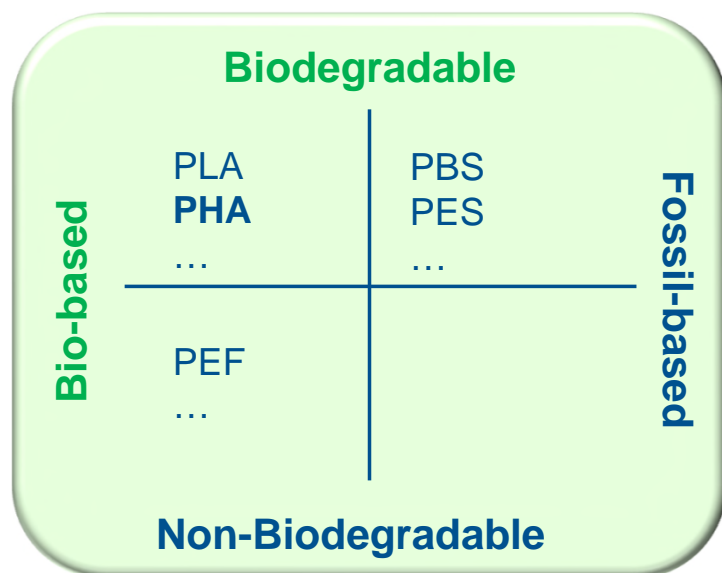


11th meeting of the Scientific Network on Food Contact Material (FCM), European Food Safety Authority, Parma, 22-24th, October, 2024

Chemical safety of polyhydroxybutyrate (PHB)

Ana Rodríguez Bernaldo de Quirós (USC)
Juana Bustos (CNA-AESAN)

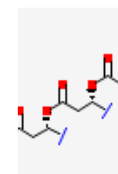
BIO-BASED AND BIODEGRADABLE POLYMERS



Polyhydroxyalkanoates (PHAs)



Aliphatic polyesters



Production by Gram
positive and Gram
negative bacteria



Polyhydroxybutyrate
Polyhydroxyvalerate

BIO-BASED AND BIODEGRADABLE POLYMERS

MIGRABIOQUANT Project

Objective: To establish an analytical methodology based on the development of methods for the identification of components present in bio-based and/or biodegradable polymers for food contact and to determine the migration.

