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SCIENTIFIC OPINION

Safety assessment of the substance calcium *tert*-butylphosphonate for use in food contact materials

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) | Claude Lambré | José Manuel Barat Baviera | Claudia Bolognesi | Andrew Chesson | Pier Sandro Cocconcelli | Riccardo Crebelli | David Michael Gott | Konrad Grob | Evgenia Lampi | Marcel Mengelers | Alicja Mortensen | Inger-Lise Steffensen | Christina Tlustos | Henk Van Loveren | Laurence Vernis | Holger Zorn | Ronan Cariou | Laurence Castle | Emma Di Consiglio | Roland Franz | Maria Rosaria Milana | Eric Barthélémy | Remigio Marano | Gilles Rivière

Correspondence: fip@efsa.europa.eu

Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids assessed the safety of calcium *tert*-butylphosphonate, which is intended to be used as a nucleating agent up to 0.15% w/w for the manufacture of polyolefin food contact materials (FCM) and articles for single and repeated use, in contact with all types of food, including infant formula and human milk. Specific migration was tested using polyethylene samples in 10% ethanol, 3% acetic acid and 95% ethanol for 2 h at 100°C, followed by 238 h at 40°C. Results for all three simulants were near or below the limit of detection of 10 µg/kg. As the solubility of the substance is far above the reported migration and above 60 mg/kg food, no assessment of the particle fraction was needed, and the conventional risk assessment was followed. The substance did not induce gene mutations in bacterial cells and structural chromosomal aberrations in mammalian cells, thus, did not raise concern for genotoxicity. The Panel considered that the use of the substance did not give rise to safety concern related to neurotoxicity for the general population, but this conclusion could not be applied to infants below 16 weeks of age, due to their specific sensitivity and the absence of dedicated data. The Panel concluded that calcium *tert*-butylphosphonate does not raise a safety concern for the consumer if it is used as a nucleating agent up to 0.15% w/w in the manufacture of polyolefin FCM that are intended to be in contact with all types of food for storage above 6 months at room temperature and below, including temperatures up to 100°C for maximum 2 h and up to 130°C for short durations. The Panel could not evaluate the safety of use to manufacture FCM for contact with infant formula and human milk.

KEYWORDS

calcium *tert*-butylphosphonate, FCM substance No 1089, food contact materials, nucleating agent, safety assessment

CONTENTS

1 | INTRODUCTION

1.1 | Background and Terms of Reference

Before a substance is authorised to be used in food contact materials (FCM) and is included in a positive list, the European Food Safety Authority (EFSA)'s opinion on its safety is required. This procedure has been established in Articles 8, 9 and 10 of Regulation (EC) No 1935/2004[1] of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food.

According to this procedure, the industry submits applications to the competent authorities of Member States, which transmit the applications to the (EFSA) for evaluation.

In this case, EFSA received from the Irish competent authority (Food Safety Authority of Ireland), an application for the evaluation of the substance calcium *tert*-butylphosphonate, with the CAS number 81607-35-4. The request has been registered in EFSA's register of received questions under the number EFSA-Q-2022-00526. The dossier was submitted on behalf of Milliken Chemical, Division of Milliken & Company, United States of America.

The Irish competent authority (Food Safety Authority of Ireland) requested the safety evaluation of the substance calcium *tert*-butylphosphonate, for inclusion in Annex I of Regulation (EU) 10/2011.

According to Regulation (EC) No 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food, EFSA is asked to carry out an assessment of the risks related to the intended use of the substance and to deliver a scientific opinion.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a confidential and a non-confidential version of a dossier following the 'EFSA Note for Guidance for the preparation of an application for the Safety Assessment of a Substance to be used in Plastic Food Contact Materials (EFSA, 2008) and the 'Administrative guidance for the preparation of applications on substances to be used in plastic food contact materials' (EFSA, 2021).

In accordance with Art. 38 of the Commission Regulation (EC) No 178/2002[2] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation and of the Decision of the EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,[3] the non-confidential version of the dossier is published on Open.EFSA.[4]

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations[3], EFSA carried out a public consultation on the non-confidential version of the application from 20 February to 12 March 2024, for which no comments were received.

Additional information was provided by the applicant during the assessment process in response to requests from EFSA sent on 23 May 2023 (see Section 5).

