Safety assessment of the substance bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate, for use in food contact materials

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Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) assessed the safety of the additive bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate (DECH), food contact materials (FCM) substance No 1079, which is intended to be used as plasticizer in poly(vinyl chloride) (PVC) film sat up to 25% w/w in contact with aqueous, acidic and low-alcohol foods for long-term storage at room temperature or below (refrigerated and frozen). The films are not intended for use in reheating food. Under the tested conditions, the substance migrated up to 0.034 mg/kg from samples of PVC films manufactured with 25% w/w DECH. Based on the reported in vitro and in vivo genotoxicity studies, the Panel concluded that the substance does not raise a concern for genotoxicity. Based on the provided toxicokinetic study, the Panel concluded that there is uncertainty on the potential for accumulation of the substance in humans. No adverse effects were observed up to the highest tested dose of 1,000 mg/kg body weight (bw) per day in repeated dose toxicity studies. Nevertheless, these data do not remove the uncertainty on the potential for accumulation in humans. Therefore, the CEP Panel concluded that the substance bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate is of safety concern for the consumer, if it is used in poly(vinyl chloride) (PVC) in contact with foods for which simulants A (10% ethanol) and B (3% acetic acid) are assigned, for long-term storage at room temperature or below. The migration of the substance should not exceed 0.050 mg/kg food.

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Keywords: bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate, plasticizer, CAS No. 84731-70-4, FCM substance No. 1079, food contact materials, safety assessment, evaluation

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Competing interests: Roland Franz declared that Fraunhofer institute at which he was employed provides advisory services to private business operators active in the sector on food contact materials. In line with EFSA’s Policy on Independence and the Decision of the Executive Director on Competing Interest Management, a waiver was granted to Roland Franz regarding his participation to the EFSA’s Working Group on Food Contact Materials (FCM WG) in accordance with Article 21 of the Decision of the Executive Director on Competing Interest Management. Pursuant to Article 21(6) of the above-mentioned Decision, the involvement of Roland Franz is authorised as member in the FCM WG, allowing him to take part in the discussions and in the drafting phase of the scientific output, but he is not allowed to be, or act as, a chairman, a vice-chairman or rapporteur of the working group.

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 following information has been provided under confidentiality and it is redacted awaiting the decision of the Commission: cis/trans ratio and manufacture details of the substance, percentage of residual impurity, tested concentrations of the in vitro mammalian cell gene mutation test, details of the study report on the toxicokinetic testing.


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1. **Introduction**

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

Before a substance is authorised to be used in food contact materials (FCM) and is included in a positive list, 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 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 Member States’ competent authorities which transmit the applications to the European Food Safety Authority (EFSA) for their evaluation.

In this case, EFSA received an application from the German Competent Authority (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL), requesting the evaluation of the substance bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate, with the Chemical Abstracts Service (CAS) number 84731-70-4 and the FCM substance No 1079. The dossier was submitted on behalf of Hanwha Chemical Co.

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 dossier in support of their application for the authorisation of bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate to be used in FCM.

Additional information was provided by the applicant during the assessment process in response to requests from EFSA sent on 30 November 2018 (see ‘Documentation provided to EFSA’).

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
- Migration of the substance
- Residual content of the substance
- Identification, quantification and migration of reaction products and impurities

**Toxicological data**
- Bacterial gene mutation test
- *In vitro* mammalian cell gene mutation test
- *In vitro* mammalian chromosomal aberration test
- *In vivo* mammalian erythrocyte micronucleus test
- Toxicokinetic study
- 90-day oral toxicity study in rats
- Combined repeated dose toxicity with reproduction / developmental toxicity screening test
- Prenatal developmental toxicity study
- Two-generation reproduction toxicity study

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

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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 are 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,2 bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate (DEHCH) is intended to be used as plasticizer in poly(vinyl chloride) (PVC) films at up to 250 mg/g (25% w/w) in contact with aqueous, acidic and low-alcohol foods for long-term storage at room temperature or below (refrigerated and frozen). The films are not intended for use in reheating food.

