s)	≤ 3 % (viscosity < 50 cp); test 1 g of the sample
pH	–	5 ≤ × ≤ 8 (1 in 100 solution)
Propylene chlorohydrins (PCHs)	≤ 0.1 mg/kg	≤ 1 mg/kg
Arsenic	≤ 3 mg/kg	–
Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1mg/kg	–
Cadmium	≤ 1 mg/kg	–

The Panel noted that these EU and JECFA specifications of the purity of HPMC (E 464) differ with regard to the level of PCHs and the maximum allowed concentrations of arsenic, mercury and cadmium (Table 1).

Commission Regulation (EU) No 231/2012 specifies a level for PCHs of no more than 0.1 mg/kg, whilst JECFA (2011) established a higher permitted level (1 mg/kg).

2.2.3. Analytical methods

The analytical method proposed by JECFA (2011) for the determination of PCH content is gas chromatography–mass spectrometry. The instrument used is a gas chromatograph with a mass selective detector in selective ion monitoring mode, an electron impact ionisation source, a pulsed-splitless injector and a data station, with column temperature programming. The column used is 30 m × 0.25 mm ID × 1.4 µm thick DB-624 film or equivalent.

The PCHs are present as two isomers: 1-chloro-2-propanol and 2-chloro-1-propanol. For the analysis, different internal and stock standard solutions of the two isomers are prepared, and the PCH content is reported in mg/kg as the sum of the two isomers.

The applicant conducted a cross-company method comparison for several batches of the additive HPMC containing different levels of PCH (low < 0.1 mg/kg, medium ca 0.1 mg/kg PCH and high > 0.1 mg/kg) and using samples with low, medium or high viscosity. The companies followed a previously established protocol to ensure that the samples were anonymous. The results are summarised in Table 2.

Table 2: Results from cross-company method comparison.

Sample	Method 1	Method 2	Method 3
1. Low PCH	Not detected	0.06	< 0.10
2. Low viscosity	0.04	0.05	0.13
3. Medium PCH	0.13	0.15	0.24
4. Medium viscosity	0.02	0.04	< 0.10
5. High PCH	0.25	0.24	0.51
6. High viscosity	Not detected	0.03	< 0.10

Propylene chlorohydrin (PCH) content (mg/kg), expressed as the sum of the two isomers (1-chloro-2-propanol and 2-chloro-1-propanol)

The companies followed three gas chromatographic methods with differences in the column type used and/or in the detection system, as follows:

- Method 1 used a DB 624-type column with mass selective detection (MSD).
- Method 2 used a wax-type column with MSD.
- Method 3 used a wax-type column with a halogen-specific detector (XSD).

The former method specified by JECFA (in 2004) used gas chromatography. For this analysis, stock and working standard solutions were prepared and a gas chromatograph with an XSD was used with linear column temperature programming; the column used was a 30m × 0.53 mm × 1 µm DB-WAX or equivalent.

In 2011, JECFA incorporated a new analytical method for the determination of PCH content, as proposed by the applicant, which replaced the method of analysis described by the former specification (JECFA, 2004) after the cross-company method comparison. The maximum permitted levels of PCH in HPMC were also increased from 0.1 mg/kg (JECFA, 2004) to 1 mg/kg (JECFA, 2011).

The Panel compared both methods and stated that the detector and the column were different. In addition, other methodological aspects of the protocol were not the same; the number of stock

standard solutions was specified in the 2011 method and an internal standard solution was used. Both PCH isomers were detected by both methods.

The applicant cited ambiguities related to the PCH limit stated by the EC and to inaccuracies of the analytical method as reasons for their proposal to raise the PCH specification limits. According to the applicant, high background noise of produced by the XSD used in the former method (JECFA, 2004) made results unreliable. Also, the wax column permitted co-elution of the PCH with unknown compounds leading to inaccuracies and higher apparent PCH concentrations. The Panel noted that the proposal to raise the PCH specification limits did not appear to be supported by the results obtained with methods 2 and 3 (Table 2). With regard to the PCH limit, the applicant stated that it was lower than necessary and inconsistent with the specified 1 mg/kg limit for other related additives, such as modified starch (E 1440 hydroxypropyl starch and E 1442 hydroxypropyl distarch phosphate).

The panel noted that for the additives E 1440, and E 1442, the limit specified for PCH is "not more than 1 mg/kg (on an anhydrous basis)", while for HPMC (E 464) it is not specified that the limit stated, of 0.1 mg/kg, is also on an anhydrous basis.

The levels of PCHs found in the samples using method 1 (equivalent to the JECFA 2011 method) were in the range of or below the authorised maximum level (0.1 mg/kg) specified by European Commission Regulation (EU) No 231/212, except for the samples with "High PCH" as expected.

The results, presented in Table 2, obtained from the comparative analysis of the different methods, demonstrate that method 3, which used an XSD (equivalent to the one stated in JECFA, 2004), detected higher levels of PCH than methods 1 and 2, which used MSD. Method 1 detected lower levels of PCH than method 2 which the applicant attributed to a lower selectivity of column elution in method 2, although it is not clear if these differences are significant.