Data submitted and used for the evaluation are:

Non-toxicological data and information

- Existing authorisation
- Chemical identity
- Description of manufacturing process of substance/FCM
- Physical and chemical properties
- Intended application
- Migration of the substance

Toxicological data

- Bacterial gene mutation test
- In vitro mammalian cell micronucleus test

[1]Regulation (EC) No 1935/2004 of the European parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. OJ L 338, 13.11.2004, p. 4–17.

[2]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p.1–48.

[3]Decision available at https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements.

[4]The non-confidential version of the dossier, following EFSA's assessment of the applicant's confidentiality requests, is published on Open.EFSA and is available at the following link: https://open.efsa.europa.eu/dossier/FCM-2022-8691.

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 (EC, 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 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

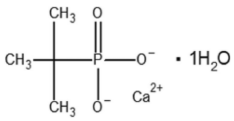
According to the applicant, the substance calcium *tert*-butylphosphonate is a powder intended to be used at up to 0.15% w/w as a nucleating agent for the manufacture of polyolefin food contact materials and articles for single or repeated use. According to the Union Guidelines on Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food (EC, 2014), nucleating agents are classified as polymer production aids. Polyolefins containing calcium *tert*-butylphosphonate are intended to be used in contact with all types of foods, including infant formula and human milk, at temperatures up to and including 130°C for short durations (< 15 min), such as microwave applications (excluding microwave susceptor), as well as longer durations of cooking and storage, where the polymer remains stable in contact with food at temperatures up to and including 100°C.[5]

The substance has not been evaluated in the past by the SCF or EFSA.

3.1 | Non-toxicological data

3.1.1 | Identity of the substance[6]

Chemical formula: $C_4H_{11}CaO_4P$, 194.2 g/mol
Chemical structure:



The substance is produced using [redacted].

The specified purity is ≥96%; the specified impurities are: [redacted], [redacted] and the closely related [redacted] <1.0%. For three provided tested batches, purity was above 99.35%, with [redacted].

[5]Technical dossier, section 'Intended application of substance'.

[6]Technical dossier, section 'Identity of the substance' and 'Physical and chemical properties of the substance'.

The particle size distribution[7] of the powder was characterised by dynamic light scattering (DLS), which did not indicate any particles smaller than 1 µm. However, DLS is not considered to be an appropriate method to measure the particle size distribution (EFSA Scientific Committee, 2021a). Solubility[7] in water at ambient temperature was determined to be [redacted] mg/L (slightly lower in 95% ethanol). The Panel noted that the ultrafiltration step using a filter with pore size in the range 3–10 kDa, as recommended in the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021a), was not conducted. Nonetheless, the estimated solubility is far above the reported migration (around or below 10 µg/kg food) and above 60 mg/kg food[8] (EFSA Scientific Committee, 2021a). Therefore, the Panel followed the conventional risk assessment approach (EFSA Scientific Committee, 2021b).

3.1.2 | Physical and chemical properties[7]

According to differential scanning calorimetry and thermographic analysis, the melting point ranges from 305 to 390°C, followed by decomposition above around 470°C, that is the substance remains solid when applied in polyolefins.

3.1.3 | Specific migration of the substance[9]

Specific migration was tested from low-density polyethylene samples of 1.27 mm thickness, compounded in an extruder at 190°C with the maximum intended amount of 0.15% w/w calcium *tert*-butylphosphonate. The test plaques (surface to volume ratio of 6 dm^2/kg food) were immersed in the food simulants 10% ethanol, 3% acetic acid and 95% ethanol for 2 h at 100°C, followed by 238 h at 40°C. Migration was measured by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection. For fatty foods, migration testing was not successful in olive oil and was carried out in 95% ethanol. Considering the solubility of the substance, 95% ethanol was considered as the worst-case alternative.

