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Safety assessment of the substance poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) for use in food contact materials

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Abstract

This opinion of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) deals with the safety assessment of poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate), Chemical Abstracts Service (CAS) No 147398-31-0 and food contact material (FCM) substance No 1059, for contact with dry/solid food. This biodegradable (co)polymer is produced by fermentation of palm oil using a genetically modified microorganism (*Cupriavidus necator*). No migration of oligomers into food simulant E (10 days at 40 and 60°C) was found at a detection limit per single oligomer of 5 μg/kg food. Migration of the degradation product crotonic acid was 8 and 25 μg/kg at the two test temperatures, respectively. The other migrating substances detected, [redacted], likely originated from or are related to the authorised substance (FCM No. 9) 'palm oil and/or palm fatty acid distillate' used as carbon source for the fermentation. At the migration levels reported, these migrants do not give rise to safety concern. No genotoxicity data are required for poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) because of its high molecular weight. The fraction below 1,000 Da is 0.5%. The major monomeric unit in the copolymer, 3-hydroxybutyric acid, is an intermediate in fatty acid metabolism. The minor monomeric unit, 3-hydroxyhexanoic acid, tested negative for bacterial gene mutations. Degradation products, which may be present in the (co)polymer, are crotonic acid and (*E*)-2-hexenoic acid. Crotonic acid is authorised for use in FCM with a specific migration limit (SML) of 0.05 mg/kg food; for (*E*)-2-hexenoic acid, no indication for genotoxicity was identified by the EFSA CEF Panel in its 2010 group evaluation of flavouring substances in FGE.05Rev2. The CEF Panel concluded that the substance poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) is not of safety concern if used alone or in blends with other polymers for contact with dry/solid food. If the SML of crotonic acid is met, migration of (*E*)-2-hexenoic acid will also not exceed 0.05 mg/kg food.

Keywords: CAS No 147398-31-0, Poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate), PHBH, food contact materials, FCM substance No 1059, dry/solid foods

Requestor: European Commission

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Note: The full opinion will be published in accordance with Article 10(6) of Regulation (EC) No 1935/2004 once the decision on confidentiality, in line with Article 20(3) of the Regulation, will be received from the European Commission. The migration test of reaction products and/or residuals from the starting substance has been provided under confidentiality and the results are redacted awaiting the decision of the Commission.

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1. Introduction

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

In accordance with Article 29(1) (a) of Regulation (EC) No 178/2002[1], the European Commission asks EFSA to supplement its Opinion published in May 2016 assessing the safety of the substance Poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) ('PHBH') for use in food contact materials, with ON 4464.[2]

The new requested Opinion will address the potential risks to the health of consumers related to the use of food contact materials manufactured with PHBH in contact with those foods for which Table 2 of Annex III to Commission Regulation (EU) No 10/2011 assigns food simulant E, for up to 40°C, and for storage up to 30 days in accordance with the test temperature and test times set out in Table 1 and Table 2 of Annex V to Regulation (EU) No 10/2011 respectively.

2. Data and methodologies

2.1. Data

The applicant (TNO Triskelion BV on behalf of Kaneka Belgium N.V.), who had previously submitted an application for use of PHBH for food contact materials (FCM) in contact with food groups 04.01 and 04.04 (EFSA-Q-2015-00414; EFSA CEF Panel, 2016), has submitted a new dossier in May 2017 requesting the evaluation of the safety of use of the substance for articles in contact with all foods (EFSA-Q-2017-00412). The information provided within that new dossier was used to address the request by the European Commission for evaluation of PHBH for FCM for those foods ('dry/solid foods') for which Table 2 of Annex III to Commission Regulation (EU) No 10/2011 assigns food simulant E (i.e. poly(2,6-diphenyl-*p*-phenylene oxide), for up to 40°C and for storage up to 30 days in accordance with the test temperature and test times set out in Table 1 and Table 2 of Annex V to Regulation (EU) No 10/2011, respectively.

Data submitted and used for the evaluation are:

Non-toxicological data and information

- Chemical identity
- Description of manufacturing process of substance/FCM
- Physical and chemical properties
- Intended use
- Existing authorisation(s)
- Identification, quantification and migration of oligomers, reaction products and impurities

Toxicological data

- Bacterial gene mutation tests
- *In vitro* mammalian chromosome aberration test
- *In vivo* mouse bone marrow micronucleus test

2.2. Methodologies

The assessment was conducted in line with the principles laid down in Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food. This Regulation underlines that applicants may consult the Guidelines of the Scientific Committee on Food (SCF) for the presentation of an application for safety assessment of a substance to be used in FCM prior to its authorisation (European Commission, 2001), including the corresponding data requirements. The dossier that the applicant submitted for evaluation was in line with the SCF guidelines (European Commission, 2001).

The methodology is based on the characterisation of the substance that is the subject of the request for safety assessment prior to authorisation, its impurities and reaction and degradation products, the evaluation of the exposure to those substances through migration and the definition of minimum sets of toxicity data required for safety assessment.