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

3.1. Non-toxicological data

Chemical formula: C_{24}H_{44}O_{4}

Chemical structure:

![Chemical structure of DEHCH](image)

3.1.1. Physical and chemical properties³

Bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate (DEHCH) has a molecular mass of 396.6 Dalton (Da). It is virtually insoluble in water (0.047 mg/L at 20°C) and highly liposoluble (Log Po/w = 8.84). Thermogravimetric analysis (TGA) demonstrated thermostability up to 300°C, which is above the temperatures used to process PVC.

DEHCH is a mixture of two cis/trans isomers (mean ratio: [value]), produced by [method] of bis(2-ethylhexyl)benzene-1,4-dicarboxylate (DEHT). The purity of the substance is > 99%. Residual DEHT (CAS No 6422-86-2; FCM No 798, total specific migration limit, SML(T) 60 mg/kg) is given as impurity at [value].

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2 Technical dossier/section 3; Appendix B-Technical dossier.
³ Technical dossier/section 2; Appendix B-Technical dossier/Annexes 1-4, 6.
Hydrolytic stability was tested in 10% ethanol and 3% acetic acid. The substance DEHCH remained unchanged after 10 days at 40°C.

3.1.2. Specific migration

Samples of PVC films (0.012 mm thickness) manufactured with 25% w/w DEHCH were submitted to specific migration tests for 10 days at 40°C with 10% ethanol and 3% acetic acid (simulants A and B in Regulation (EU) No 10/2011). After heptane extraction of the migration solutions, DEHCH, DEHT and 2-ethylhexanol (2-EH, CAS No 104-76-7; FCM No 209, specific migration limit, SML 30 mg/kg) were analysed by Gas Chromatography-Mass Spectrometry–Selected Ion Monitoring (GC-MS-SIM). Migration of DEHCH, DEHT and 2-EH measured in 10% ethanol was 0.034 mg/kg, <0.0085 mg/kg and 0.0060 mg/kg, respectively. Migration in 3% acetic acid was 0.023 mg/kg, <0.0085 mg/kg and 0.028 mg/kg, respectively.

The migration of DEHT and 2-EH, which are substances listed in Regulation (EU) No 10/2011, was therefore well below their respective migration limits (SML(T) 60 mg/kg and SML 30 mg/kg).

3.2. Toxicological data

3.2.1. Genotoxicity

3.2.1.1. Bacterial reverse mutation test

DEHCH (> 99% purity) was tested in a bacterial reverse mutation test (Ames test) in Salmonella typhimurium (TA98, TA100, TA1535, TA1537) and Escherichia coli (WP2uvrA). The assay was performed following Organisation for Economic Co-operation and Development OECD Test Guideline 471 (1997) and Good Laboratory Practice, using the plate incorporation method and ethanol as solvent for the test substance. In the preliminary dose-range finding study (4–2,500 µg/plate), no cytotoxicity was observed at any dose level; precipitation was observed at doses ≥ 500 µg/plate. The main experiment was performed twice, using triplicate plates with 15.6, 31.3, 62.5, 125, 250 and 500 µg/plate, in the presence and absence of metabolic activation (S9 mix).

In both experiments, DEHCH did not induce reproducible and/or dose-related increases of revertants. Positive controls elicited the expected positive response. Based on these findings, the Panel concluded that the substance is not mutagenic under the conditions of this study.

3.2.1.2. In vitro mammalian cell gene mutation test

DEHCH (> 99% purity) was tested in an in vitro cell gene mutation assay (in vitro mammalian cell mutation test in mouse lymphoma cell L5178Y/TK+/−). The assay was performed following OECD Test Guideline 490 (2015) and Good Laboratory Practice. In the first experiment, cells were treated for 4 h in the presence and absence of metabolic activation; in the repeat experiment, cells were treated for 24 h without metabolic activation (S9 mix).