Migration was measured after 2 h at 100°C and after 22, 94 and 238 h at 40°C. In all food simulants and at all time points, it was near to or below the limit of detection (8 µg/kg in 10% and 95% ethanol, 10 µg/kg in 3% acetic acid). A maximum migration was observed at around 8 µg/kg after 24 h when using 10% ethanol and at about 10 µg/kg after 96 h with 3% acetic acid, and then constant over the remaining testing period. With 95% ethanol, all results were below the limit of detection. Therefore, the Panel agreed that no testing at 60°C was required to cover contact above 30 days at room temperature or below, including hot-fill conditions. Contact up to 130°C for short durations (e.g. below 15 min) are covered by the migration for 2 h at 100°C. The low migration of the substance was confirmed by high-performance liquid chromatography–mass spectrometry (HPLC-MS). The migration solutions were also analysed for total phosphorus content by inductively coupled plasma-mass spectrometry (ICP-MS), and the results were [redacted] with the low migration of the substance itself (as measured using HPLC-UV and HPLC-MS), and this ICP-MS analysis also indicated that the migration of any impurities and possible reaction products containing phosphorus was very low too.

3.1.4 | Migration of impurities related to the substance[9]

No data were provided on the migration of the impurity [redacted]. However, based on its specification (< 1.0%), migration pro rata to 10 µg calcium *tert*-butylphosphonate/kg food was estimated to be 0.1 µg/kg food in 3% acetic acid.

3.2 | Toxicological data[10]

In accordance with the EFSA Note for Guidance (EFSA CEF Panel, 2008), to assess the genotoxic potential, the applicant submitted a bacterial reverse mutation assay (Ames test) and an in vitro mammalian cell micronucleus test conducted on calcium *tert*-butylphosphonate.

3.2.1 | Bacterial reverse mutation assay

A bacterial reverse mutation assay was conducted in Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 *uvrA* to assess the mutagenicity of calcium *tert*-butylphosphonate (purity 98.4%). The study was in

[7]Technical dossier, section 'Physical and chemical properties of the substance'.

[8]60 mg/kg food is the upper overall migration limit foreseen in Reg. (EU) 10/2011.

[9]Technical dossier, section 'Data on migration of substance'.

[10]Technical dossier, section 'Toxicological data'.

compliance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) 471 (OECD, 1997) and the Good Laboratory Practice (GLP) principles.

Calcium *tert*-butylphosphonate was poorly soluble in water and organic solvents compatible with the assay; a fine suspension was formed in dimethyl formamide, which was selected as the vehicle.

Two separate experiments were conducted: the first one using the plate incorporation method, and the second one using the preincubation procedure. Both assays were performed in the absence and presence of metabolic activation by phenobarbital/β-naphthoflavone-induced rat liver S9 fraction (S9-mix). In the first experiment, the test item was tested at eight concentrations ranging from 1.5 to 5000 µg/plate; in the second experiment, six concentrations in the range of 15–5000 µg/plate were applied. All concentrations, as well as negative (vehicle) and positive controls, were evaluated on triplicate plates.

Precipitate of the test item was observed in the first experiment at 5000 µg/plate and in the repeat experiment at 1500 and 5000 µg/plate, with and without S9-mix. No evidence of toxicity and no increase in revertant colonies were observed at any concentration in any bacterial tester strain, with or without metabolic activation. Both vehicle and positive controls were within the respective historical control ranges.

The results of the study are considered negative, that is the test item did not induce gene mutations under the conditions of the study. Based on the 'Harmonised approach for reporting reliability and relevance of genotoxicity studies' (EFSA, 2023), the Panel considered the study reliable without restrictions and the results of high relevance.

3.2.2 | In vitro mammalian cell micronucleus test

Calcium *tert*-butylphosphonate (purity 98.4%) was tested in the in vitro micronucleus assay in human peripheral blood lymphocytes. The test was carried out following GLP principles. The cytokinesis block micronucleus assay protocol was applied. Positive controls were cyclophosphamide, mitomycin C and demecolcine. The test item, being insoluble in culture medium, was suspended in DMSO.

Duplicate lymphocyte cultures from healthy donors were treated for 4 h with and without phenobarbital/β-naphthoflavone-induced rat liver S9, followed by 24 h recovery in the presence of cytochalasin B (CytB), or for 24 h without S9, followed by further 24 h with CytB. The following concentrations, 50, 100 and 200 µg/mL, were tested in all experiments. The maximum concentration was selected based on the results of a range-finder experiment in order to achieve visible precipitation at the highest tested dose, as recommended by guidelines for poorly soluble test chemicals.