The Panel requested data on the measured residual levels from the applicant, which were provided in November 2014. Data on two products were submitted; seven independent batches were analysed for product 1, and PCH levels were reported to be between 0.02 and 0.09 mg/kg, and the other five independent batches were analysed for product 2, and PCH levels were reported to be between 0.04 and 0.07 mg/kg.

According to the results (Table 2), the new method, incorporated into the 2011 JECFA specifications for the analysis of PCHs, leads to the detection of lower levels of PCH than the former method. The Panel noted that this appeared to be mainly as a result of the use of a more selective detector and column. Therefore, the Panel concluded that the proposed increase in the limit of PCHs specified by Commission Regulation (EU) No 231/2012 is not be justified by the change in the method of analysis or by the actual PCH levels in commercial samples resulting from current HPMC manufacturing methods.

2.2.4. Exposure estimate

The applicant estimated the exposure (mean and high level) to PCH for the five standard age groups (toddlers, children, adolescents, adults and the elderly) by applying the FAIM template (EFSA, 2013). The mean and 95th percentile food consumption statistics used in the FAIM were calculated by EFSA using the EU Comprehensive Food Consumption Database.

The upper level of the range of mean and high level dietary exposure estimates amongst dietary surveys available within the FAIM template, provided by the applicant for PCHs, are summarised in Table 3.

Table 3: Summary of the total anticipated estimated dietary exposures based on a maximum limit of 1 mg/kg of PCH in HPMC (E 464), as proposed by the applicant

Population groups	Mean (µg/kg bw per day)	High level (µg/kg bw per day)
Toddlers (12–35 months)	0.3	0.7
Children (3–9 years)	0.3	0.5
Adolescents (10–17 years)	0.2	0.3
Adults (18–64 years)	0.2	0.3
The elderly (> 65 years)	0.1	0.2

The Panel note that, even if it is assumed that all processed foods contain the HMPC food additive at the maximum reported use level, and that the PCH residual is at the proposed 1 mg/kg specification limit in all HMPC food additives, the exposure estimates provided to support the change in the specifications were based on use levels of HPMC in only limited examples of its authorised uses (around 20 out of 70). The Panel identified other sources of PCH impurities in food from authorised uses of hydroxypropyl cellulose (E 463), E 1440 and E 1442. The Panel considers that uses of E 463, E 464, E 1440 and E 1442 would not be additive as they have the same technological function as food additives and are unlikely to be used in combination in foods. The Panel noted that the specifications for E 1440 and E 1442 allow up to 1 mg/kg PCH on an anhydrous basis.

The Panel identified the sources of uncertainty of the exposure estimate made by the applicant and their influence on FAIM results and concluded, as a whole, that the total estimated uncertainty from the proposed uses regarding the authorisation of uses (around 20 out of 70) would lead to an underestimation of the calculated exposures to PCH impurities from E 464.

2.2.5. Biological and toxicological data

Absorption, distribution, metabolism and excretion data for PCH

No ADME data were submitted to EFSA by the applicant; however, the Panel noted that in the report of the National Toxicology Program (NTP) study (NTP, 1998) the following data were described:

"Data from limited metabolism studies indicate that propylene chlorohydrins are partly excreted in the conjugated form in the urine of laboratory animals. Following oral administration of 140 mg/kg 2-chloro-1-propanol to rabbits, 11 % of the dose was excreted in the urine as its glucuronic acid conjugate (Williams, 1959). In a study of the metabolism of 1-halogenopropanes (Barnsley, 1966), 1-chloro-2-propanol (0.5 mL) was administered as a 3.3 % (w/v) solution in arachis oil. 2-Hydroxypropyl mercapturic acid (N-acetyl-S-(2-hydroxypropyl)-L-cysteine) was identified as a urinary metabolite of 1-chloro-2-propanol in the urine of male rats (strain not specified) 24 hours after a single subcutaneous injection. Jones and Gibson (1980) conducted in vitro studies of the metabolism of 1,2-dichloro propane using the methods of Barnsley (1966), proposed 1-chloro-2-propanol as an intermediate metabolite to explain the presence of the N-acetyl-S-(2-hydroxypropyl)-cysteine found in the urine of male rats injected subcutaneously with 1,2-dichloropropane. Jones and Gibson (1980) also conducted in vivo studies in male Sprague-Dawley rats. Following subcutaneous injection of 100 mg [36Cl]–1-chloro-2-propanol to a single rat, 4 % of the administered radiolabel was excreted unchanged in the expired air. In addition, two major urinary metabolites identified in rats dosed orally with 1-chloro-2-propanol (100 mg/kg per day for 4 days) were N-acetyl-S-(2-hydroxypropyl)-cysteine and β-chlorolactate.