Analytical Strategy



✓ Materials characterization



✓ Non-targeted methods: analysis of volatile & semi-volatile compounds

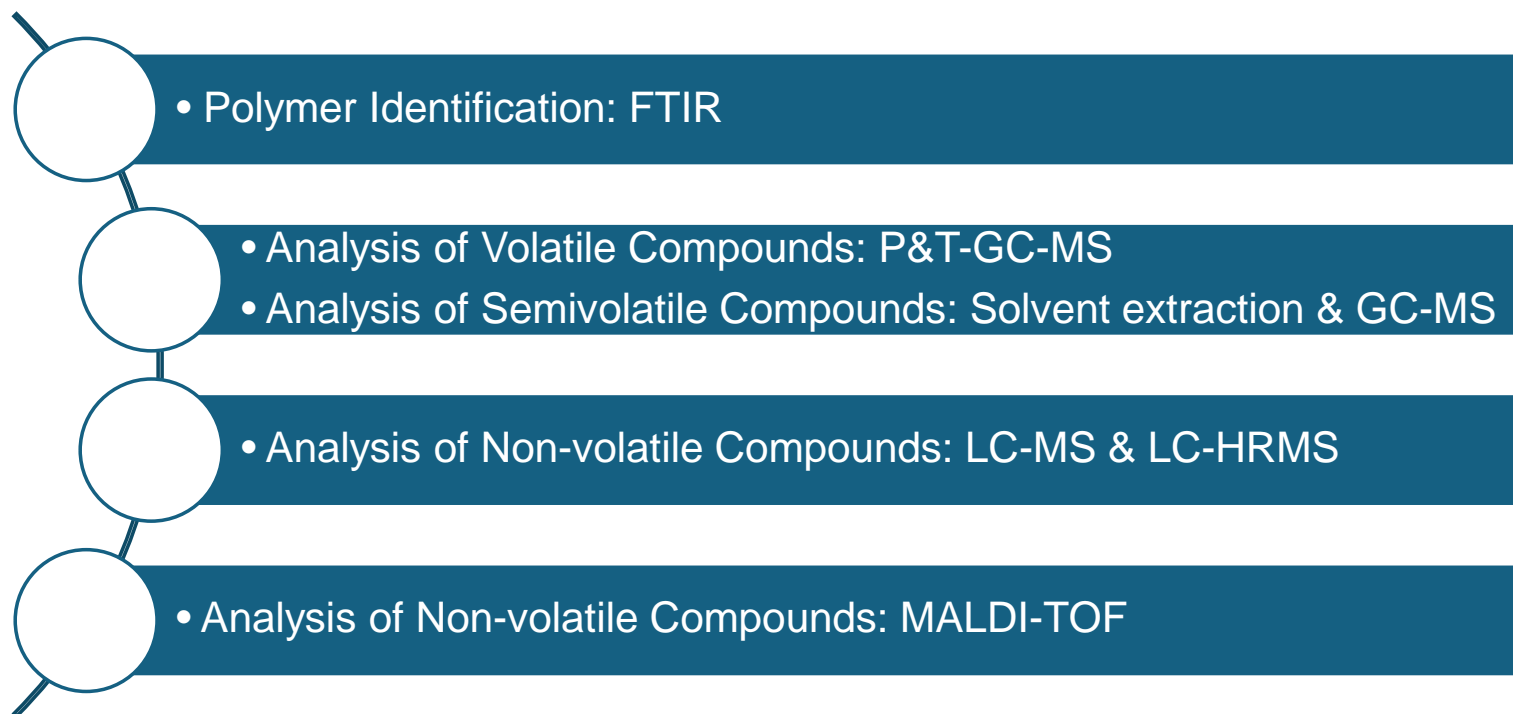


✓ Non-targeted methods: analysis of non-volatile compounds



✓ Migration assays

POLYHYDROXYBUTYRATE: CHEMICAL CHARACTERIZATION



POLYMER IDENTIFICATION-FTIR



Material



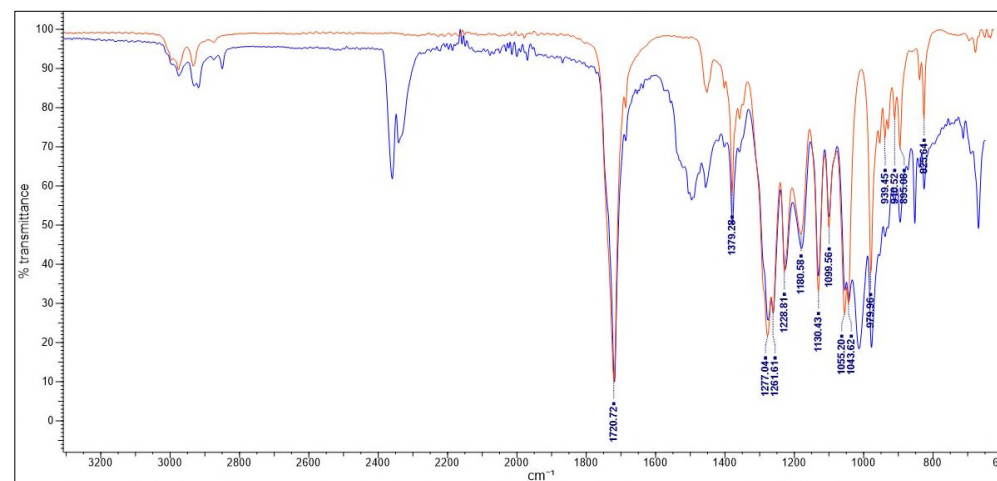
FTIR

Polymer
analysis

Polymer
identification

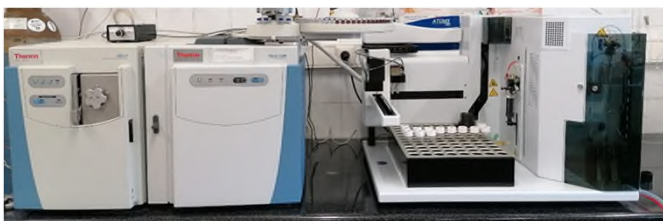
IR Spectral
Libraries

**Libraries of
Polymers & Related
Compounds**



CHEMICAL CHARACTERIZATION: VOLATILE COMPOUNDS

■ Non-targeted screening- P&T GC-MS



P&T-GC-MS

Compounds		Remarks
Alkanes	Hexane Nonane Decane Dodecane Tetradecane	Carbon source in the production of PHAs PHB extraction
Ketones	2-Pentanone Cyclohexanone	VOC identified in PHB production PHB extraction from bacterial cells
Acids	4-hydroxybutyric acid	Production of PHB
Esters	Methyl butyrate Methyl-2-methyl butyrate N-butyl acetate	Production of PHB
Others	gamma-Butyrolactone Styrene	Production of PHB To retard the thermal degradation of PHB

Summary of some compounds tentatively identified

CHEMICAL CHARACTERIZATION: SEMI-VOLATILE COMPOUNDS

Non-targeted screening- Solvent Extraction and GC-MS

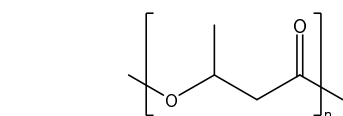
Compounds (MeOH Extracts)		Remarks
Acids	Butanoic acid; Heptanoic acid Octanoic acid; Crotonic acid Dodecanoic acid; Tetradecanoic acid; Oleic acid; Pentadecanoic acid; Hexadecanoic acid Octadecanoic acid	Production of PHB Degradation product