Micronuclei were scored in 2000 binucleated cells per concentration (1000 from each culture) for the test item and positive controls, and in 4000 binucleated cells (from 4 cultures) for the vehicle controls. The Cytokinesis-Block Proliferation Index, as an index of toxicity, was determined in 500 cells per culture.

Visible precipitation was observed at the end of the incubation period at the highest concentration in all cultures. No increase in binucleated cells containing micronuclei and no dose-related toxicity were observed in treated cultures compared to vehicle controls under any experimental condition. A clear-cut and statistically significant response was induced by the positive control substances.

The results of the study were considered negative, that is the test item did not induce the formation of micronuclei under the conditions of the study. The Panel noted that the experiment with extended treatment did not follow the protocol recommended in the OECD TG 487, as a 24-h incubation with the test article was performed without CytB, which was added in the subsequent 24-h recovery period in order to avoid any possible interaction between CytB and the test article. This modified protocol was in line with more recent recommendations for the conduct of the micronucleus test with cultured human lymphocytes (Whitwell et al., 2019) and the EFSA recommendations for the conduct of the in vitro micronucleus assay with nanomaterials because of the known inhibition of endocytosis by CytB (EFSA Scientific Committee, 2021b). The application of this modified protocol was considered acceptable for the testing of calcium *tert*-butylphosphonate also in view of the low solubility, with formation of precipitate, of the test item in culture medium.

The Panel considered the study reliable without restrictions and the results of high relevance.

3.3 | Discussion

The solubility of the substance is above 60 mg/kg food[11] and far above the measured migration. In accordance with the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles, including nanoparticles (EFSA Scientific Committee, 2021a), no additional assessment for the possible fraction of small particles including nanoparticles, was needed; hence, conventional risk assessment was followed.

In all food simulants and at all time points, the migration of the substance was near to or below the limit of detection (up to 10 µg/kg).

[11] 60 mg/kg food is the upper overall migration limit foreseen in Reg. (EU) 10/2011.

Calcium *tert*-butylphosphonate did not raise concern for genotoxicity, based on the negative results obtained in the two recommended in vitro genotoxicity assays. The Panel considered that this conclusion also applies to its impurity, [redacted], due to its structural similarity.

Organophosphorus compounds require special attention with regard to their potential to trigger neurotoxicity. However, the migration of the calcium *tert*-butylphosphonate results in an exposure of the consumers which is below the threshold of toxicological concern for organophosphates, that is 18 µg/person per day (EFSA Scientific Committee, 2012). Therefore, the Panel considered that the use of the substance does not give rise to a safety concern related to neurotoxicity for the general population. This conclusion could not be applied to infants below 16 weeks of age due to their specific sensitivity (EFSA Scientific Committee, 2017) and to the absence of dedicated data to rule out neurotoxicity, that is addressing whether or not the substance has a potential to act as an organophosphate. Hence, the Panel could not evaluate the safety of use of the substance to manufacture polyolefin materials and articles for contact with infant formula and human milk.

4 | CONCLUSIONS

The CEP Panel concluded that the substance calcium *tert*-butylphosphonate is not of safety concern for the consumer, if it is used as a nucleating agent up to 0.15% w/w in polyolefin materials and articles intended for contact with all types of food for storage above 6 months at room temperature and below, including temperatures up to 100°C for maximum 2 h and up to 130°C for short durations. The Panel could not evaluate the safety of use of the substance to manufacture polyolefin materials and articles for contact with infant formula and human milk.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Dossier 'Calcium tert-butylphosphonate'. August 2022. Submitted on behalf of Milliken Chemical, Division of Milliken & Company, United States of America.

Additional information, October 2023. Submitted on behalf of Milliken Chemical, Division of Milliken & Company, United States of America.

ABBREVIATIONS

CAS	chemical abstracts service
CytB	cytochalasin B
DLS	dynamic light scattering
FCM	food contact materials
GLP	good laboratory practice
HPLC	high-performance liquid chromatography
ICP	inductively coupled plasma
MS	mass spectrometry
OECD	Organisation for Economic Co-operation and Development
SCF	Scientific Committee on Food

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

Irish competent authority (Food Safety Authority of Ireland)

QUESTION NUMBERS

EFSA- Q-2022-00526

PANEL MEMBERS

José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, and Holger Zorn.

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†Deceased.