The disposition of 1-chloro-2-propanol was studied in male F344/N rats (aged 11 to 13 weeks) exposed by nose-only inhalation to approximately 8 or 80 ppm [^{14}C]–1-chloro-2-propanol for 6 hours (Bond et al., 1988). The two major routes of ^{14}C elimination were urinary and respiratory, which together accounted for about 80 % of the total radiolabel recovered 70 hours after the end of the 6-hour exposure. At both exposure concentrations, most of the radiolabel was eliminated in urine during the first day after exposure. Half-lives for elimination were 3.9 hours for breath and 5 hours for urine. The results indicated that metabolism of 1-chloro-2-propanol was rapid and related linearly to the inhaled exposure concentration. Following inhalation, 1-chloro-2-propanol was widely distributed to tissues, rapidly metabolised, and eliminated. One hour after the end of the 6-hour exposure period, the kidney, liver, trachea, and nasal turbinates of 8 ppm rats contained 30 to 50 nmol ^{14}C/g tissue, and those of 80 ppm rats contained 200 to 350 nmol ^{14}C/g tissue; other tissues contained less than 150 nmol ^{14}C/g tissue. The tissue elimination of ^{14}C was biphasic at both exposure concentrations, with a short-term elimination half-life of 1 to 4 hours and a long-term elimination half-life of 40 to 126 hours. No evidence of unmetabolized 1-chloro-2-propanol was seen in the tissues examined. Biliary excretion was a major route of elimination of 1-chloro-2-propanol; approximately 30 % of the administered radiolabel was excreted in the bile within 10 hours. Three hours after exposure of rats to 80 ppm, two major metabolites were detected in the urine and three major metabolites were detected in liver. In both cases, one of these metabolites was identified as N-acetyl-S-(hydroxypropyl)-cysteine and/or S-(2-hydroxypropyl)cysteine, which is consistent with data obtained by Barnsley (1966) and

Jones and Gibson (1980). These metabolites appear to be derived from the conjugation of 1-chloro-2-propanol with glutathione. Bond et al. (1988) also investigated whether the exhaled radiolabelled CO_2 was derived from the second and/or third 2 carbon atoms using 1-chloro-2-propanol labelled with ^{14}C either at both carbons or only at the second carbon atom. Less $^{14}CO2$ was detected in rats exposed to [2–^{14} C]–1-chloro-2-propanol than in rats exposed to [2,3- ^{14}C]–1-chloro-2-propanol, indicating that the second and third carbon atoms are both metabolised, at least in part, to CO_2.

No information on the absorption, distribution, metabolism, or excretion of 1-chloro-2-propanol in humans was found in the literature."

The Panel noted that ADME data were mainly confined to 1-chloro-2-propanol. These data indicated extensive absorption and metabolism of this compound with 80 % elimination in urine and expired air within 24 to 70 hours. The presence of cysteine and N-acetylcysteine conjugates indicates the potential formation of reactive intermediate(s) during the metabolism of 1-chloro-2-propanol.

Toxicological data for PCH

Genotoxicity

The applicant did not provide a summary of genotoxicity studies. In the dossier, the applicant stated that "propylene chlorohydrins are not genotoxic".

The Panel noted that several *in vitro* and two *in vivo* genotoxicity studies, using a mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (72:25 %), were available. There was one *in vitro* study of 1-chloro-2-propanol using bacteria. The Panel prepared a summary of these studies.

In vitro assays with bacteria

A mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) was tested by Rosenkranz et al. (1975) at levels of 0, 1.1, 2.2, 5.5, 11.0, 16.5 and 22 mg/plate in *Salmonella typhimurium* tester strains TA1535 and TA100, only in the absence of metabolic activation, and was found positive in *S. typhimurium* TA 1530.

Carr and Rosenkranz (1978) tested 1-chloro-2-propanol (purity not stated) at levels of 0, 0.1, 1, 2, 5, and 10 µl/plate in *S. typhimurium* TA 1535, in the absence and presence of an S9 microsomal fraction derived from arochlor-induced rat livers, and found a positive result in this strain. 1-Chloro-2-propanol was also tested in *S. typhimurium* strain TA 100 at 0 and 2 µl/plate, in the absence of S9, and found to have weak mutagenic effect. No effects were observed after testing 1-chloro-2-propanol in *S. typhimurium* strain TA 1538 at 0, 5 and 10 µl/plate.

Biles and Piper (1983) tested a mixture of 1-chloro-2 propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) at levels of 0, 527, 5 270, 10 500, 52 700 and 16 250 µg/plate in *S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100, in the absence and presence of an S9 microsomal fraction derived from arochlor-induced rat livers. The highest dose was toxic for the bacteria. The results obtained with TA 1535 and TA 100 indicated clear dose-related mutagenic responses with and without S9 activation. There was a marked enhancement of the mutagenic response in TA 1535 when S9 was added. TA 1538, TA 1537 and TA 98 showed no mutagenic responses.