In a range-finding preliminary test in the presence (4 h treatment) and absence (4 h and 24 h treatment) of metabolic activation, no toxic effect (< 50% survival) was observed up to the highest tested dose of 2,000 µg/mL. The dose selection for the main experiments was based on precipitation of the test item at the end of the treatment period: in the first experiment, tested concentrations were 0.001 µg/mL with and without metabolic activation; in the second experiment, tested concentrations were 0.0025 µg/mL. Two parallel cultures were used per test concentration. Ethanol was used as solvent. In both experiments the mutation frequency in treated cultures did not exceed the threshold of 126 (global evaluation factor according to OECD Test Guideline 490) above the corresponding solvent control at any tested concentration. No relevant change in the ratio of small vs. large colonies was observed up to the maximal tested concentration.

Overall, the Panel concluded that DEHCH is not mutagenic in the mouse lymphoma assay under the conditions of this study.

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4 Technical dossier/section 2; Appendix B – Technical dossier/Additional data/Annex 16.
5 Technical dossier/sections 5&6; Appendix B –Technical dossier/Annex 5.
7 Technical dossier/section 8.1.1; Appendix B –Technical dossier/Annex 7.
8 Technical dossier/section 8.1.6.1; Appendix B –Technical dossier/Annex 8.
3.2.1.3. *In vitro* mammalian chromosomal aberration test

DEHCH (> 99% purity) was tested in an *in vitro* chromosome aberration test in Chinese hamster lung cells (CHL) following OECD Test Guideline 473 (1997) and Good Laboratory Practice. In the preliminary dose range-finding study, doses from 9.8 to 2,500 µg/mL were tested. Ethanol was used as solvent. No cytotoxicity was observed up the highest tested dose; precipitation of the test item was observed at concentrations ≥ 312 and ≥ 78.1 µg/mL in the presence and absence of metabolic activation (S9 mix), respectively. Based on these findings, in the main experiment, DEHCH was tested in the presence of metabolic activation for 6 hours at 39.1, 78.1, 156.3 and 312.5 µg/mL, and in the absence of metabolic activation for 6 h and 22 h, respectively, at 9.8, 19.5, 39.1, and 78.1 µg/mL. Chromosomal aberrations were analysed scoring 100 metaphases per tested concentration. There was no significant increase in the number of metaphases with structural aberrations at any tested concentration. In the positive control groups, there were significant increases in the number of aberrant metaphases.

The Panel concluded that DEHCH is not clastogenic under the tested conditions of this study. However, the Panel noted that the number of scored metaphases, although acceptable at the time the study was performed in 2009, is lower than currently recommended (OECD Test Guideline 473, 2016).

3.2.1.4. *In vivo* mammalian erythrocyte micronucleus test

DEHCH (> 99% purity) was tested in the *in vivo* mammalian erythrocyte micronucleus test. The test was performed according to OECD Test Guideline 474 (1997) and Good Laboratory Practice. DEHCH was dissolved/suspended in corn oil. It was administered by gavage twice at 24 h interval to 7-week-old male and female CD1(ICR) mice at 500, 1,000 and 2,000 mg/kg body weight (bw), using six animals per sex and dose. Micronucleated polychromatic erythrocytes (MNPCe) were counted in 2,000 polychromatic erythrocytes (PCE) per animal. There was no statistically significant increase of MNPCeS at any dose compared to the controls.

No effect was observed on the ratio of PCE to the sum of PCE and normochromatic erythrocytes (NCE), indicating absence of bone marrow exposure. However, the bioavailability of the test item after single oral gavage administration was demonstrated in a toxicokinetic study in rats (see section 3.2.2).

In conclusion, the Panel evaluated DEHCH as not clastogenic and aneugenic *in vivo* under the test conditions of this study.

3.2.1.5. Conclusions on genotoxicity

DEHCH was tested in a battery of *in vitro* and *in vivo* genotoxicity assays, performed according to Good Laboratory Practice (GLP) and the OECD guidelines. DEHCH was negative in adequately conducted bacterial and mammalian cell gene mutation tests and in the *in vivo* micronucleus test; negative results were obtained in a limited *in vitro* chromosomal aberration assay. The Panel noted that the battery of studies available is not aligned with the more recent test requirements, as an *in vitro* micronucleus assay is missing (EFSA Note for Guidance, 2017), however, the data package available is considered sufficient to rule out a genotoxic concern for DEHCH, since all relevant genotoxicity end-points (gene mutation, clastogenicity and aneugenicity) were adequately addressed.