To establish the safety from ingestion of migrating substances, the toxicological data indicating the potential hazard and the likely human exposure data need to be combined. Exposure is estimated from

[1] OJ L 13, 1.2.2002. p. 1.

[2] EFSA Journal 2016; 14(5):4464; concerning Poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) ('PHBH'), Chemical Abstracts Service (CAS) No 147398-31-0, EFSA-Q-2015-00414, FCM No 1059.

studies on migration into food or food simulants and considering that a person may consume daily up to 1 kg of food in contact with the relevant FCM.

As a general rule, the greater the exposure through migration, the more toxicological data is required for the safety assessment of a substance. Currently, there are three tiers with different thresholds triggering the need for more toxicological information as follows:

a) In case of high migration (i.e. 5–60 mg/kg food), an extensive data set is needed.
b) In case of migration between 0.05 and 5 mg/kg food, a reduced data set may suffice.
c) In case of low migration (i.e. < 0.05 mg/kg food), only a limited data set is needed.

More detailed information on the required data is available in the SCF guidelines (European Commission, 2001).

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

3. Assessment

According to the applicant, the substance poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate), CAS No 147398-31-0, is a biodegradable (co)polymer used as a 100% material or compounded together with other bio-based/biodegradable materials, such as starch, polylactic acid polymer, polybutyrate adipate terephthalate, polybutylene succinate and plasticisers, to produce packaging articles, such as bags or trays, at a maximum process temperature of 200°C. According to the request, the use of the substance is assessed for films or sheets intended to be in long term contact, i.e. 6 months or more, with dry/solid foods at room temperature or below, including hot-fill and/or short heating up conditions.

3.1. Non-toxicological data

Chemical formula: H[C4H6O2]x[C6H10O2]yOH (Figure 1)

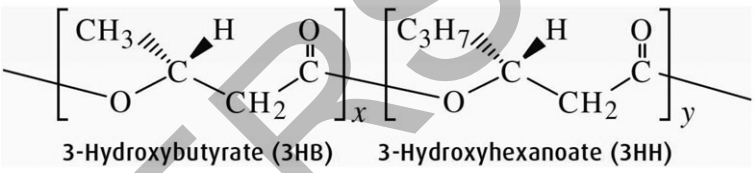

Figure 1: Chemical structure of poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate)

The substance poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) is a biodegradable (co)polymer produced by fermentation of palm oil and/or palm oil fatty acid distillate as carbon source using genetically modified microorganisms (*Cupriavidus necator*). Before isolating the (co)polymer, the microorganisms are inactivated by heat treatment. The (co)polymer is obtained at a purity of higher than 98%, consisting of 80–99% 3-hydroxybutyrate (3HB) and 1–20% 3-hydroxyhexanoate (3HH).

The molecular weight of the substance ranges from 10,000 to 1,000,000 Da with Mw = 518,000 Da, Mn = 182,000 Da. The fraction below 1,000 Da amounts to 0.5%, with the major part being above 500 Da.

The substance has a melting point range of 120–150°C, depending on the co-monomeric composition. It is thermally stable up to 250°C and, therefore, stable under the manufacturing conditions of maximum 200°C. The (co)polymer is stable at room temperature against hydrolysis in water. It is insoluble in water and ethanol, sparingly soluble in toluene, chloroform and tetrahydrofuran (THF).

Since the carbon source for the fermentation is palm oil, which is already listed as FCM No 9 under Regulation (EU) No 10/2011 without a specific restriction, specific migration of this starting substance was not tested.

The overall migration (OM) was tested using simulants 10% ethanol, 3% acetic acid and olive oil at conditions of 10 days at 60°C and, in all cases the migration was lower than 6 mg/kg. The Panel noted that these simulants and their associated test conditions are too severe for the dry/solid food applications envisaged and considered the overall migration test results as informative and indicative of the material inertness.

As required by Regulation (EU) No 10/2011 for contact with dry/solid foods, migration of oligomers and other potential reaction products was tested using simulant E. Under test conditions of 10 days at 40°C and 60°C and using liquid chromatography-mass spectrometry (LC–MS), no migration of oligomers was found at a detection limit per single oligomer of 5 μg/kg food.

The migration solutions from the simulant E test were also analysed by gas chromatography–mass spectrometry (GC–MS) in order to investigate the presence and identity of other migrants such as residuals from the starting substance or reaction products. From the test at 40°C which was considered relevant versus the intended application, semiquantitative analysis of the food simulant E solvent extracts, assuming uniform response factors, the following migrants and migration levels were found:

[redacted]

These substances were concluded to originate from or to be likely related to the source material 'palm oil and/or palm fatty acid distillate' already authorised under Regulation (EU) No 10/2011.

Thermolysis of the end groups, the 3-hydroxy carboxylic acids, can lead to the formation of crotonic acid ((*E*)-2-butenoic acid) and (*E*)-2-hexenoic acid. Crotonic acid is listed in Regulation (EU) No 10/2011 under FCM substance No 467 with a specific migration limit (SML) of 0.05 mg/kg food; (*E*)-2-hexenoic acid is listed as flavouring in Regulation (EC) No 872/2012 under Fl. No 08.119.