1-Chloro-2-propanol and an isomeric mixture of 1-chloro-2-propanol and 2-chloro-1-propanol of unknown proportions were tested by Pfeiffer and Dunkelberg (1980) at levels of up to 0–160 µmol/plate and were found positive for mutagenic effects in *S. typhimurium* TA 1535 and TA 100, but not in TA 1537 and TA 98, in the absence of S9. The isomeric mixture was more potent in *S. typhimurium* TA 1535 than in TA 100. The effect in *S. typhimurium* TA 100 was comparable for 1-chloro-r-propanol alone and an isomeric mixture of 1-chloro-2-propanol and 2-chloro-1-propanol.

Zeiger et al. (1987) reported the results of mutagenicity testing of PCH carried out in two laboratories under the framework of the carcinogenicity bioassay performed at the National Cancer Institute (NCI) (NTP, 1998). The test material, a mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively), was clearly mutagenic in *S. typhimurium* TA 1535 in the absence and presence of S9, weakly positive or equivocal in TA100 and negative in strains TA 97, TA 98, TA 1537, with and without metabolic activation by a liver S9 fraction from arochlor-induced rats.

In vitro assays with mammalian cells

Galloway et al. (1987) reported the results of *in vitro* cytogenetic assays with PCH. A mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) was tested in assays for chromosomal aberration and sister chromatid exchange (SCE) induction in Chinese hamster ovary cells, with and without metabolic activation by a liver S9 fraction from arochlor-induced rats. Clearly positive results were obtained in both the SCE (at 167–4 000 µg/mL) and chromosomal aberration (at 3 000–5 000 µg/mL) tests, with and without S9.

Biles and Piper (1983) reported results from a L5178Y mouse lymphoma assay. An initial toxicity assay was conducted with seven concentrations of a mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively), ranging from 1.0 to 9 960 µg/mL, both in the absence and presence of S9. The mutagenicity test was conducted using 15 concentrations (ranging from 4 506–10 241 µg/mL) in cultures, in the absence and presence of S9. Non-toxic mutagenic doses ranged from 5 000 to 10 000 µg/mL. Although the mixture of 1-chloro-2-propanol was mutagenic in the TK+/– mouse lymphoma assay with and without S9, a linear dose–response relationship was not observed.

Kerckhaert et al. (1996) tested a mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) in the Syrian hamster embryo cell transformation assay at doses of 0, 1 000, 2 000, 3 000, 4 000 and 5 000 µg/mL and observed a positive result.

Gibson et al. (1997) tested a mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) in the micronucleus test in Syrian hamster embryo cells at doses of 0, 3 000, 3 500 and 4 000 µg/mL and observed a weak (1.5- to 2.5-fold) dose-related increase in micronuclei.

Drosophila tests

Foureman et al. (1994) reported the results of tests in *Drosophila* performed in connection with the NTP bioassay. The same mixture of 1-chloro-2-propanol and 2-chloro-1-propanol tested in other NTP studies (with an isomer ratio of 75:25) was tested in the sex-linked recessive lethal (SLRL) and chromosomal reciprocal translocation (RT) tests in *Drosophila melanogaster*. The SLRL test was performed by feeding (as a sucrose solution at 200 ppm for three days) and by injection (about 0.2–0.3 µl/fly of a 1 000 ppm solution in saline). The RT test was performed by injection only, at the same dose as used in the SLRL test. The results of the SLRL assay suggested that 1-Chloro-2-propanol is weakly mutagenic, but only by the injection route, and did not induce reciprocal translocations.

In vivo assays with mammals

Biles and Piper (1983) described a cytogenetic assay performed in rat bone marrow with a mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (ratio of 75:25). Rats were dosed orally by gavage with 0, 10, 31 and 100 mg/kg body weight (bw) per day for five days. At the highest dose, the body weight gain was not significantly decreased and no other effects on body weight gain were observed. The mean mitotic index was not different among the groups. There was a significantly dose-related increase in the number of aberrations, mostly breaks.

Another *in vivo* assay was carried out in conjunction with a preliminary sub-chronic oral toxicity study (33–3 000 ppm in drinking water) on chloropropanol (isomer ratio of 75:25) performed at the NCI (MacGregor et al., 1990). At the end of the 14-week exposure period, blood samples were obtained from male and female B6C3F1 mice, blood smears prepared and, after staining with a chromatin-specific fluorescent dye, micronuclei scored in 2 000 polychromatic erythrocytes and 10 000 normochromatic erythrocytes in each of 10 animals per group. No increase in micronuclei in either cell type was observed in treated mice compared with control animals. The Panel noted that this study was primarily designed for toxicity testing, and that the range of dosages applied may be inadequate for genotoxic hazard identification. The Panel also noted that no positive controls were performed. Therefore, the Panel concluded that the results of this study should be considered inconclusive for risk assessment.

Acute oral toxicity

The applicant provided a reference (Bevan, 2001) in which the oral dose required to kill 50 % of the study population (LD_{50}) was mentioned in a table. These data were presented by the applicant in

Table 1 of Attachment 5 "Summary of Oral Toxicity Data for Propylene Chlorohydrins (PCH)". In this table several studies in rats reporting PCHs with LD_{50} values of 100–300 and 381 mg/kg bw and 0.22 mL/kg bw (no density value available) were reported.