Esters	Methyl-4-hydroxybutyrate Methyl crotonate Methyl 4-methoxybutyrate Hexadecanoic acid, methyl ester	Methyl ester of the monomers of PHAs Degradation product PHB derivative
Others	Acetyl triethyl citrate Acetyl tributyl citrate (ATBC) Tributyl citrate	Additives, plasticizers

Compounds (Hexane-Acetone Extracts)		Remarks
Acids	Dodecanoic acid; Tetradecanoic acid; Oleic acid; Pentadecanoic acid; Hexadecanoic acid; 9-Hexadecenoic acid; 10-Heptadecenoic acid	Production of PHB
Others	Acetyl tributyl citrate (ATBC) Tributyl citrate	Additives, plasticizers

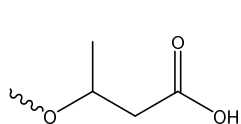
Summary of some compounds tentatively identified

CHEMICAL CHARACTERIZATION: NON-VOLATILE COMPOUNDS

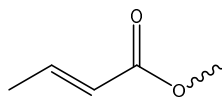
- Non-targeted screening- Solvent Extraction and LC-MS & LC-HRMS



PHB



Carboxylic chain-end



Crotonyl chain-end

LC-MS

Serie Oligomers Hydroxyl-terminated
From trimer (n=3) up to undecamer (n=11)

Serie Oligomers Crotyl-terminated
From trimer (n=3) up to undecamer (n=11)

- MALDI-TOF

INSTRUMENT & CONDITIONS

- Ultraflex III TOF/TOF mass spectrometer
- Positive reflector mode
- m/z 80-2500

MALDI-TOF

Serie Oligomers Hydroxyl-terminated

Serie Oligomers Crotyl-terminated

Lestido-Cardama et al. (2024). Tentative identification of non-volatile compounds in a biodegradable bio-based packaging material using a non-targeted method. Proceedings 11^o Shelf Life International Meeting, Reggio Emilia, 20-23 May, 2024.