The specific migration of crotonic acid into food simulant E after 10 days at 40°C and 60°C was 8 and 25 μg/kg, respectively. From the compositional ratio of C4 to C6 units in the copolymer and due to the higher molecular weight of the hexenoic acid, the relative migration of both α,β-unsaturated acids will always be lower for the C6 acid.

3.2. Microbiological information

Given the heat treatment and the applied purification steps at the end of the production process and the heat treatment during manufacturing of the final FCM, the use of *Cupriavidus necator* as producing organism is considered to be of no safety concern.

3.3. Toxicological data

No genotoxicity data are required for poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) as the substance is a large polymer with a molecular weight ranging between 10,000 and 1,000,000 Da, which are unlikely to be absorbed in the gastrointestinal tract. Nevertheless, the applicant provided a complete set of genotoxicity tests, i.e. a bacterial gene mutation test, an *in vitro* chromosome aberration test and an *in vivo* micronucleus bone marrow test. The *in vitro* tests were negative but limited due to the precipitation of the compound in the culture medium. No increase in micronuclei frequency was observed in the *in vivo* test in mice, but no evidence was provided for the exposure of the bone marrow. The fraction below 1,000 Da amounts to 0.5%, with the major part being above 500 Da.

Considering that the (co)polymer is requested for use only with dry/solid foods, no contact with an aqueous phase, and therefore, no hydrolysis to the two constituent hydroxy acids and no subsequent migration are to be expected.

Nevertheless, the Panel noted that the major unit of this polymer, 3-hydroxybutyric acid, is an intermediate in fatty acid metabolism. For the minor unit of this polymer, 3-hydroxyhexanoic acid, a bacterial gene mutation test according to OECD TG471 was provided. It was tested for gene mutation in four histidine-requiring strains TA98, TA100, TA1535 and TA1537 of *Salmonella* Typhimurium, and in *Escherichia coli* strain WP2 uvrA both in the absence and in the presence of metabolic activation up to 5,000 μg/plate. The results of the study were considered negative.

According to the migration studies with the food simulant E, no oligomers were identified at a detection limit per single oligomer of 5 μg/kg food. Other than oligomers, several substances were found to migrate into food simulant E and it was concluded that they originate or are likely related to the already authorised substance (FCM No 9) 'palm oil and/or palm fatty acid distillate' used as carbon source for the fermentation. Given the nature and origin of these substances, their migration at the levels reported does not give rise to a safety concern.

Concerning the two degradation products that have been identified, crotonic acid is authorised under Regulation (EU) No 10/2011 for use in FCM with a SML of 0.05 mg/kg. For (*E*)-2-hexenoic acid, no indication for genotoxicity was identified based on limited genotoxicity data from structurally related

substances used in the group evaluation of flavouring substances in FGE.05Rev2 (EFSA CEF Panel, 2010). Given the structural similarity between crotonic acid and (*E*)-2-hexenoic acid, for the latter substance also an SML of 0.05 mg/kg food could be applicable. As the migration of (*E*)-2-hexenoic acid would be lower than that of crotonic acid, it will not exceed 0.05 mg/kg, if the migration limit for crotonic acid is met.

4. Conclusions

Having considered the above-mentioned data, the CEF Panel concluded that the substance poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate) is not of safety concern for the consumer if it is used either alone or blended with other polymers in contact with (dry/solid) foods for which Table 2 of Annex III to Commission Regulation (EU) No 10/2011 assigns food simulant E, under contact conditions up to 6 months or more at room temperature or below, including hot-fill or short heating up phase. The specific migration of the degradation product crotonic acid should not exceed 0.05 mg/kg food. As the migration of (*E*)-2-hexenoic acid can be expected to be always lower than that of crotonic acid, no individual restriction is necessary.

Documentation provided to EFSA

1) Poly((*R*)-3-hydroxybutyrate-co-(*R*)-3-hydroxyhexanoate). May 2017. Submitted by TNO Triskelion BV on behalf of Kaneka Belgium N. V.
2) Mandate received from the European Commission on 9 June 2017 and reception acknowledged by EFSA on 30 June 2017.
3) Additional data. August 2017. Submitted by Kaneka Belgium N. V.
4) Additional data. March 2018. Submitted by Kaneka Belgium N. V.

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European Commission, 2001. Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation. Available online: http://ec.europa.eu/food/fs/sc/scf/out82_en.pdf

Abbreviations

3HB	3-hydroxybutyrate
3HH	3-hydroxyhexanoate
CAS	Chemical Abstracts Service
CEF Panel	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
FCM	food contact material(s)
GC	gas chromatography
LC	liquid chromatography
MS	mass spectrometry
OM	overall migration
SCF	Scientific Committee on Food
SML	specific migration limit
THF	tetrahydrofuran