Short-term and sub-chronic toxicity

The applicant provided references to 14-week studies in mice and rats. These studies were presented by the applicant in Table 1 of Attachment 5 "Summary of Oral Toxicity Data for Propylene Chlorohydrins (PCH)". The Panel also identified two 14-day studies in mice and rats.

The NTP (1998) performed a 14-day oral study of PCHs in drinking water in B6C3F1 mice (10 animals/sex/group) at dose levels of 0, 100, 330, 1 000, 3 300 and 10 000 mg technical grade 1-chloro-2-propanol/L (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) equal to 0, 20, 60, 175, 430 or 630 mg/kg bw per day in males and 0, 25, 95, 290, 640 or 940 mg/kg bw per day in females. One male mouse in the 10 000 mg/L group died before the end of the study. Mean body weight gains of mice from the 10 000 mg/L group were significantly lower than those of the controls. Water consumption was significantly decreased in the two high dose groups. Male and female liver weights of the 1 000, 3 300 and 10 000 mg/L animals were significantly increased and thymus weights of the 10 000 mg/L mice were significantly lower than those of the controls. Exposure at levels of 1 000 mg/L increased relative liver weights in females and increased the vacuolization of hepatocyte cytoplasms in both males and females. The no observed adverse effect level (NOAEL) derived from the study was 60 mg/kg bw per day (330 mg/L drinking water).

The NTP (1998) also performed a 14-day oral study of PCHs in drinking water in F344/N rats (10 animals/sex/group) at dose levels of 0, 100, 330, 1 000, 3 300 and 10 000 mg technical grade 1-chloro-2-propanol/L (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) equal to 0, 15, 45, 140, 260, or 265 mg/kg bw per day. Two of the high dose females died before the end of the study. Body weights and water intake of the 3 300 and 10 000 mg/L group were decreased. The thymus weights of 10 000 mg/L rats were significantly lower than those of the controls. Exposure to 1-chloro-2-propanol caused cytoplasmic alterations and degeneration of the acinar cells and fatty change in the pancreas, atrophy of the bone marrow, and atrophy and extra medullary haematopoiesis of the spleen in males and females. The NOAEL derived from the study was 45 mg/kg bw per day (330 mg/L drinking water).

Philips and Dubois (1981) performed an oral 21-day study in rats (most probably by gavage) at doses of 0, 7.6, 25.4 and 76 mg chloropropanol/kg bw per day (75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) (number of animals not specified). Haematology, urine analysis and clinical chemistry were performed. At sacrifice, 21 tissues were examined histopathologically. No treatments related to mortality, effects on body weight, haematology, clinical chemistry, urine analysis or histopathology were reported. The Panel noted that only an abstract summarising this study was available.

The NTP (1998) performed a 14-week oral study in drinking water in B6C3F1 mice (10 animals/sex/group) at dose levels of 0, 33, 100, 330, 1 000 or 3 300 mg technical grade 1-chloro-2-propanol/L (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) equal to 0, 5, 15, 50, 170 or 340 mg/kg bw per day in males and 0,7, 20, 70, 260 or 420 mg/kg bw per day in females. One male from the 330 mg/L group died before the end of the study. No treatment-related effects on body weights were observed. Minimal anaemia was observed in the 3 300 mg/L males. Epididymis weights of the high dose males were significantly increased. Kidney weights of the high dose animals, liver weights of 1 000 mg/L males and of all exposed groups of females, and thymus weights of 1 000 and 3 300 mg/L females were increased. The incidences of pancreatic acinar cell degeneration and fatty change in 3 300 mg/L males and females, and cytoplasmic vacuolization of the liver in all groups of exposed females, were significantly higher than in controls. The severities of renal tubule cytoplasmic vacuolization were greater in 1 000 and 3 300 mg/L males than in the controls. The Panel considered that the NOAEL of the study was 50 mg/kg bw per day (330 mg/L drinking water).

The NTP (1998) also performed a 14-week oral study in drinking water in F344/N rats (10 animals/sex/group) at dose levels of 0, 33, 100, 330, 1 000 or 3 300 mg technical grade 1-chloro-2-propanol/L (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) equal to 5, 10, 35, 100, or 220 mg/kg bw per day. No mortalities occurred. Mean body weight gains and water

consumption of the high dose rats were significantly lower than those of the controls. A minimal to mild anaemia was observed in exposed female rats. The cauda epididymis and the epididymis weights of the high dose males were significantly decreased. The percentage of abnormal sperm in 3300 mg/L (high dose) males and the concentration of epididymal sperm in 330 mg/L males were significantly higher than in controls. The incidences of acinar cell degeneration and fatty change of the pancreas in 1 000 and 3 300 mg/L rats, hepatocytic metaplasia of the pancreatic islets in 3300 mg/kg diet females, cytoplasmic vacuolization of the liver in 100, 1 000 and 3 300 mg/L males, and renal tubule epithelium regeneration in 3 300 mg/L females were increased compared with the controls. The Panel considered that the NOAEL of the study was 35 mg/kg bw per day (330 mg/L drinking water).