3.2.2. Toxicokinetic

A toxicokinetic study was performed according to GLP and OECD Test Guideline 417 (2013) in Sprague–Dawley (SD) rats with non-radiolabelled DEHCH (> 99% purity), after single oral administration by gavage at 60 mg/kg bw (low dose).
After single oral administration, DEHCH is excreted in urine and faeces, not reaching a mass balance. The tissue distribution data showed higher concentrations of DEHCH in spleen and heart. The Panel considered that the data provided may indicate a potential for accumulation in humans. In the absence of an adsorption, distribution, metabolism and excretion (elimination) (ADME) study performed with radiolabelled compound and repeated administrations, that was requested to the applicant, the uncertainty about the potential accumulation of the parent compound and metabolites in humans could not be removed.

3.2.3. Concluding remarks on toxicity

Based on the results of the genotoxicity studies and the toxicokinetic study, the Panel noted that

— the substance is not genotoxic;
— the limitations in the toxicokinetic study means that there is uncertainty on the potential for accumulation of the substance in the body.

In addition to the genotoxicity and toxicokinetic studies, the following studies were provided by the applicant and evaluated by the Panel: a repeated dose 90-day oral toxicity study, a combined repeated dose toxicity study with a reproduction/development toxicity screening test, a prenatal developmental toxicity study and a two-generation reproduction toxicity study. The Panel noted that no adverse effects were observed up to the highest tested dose of 1,000 mg/kg bw per day. Nevertheless, those additional toxicological data do not remove the uncertainty on the potential for accumulation in humans. Therefore, bearing in mind that the substance does not raise concern for genotoxicity, the highest migration observed was 0.034 mg/kg, and in line with the SCF guidelines (European Commission, 2001), the Panel considers that the specific migration of DEHCH should not exceed 0.05 mg/kg food.

4. Conclusions

Based on the above-mentioned data, the CEP Panel concluded that the substance bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate is not of safety concern for the consumer, if the substance is used in PVC in contact with foods for which simulants A (10% ethanol) and B (3% acetic acid) are assigned, for long-term storage at room temperature or below. The migration of the substance should not exceed 0.050 mg/kg food.

Documentation provided to EFSA

1) Dossier 'Bis(2-ethylhexyl)-cyclohexane-1,4-dicarboxylate'. July 2018. Submitted by Hanwha Chemical Co, Korea.

References


12 Technical dossier/section 8.2.1; Appendix B -Technical dossier/Annex 11.
13 Technical dossier/section 8.2.3.1; Appendix B -Technical dossier/Annex 12.
14 Technical dossier/section 8.2.3.2; Appendix B -Technical dossier/Annex 13.
15 Technical dossier/section 8.2.3.3; Appendix B -Technical dossier/Additional data/Annex 17.

**Abbreviations**

- **ADME**: Adsorption, distribution, metabolism and excretion (elimination)
- **BVL**: Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
- **bw**: body weight
- **CAS**: Chemical Abstracts Service
- **CEP Panel**: EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
- **CHL**: Chinese hamster lung cells
- **Da**: Dalton
- **DEHCH**: bis(2-ethylhexyl)cyclohexane-1,4-dicarboxylate
- **DEHT**: bis(2-ethylhexyl)benzene-1,4-dicarboxylate
- **2-EH**: 2-ethylhexanol
- **EU**: European Union
- **FCM**: food contact materials
- **GC-MS-SIM**: Gas Chromatography-Mass Spectrometry–Selected Ion Monitoring
- **GLP**: good laboratory practice
- **LC-MS**: Liquid Chromatography-Mass Spectrometry
- **MNPCE**: micronucleated polychromatic erythrocytes
- **NCE**: normochromatic erythrocytes
- **OECD**: Organisation for Economic Co-operation and Development
- **PCE**: polychromatic erythrocytes
- **Po/w**: octanol/water partition coefficient
- **PVC**: poly(vinyl chloride)
- **SCF**: Scientific Committee on Food
- **SD**: Sprague-Dawley
- **SML**: specific migration limit
- **SML(T)**: total specific migration limit
- **TGA**: thermogravimetric analysis
- **w/w**: weight by weight