Lestido-Cardama et al. (2024). Non-target analysis of a biodegradable bio-based food packaging material by LC-ESI-Q-Exactive. Proceedings, 20th Annual Workshop On Emerging High-Resolution Mass Spectrometry (Hrms) And Lc-Ms/Ms Applications In Environmental Analysis And Food Safety, Barcelona, 7-8 October 2024.

CONCLUDING REMARKS



- ✓ The chemical composition of a bio-based and biodegradable polymer based on polyhydroxybutyrate was investigated using a battery of analytical techniques including GC-MS, LC-MS, LC-HRMS and MALDI-TOF.
- ✓ A great variety of volatile and semi-volatile compounds were tentatively identified. Most of the compounds detected were related to the production and degradation of PHB. Additives plasticizers such as ATBC were also detected.
- ✓ Two series of oligomers, namely hydroxyl-terminated and crotyl-terminated were tentatively identified.
- ✓ Future work will be focused on testing migration and exposure assessment.

EFSA SCIENTIFIC OPINION: PHBH

Poly((R)-3- hydroxybutyrate-co-(R)-3-hydroxyhexanoate) (PHBH)

Characterization of the substance for safety assessment (prior to authorization)

Non-toxicological data and information

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

Toxicological data

- Bacterial gene mutation tests
- In vitro mammalian cell gene mutation test
- In vivo mouse bone marrow micronucleus test
- repeated dose 90-day oral toxicity study in rats
- Fluid tests simulating gastric and intestinal pH conditions
- Information on accumulation in rats

SCIENTIFIC OPINION

ADOPTED: 5 December 2018
doi: 10.2903/j.efsa.2019.5551

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

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),
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Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel) assessed the safety of poly((R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate) (PHBH), CAS No 147398-31-0 and food contact material (FCM) substance No 1059. This biodegradable copolymer is produced by fermentation of palm oil using a genetically modified microorganism (*Cupriavidus necator*). Overall migration was up to 5.4 mg/kg. Oligomers are hydroxyl-terminated or with crotyl- and hexenyl end-groups from dehydration of hydroxyl end-groups. In the absence of calibration standards, the total oligomer migration was set at the overall migration values. Other degradation products are crotonic acid and (E)-2-hexenoic acid. Crotonic acid is authorised for use in FCMs 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 CEP Panel in its group evaluation of flavouring substances in FCE/DSRev2 (EFSA CEP Panel, 2010b). The other migrating substances detected, [redacted] are from the authorised substance 'palm oil and/or palm fatty acid distillate' (FCM substance No 9) used as a carbon source for the fermentation and do not give rise to safety concern. A PHBH oligomer mixture was synthesized to simulate that migrating. It did not give rise to concern for genotoxicity. From the repeated dose 90-day oral toxicity study in rats, the Panel identified the no-observed-adverse-effect level (NOAEL) at the highest dose tested in males, 1,364 mg/kg body weight (bw) per day. The Panel concluded that the potential for bioaccumulation of oligomers is low. Overall, the CEP Panel concluded that the substance PHBH is not of safety concern for the consumer if it is used alone or blended with other polymers in contact with all kinds of food during more than 6 months at room temperature or below, including hot-fill or a short heating up phase. The specific migration of all oligomers < 1,000 Da should not exceed 5 mg/kg food. The migration of crotonic acid should not exceed the SML of 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.

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Keywords: CAS No 147398-31-0, poly((R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate), food contact materials, FCM substance No 1059, all foods

* Member of the former Working Group on "Food Contact Materials" of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEP).