Chronic toxicity and carcinogenicity

The applicant provided references for two-year carcinogenicity studies in mice and in rats. These studies were presented by the applicant in Table 1 of Attachment 5 "Summary of Oral Toxicity Data for Propylene Chlorohydrins (PCH)".

In the NTP study (1998), 50 male and 50 female B6C3F1 mice were given drinking water containing 0, 250, 500 or 1 000 mg 1-chloro-2-propanol/L (technical grade 1-chloro-2-propanol (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol)), equal to mean doses of approximately 45, 75 or 150 mg/kg bw per day for males and 60, 105 or 210 mg/kg bw per day for females during the first several months of the study and 25, 50 or 100 mg/kg bw per day for the remainder of the two-year study, for up to 105 weeks. No haematology, clinical chemistry or urine analyses were performed in this study. Multiple organs were histopathologically examined. Survival of all exposed groups was similar to that of the controls. The mean body weights of all exposed mice were similar to those of the controls throughout the course of the study. The water consumption of all exposed groups was similar to that of the controls. No treatment-related neoplasms or non-neoplastic lesions were observed in this study.

Also in the NTP study (1998), 50 F344/N rats were given drinking water containing 0, 150, 325 or 650 mg 1-chloro-2-propanol/L (technical grade 1-chloro-2-propanol (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol)), equal to approximately 15, 30 or 65 mg/kg bw per day during the first several months of the study and 8, 17 or 34 mg/kg bw per day for the remainder of the two-year study, for up to 105 weeks. No haematology, clinical chemistry or urine analyses were performed in this study. Multiple organs were histopathologically examined. The survival of all exposed groups was similar to that of the controls. The mean body weights of exposed rats were similar to those of the controls throughout most of the study. The water consumption of all exposed groups was similar to that of the controls. No treatment-related neoplasms or non-neoplastic lesions were observed in this study.

Reproductive and developmental toxicity

A study using reproductive assessment by continuous breeding and one oral preliminary study with chloropropanol were provided by the applicant. These studies were presented by the applicant in Table 1 of Attachment 5 "Summary of Oral Toxicity Data for Propylene Chlorohydrins (PCH)".

The exposure levels in the continuous breeding study, using Sprague–Dawley rats (Chapin and Sloane, 1997), were 0, 0.03, 0.065 and 0.13 % technical grade 1-chloro-2-propanol (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) in drinking water, equivalent to 0, 27, 58 and 117 mg 1-chloro-2-propanol/kg bw per day (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol), as determined using the default factor of 0.09 for sub-chronic studies in drinking water described by EFSA guidelines (EFSA Scientific Committee, 2012a). In the F0 generation, no changes in the number of litters per pair or the number or weight of live pups per litter were observed. Postpartum dam weights were reduced for the mid-dose (7 %) and high-dose females (15 %). Sire weights were only decreased in the high-dose group. These decreases were consistent with the 10 and 30 % reduction in water consumption for the mid- and high-dose groups, respectively. The last litter from all groups of the F0 generation was reared until weaning at postnatal day 21. While pup viability was unaffected by the test substance, pup weight of the F1 pups was reduced by 9 and 20 % in the mid- and high-dose groups, respectively. When adult F1 males and females of the control and high-dose groups were mated within the group, no effects on reproduction were observed. The only reproduction-related effects in the F1 males were decreased absolute testis

weight and increased relative epididymis weight. In the high-dose F1 female and male animals, body weights and kidney weights were decreased. However, the Panel noted that these effects were observed along with decreased adult weights of the male and female F1 animals. Some parameters were only tested in the high-dose group and not in the mid- and low-dose groups of this study (e.g. F1 females of these groups were not mated and therefore no parameters could be measured in F2 pups). Therefore, the Panel noted that there is some uncertainty regarding the NOAEL of 58 mg 1-chloro-2-propanol/kg bw per day (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol) proposed by the applicant.

Chloropropanol (75 % 1-chloro-propanol, 25 % 2-chloro-1-propanol) was administered by gavage to five mated (four or five pregnant) animals/group, from gestation day (GD) 6 to 15, at dose levels of 8, 20, 50 and 125 mg/kg bw per day (Philips, 1980). A Cesarean section was performed at GD 20. Mean number of corpora lutea, implantations and live fetuses were similar among the groups. Except for the 20 mg/kg bw per day group, where the number of resorptions was increased, the number of resorptions was similar in all groups. Two fetuses of two different litters of the 50 mg/kg bw per day group showed major malformations (both had shortened lower jaws and one, in addition, had a reduced number of digits on the left foreleg, bifurcation of the sternum and ocular malformation). The Panel noted that the study report stated that only external examinations were performed; however, the Panel considered that it would be difficult to diagnose bifurcation of the sternum without skeletal staining. The authors of the study concluded that chloropropanol may be a weak teratogen. The Panel noted that this is only a preliminary study with a low number of animals. Therefore, a full prenatal developmental study by gavage, performed in accordance with current Organisation for Economic Cooperation and Development (OECD) guidelines, would be necessary to make any conclusions regarding developmental toxicity.

The Panel noted that the applicant, in Attachment 5 of the technical dossier, stated that "No malformations were observed upon gross examination of the foetuses", whereas the authors of this preliminary study noted that 1-chloro-2-propanol should be considered as a weak teratogen.

2.3. Methodologies

The current "Guidance for submission for food additive evaluations" (EFSA ANS Panel, 2012) was followed by the ANS Panel for the evaluation of the change in the specifications for the already authorised food additive HPMC (E 464). In addition, an estimate of the exposure to PCH, according to the requested FAIM approach, was provided.

3. Assessment

The Panel was asked to evaluate the safety of a proposed change in the specifications for PCH residual levels in HPMC. The Panel received a dossier from the applicant and subsequently requested additional data. The original dossier stated that substantial toxicological data were available for PCH and that safety data from JECFA (1969–1973) should be taken into account. In its initial discussions, the Panel was made aware of published studies which appeared to contradict the statements of the applicant. Therefore, in agreement with the EFSA guidance for submission for food additives evaluation (EFSA ANS Panel, 2012), the Panel requested data regarding the levels of PCH in at least five representative batches of HPMC that were independently produced.

The applicant cited ambiguities related to the PCH limit stated by the EC and to inaccuracies of the analytical method used for measuring PCH levels as reasons for their proposal to raise the PCH specification limits. According to the applicant, high background noise produced by the XSD used in the former analysis method made results unreliable. Also, the wax column permitted co-elution of the PCH with unknown compounds leading to inaccuracies and higher apparent PCH concentrations. The Panel noted that the proposal to raise the PCH specification limits did not appear to be supported by the results obtained with methods 2 and 3.

In response to the Panel's request for data on PCH levels in at least five batches, results were provided for two HPMC products (seven batches for one product and five for the other). All of the reported values were below the current specification of 0.1 mg/kg. The Panel noted that these results appear to contradict the stated technical need for the change in specification.

The Panel noted that the requested literature search had not identified the ADME references cited in the NTP report. The data on ADME were mainly on 1-chloro-2-propanol and limited data on 2-chloro-1-propanol or the mixture of both isomers were available. The Panel noted that the ADME data indicated that an active intermediate might be formed, which appeared to be detoxified by glutathione conjugation leading to cysteine conjugate excretion.

The Panel does not agree with the applicant's statement in the dossier that "propylene chlorohydrins are not genotoxic". The Panel noted that 1-chloro-2-propanol and the mixture of 1-chloro-2-propanol and 2-chloro-1-propanol (75 % and 25 %, respectively) showed mutagenic properties in S. Typhimurium strains TA 1535 and TA 100 in the absence and presence of an S9 fraction. The mixture of 1-chloro-2-propanol isomers was mutagenic and clastogenic in cultured mammalian cells, with and without metabolic activation with S9, and induced micronuclei and morphological transformation in hamster embryo cells. The same mixture was also weakly positive in a mutation test using *Drosophila* germ cells and clastogenic in rat bone marrow *in vivo.* Based on the available results, the Panel concluded that data on PCH (both 1-chloro-2-propanol and the mixture of 1-chloro-2-propanol and 2-chloro-1-propanol) were indicative of a genotoxic hazard

The Panel also noted that there is some additional uncertainty concerning the proposed NOAEL of 58 mg/kg bw for 1-chloro-2-propanol (approximately 75 % 1-chloro-2-propanol and 25 % 2-chloro-1-propanol), as some parameters in the reproductive toxicity study were tested only in the high-dose group and not in the mid- and low-dose groups.

The Panel concluded that the NOAELs for sub-chronic toxicity and from the NTP carcinogenicity study were similar (around 35 mg/kg bw per day in the rat). The Panel considered that the lack of neoplastic effects in this study at all three dose levels tested in mice and rats demonstrated a lack of carcinogenic potential. However, it was not possible to derive a $BMDL_{10}$ as there were no relevant lesions at any dose level. Furthermore, the Panel considered that using a margin of exposure (MOE) approach was not applicable because PCHs do not fulfil the basic criteria of being both genotoxic and carcinogenic required for this method to be applicable (EFSA Scientific Committee, 2012b).