EFSA SCIENTIFIC OPINION: PHBH



PHBH SAFETY ASSESSMENT

Intended use	To be used in FCM in contact with all kinds of food
Migration tests (10 days/60°C) (10 days/20°C) (10 days/40°C)	<ul style="list-style-type: none"> ▪ Simulant E (modified polyphenylene oxide) (dry foods) ▪ 10% ethanol (water and aqueous foods) ▪ 3% acetic acid (acidic foods) ▪ olive oil, isooctane and 95% ethanol (fatty foods)
Analytical Techniques	<ul style="list-style-type: none"> ▪ GC-MS ▪ LC-MS

EFSA SCIENTIFIC OPINION: PHBH

Migration Studies

- **Overall migration:** 10% ethanol (5.4 mg/kg); 3% acetic acid (3.0 mg/kg); olive oil (not detectable (< 6 mg/kg); **X** isooctane (41 mg/kg); **X** 95% ethanol (17 mg/kg).
The most representative simulant: **10% ethanol**
- **Simulant E → GC-MS:** substances originating from or to be likely related to the source material 'palm oil and/or palm fatty acid distillate' (authorized under Regulation (EU) No 10/2011)
- **Other migrating substances:** degradation products (e.g., crotonic acid and (E)-2-hexenoic acid.) **The migration of crotonic acid was below its SML (0.05 mg/kg).**
- **Oligomers:** hydroxyl (OH-)-terminated (dimer n=2 - n=12)
crotyl- terminated (dimer n=2 - n=12)
Smaller oligomers (n=2, 3, 4, 5) dominated in the 10% ethanol migration solution
Estimated quantification OH-terminated (2.3 mg/kg)
(n=2,3,4,5) crotyl-terminated (1.7 mg/kg)
Specific migration of all oligomers < 1,000 Da should not exceed 5 mg/kg food



EFSA SCIENTIFIC OPINION: PHBH

Toxicological data

➤ *Evaluation of the substance itself:*

Genotoxicity tests → The results of the study were considered negative

➤ *Evaluation of the migrating oligomers:*

PHBH migrating oligomers **do not give rise to concern for genotoxicity**

-repeated dose 90-day oral toxicity study in rats: no-observed-adverse-effect level (NOAEL) at the highest level tested in males, 1,364 mg/kg bw per day

-The potential for bioaccumulation is low

➤ *Evaluation of the migrating impurities*


Substances originating from or to be likely related to the source material 'palm oil and/or palm fatty acid distillate. Considering the nature and origin of these substances, their migration at the levels reported **does not give rise to a safety concern**

➤ *Evaluation of the dehydration products*

The potential migration of crotonic acid ((E)-2-butenic acid) and (E)-2-hexenoic acid, and the content of these two substances bound into the oligomer chains but likely to be released by hydrolysis following migration and ingestion, **does not give rise to a safety concern**



Bio-based/biodegradable materials: Polyhydroxyalkanotes

 PHBH SAFETY ASSESSMENT	SCIENTIFIC STUDY ON THE CHEMICAL CHARACTERIZATION OF PHB
<p>poly((R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate) (PHBH)</p> <p>Migration</p> <ul style="list-style-type: none"> • Overall migration (up to 5.4 mg/kg) • hydroxyl (OH-)-terminated and crotyl-terminated oligomers (n=2 -12) • Other degradation products: crotonic acid (below SML (0.05 mg/kg)) and (E)-2-hexenoic acid • Other migrating compounds: substances from the authorized substance 'palm oil and/or palm fatty acid distillate' (FCM substance No 9) 	<p>polyhydroxybutyrate (PHB)</p> <p>Chemical analysis and characterization</p> <ul style="list-style-type: none"> • Polymer identification FTIR • <i>Volatile compounds</i>: hexane, decane, 4-hydroxybutyric acid, methyl butyrate, etc. • <i>Semi-volatile compounds</i>: butanoic acid, crotonic acid, methyl crotonate, ATBC, etc. <p>Compounds related with the production and degradation of PHB, additives plasticizers</p> <ul style="list-style-type: none"> • <i>Non-volatile compounds</i>: hydroxyl-terminated and crotyl terminated oligomers
<p>Toxicological data</p> <p>PHBH is not of safety concern for the consumer if it is used alone or blended with other polymers in contact with all kinds of food during more than 6 months at room T or below</p>	<p>Future work</p> <p>Migration tests and exposure assessment</p>



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MIGRABIOQUANT Project



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