It should be noted that, in the Attachment 5 "Propylene Chlorohydrin Residues in Hydroxypropyl Methyl Cellulose" of the technical dossier provided by the applicant, it is stated that "No malformations were observed upon gross examination". The author (Phillips, 1980) of this study, however, concluded that chloropropanol may be a weak teratogen The Panel also noted that, although this is only a preliminary study with a low number of animals, the author conclusion is valid. Therefore, in the absence of a full prenatal developmental study by gavage, performed in accordance with the current OECD guidelines, the Panel cannot make any conclusions on developmental toxicity. The EFSA

Scientific Committee (2011) considered that clear evidence of genotoxicity in somatic cells *in vivo* should be regarded as an adverse event per se since genotoxicity is also implicated in other degenerative diseases. The Panel noted that when evidence of genotoxicity is less clear, using a weight of evidence evaluation, negative carcinogenicity data can be considered more conclusive on a case-by-case basis. However, since *in vivo* genotoxicity has occasionally been associated with teratogenic potential and given the results of the Philips study (1980), the Panel considered that the carcinogenicity data were not sufficient to make the conclusion that there was no genotoxic risk in this case.

The Panel noted that the exposure estimates provided by the applicant to support the change in specifications were based on the normal use levels of HPMC for a limited number of its authorised uses (around 20 out of 70). The Panel would only be able to compare the exposure from these selected uses with the available toxicological database. The Panel considered this assumption as being driven by huge uncertainties knowing that there are other authorised uses of HPMC and that there are also other sources of PCH exposure from other cellulosic or hydroxypropyl food additives. If these uncertainties are confirmed, the anticipated exposure to PCH would be higher than estimated and, therefore, any conclusions based on the comparison of the exposure with a toxicological reference point ($BMDL_{10}$ or NOAEL) are likely to result in a significant underestimation of the risk.

The Panel used an MOE approach only for residuals or contaminants in a food additive that are genotoxic and carcinogenic, which is not the case for PCH; in addition, there are currently no accepted MOE approaches for compounds which are only genotoxic. Instead, it would be possible to compare exposure to a residual with the threshold of toxicological concern (TTC) for genotoxic effects of 0.15 µg/person per day. The Panel noted that the exposure estimated using the current specification of 0.1 mg PCH/kg HPMC would be around this value. Consequently, at the requested new specification of 1 mg/kg, and without considering the fact that only some of the authorised uses (around 20 out of 70 from the legislation) were proposed, the TTC would be exceeded. Therefore, the Panel was unable to conclude that there would not be a genotoxic risk associated with the requested new specification of 1 mg PCH/kg HPMC.

4. Conclusions

The ANS Panel was asked to provide an opinion on the safety of increasing the PCH levels in HPMC from 0.1 to 1 mg/kg. PCH is a residue formed during hydroxypropylation of starches and 1 mg/kg is the current limit defined by JECFA. The applicant provided supporting analytical and toxicity data for PCH.

Based on the following considerations:

- exposure is likely to be underestimated since data were only provided for a limited number of authorised uses and other sources of PCH were not considered;
- information on ADME was not provided despite the request for a literature search;
- the statement, by the applicant, that propylene chlorohydrins "are not genotoxic" is not correct;
- no carcinogenic effects were observed in relevant chronic/carcinogenicity studies and it was impossible to derive a $BMDL_{10}$;
- a weak teratogenic action was reported, but the limited studies available were insufficient to reach a conclusion regarding developmental toxicity;
- an MOE approach to impurities, as suggested by EFSA guidance (EFSA Scientific Committee, 2012b), was not possible;
- when using a TTC approach to the consideration of the genotoxic substance, the Panel was unable to make any conclusion regarding the absence of risk;
- analytical data provided for a total of 12 different batches of HPMC show that PCH levels are in compliance with the current specifications of 0.1 mg/kg.

the Panel concluded that the available data are insufficient to support a change in the specification from 0.1 to 1 mg PCH/kg HPMC.

Documentation provided to EFSA

1. Request for a change on the specifications of E 464 to the European Commission for an Amendment of Annex II to Regulation (EC) 1333/2008 concerning PCH levels. October 2013. Dossier submitted by Organisation des Fabricants de produits Cellulosique Alimentaires (OFCA).

2. Attachment 5 "Propylene Chlorohydrins residues in Hydroxypropyl Methylcellulose – Summary of Oral Toxicity Data for Propylene Chlorohydrins (PCH)", Dow Europe Gmbh, 2008.

3. "Potential Dietary Exposure to PCH Residue in HPMC Food Additive". November 2014. Dossier submitted by Organisation des Fabricants de produits Cellulosique Alimentaires (OFCA).

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Abbreviations

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ANS Panel	Panel on Food Additives and Nutrient Sources added to Food
$BMDL_{10}$	lower benchmark dose level
bw	body weight
EU	European Union
FAIM	Food Additives Intake Model
FAO	Food and Agriculture Organization of the United Nations
GD	gestation day
HPMC	hydroxypropyl methyl cellulose
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	lethal dose 50
MOE	margin of exposure
MSD	mass selective detection
NCI	National Cancer Institute
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Cooperation and Development
OFCA	Organisation des Fabricants de produits Cellulosiques Alimentaires
PCH	propylene chlorohydrin
RT	reciprocal translocation
SCE	sister chromatid exchange
SLRL	sex-linked recessive lethal
TTC	threshold of toxicological concern
WHO	World Health Organization
XSD	halogen-specific detector