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SCIENTIFIC OPINION

Safety assessment of the substance N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) for use in plastic food contact materials

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The EFSA Panel on Food Contact Materials assessed the safety of N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) to be used at up to 650 mg/kg in polyethylene terephthalate (PET) to scavenge acetaldehyde (AA). Final articles are intended for contact with aqueous, acidic and low-alcoholic beverages for long-term storage at room temperature and below. The migration of the substance from PET bottles into 20% ethanol was 0.0038 mg/kg food. The Panel calculated the potential migration of the summed reaction products not to exceed 0.02 mg/kg food. From experimental studies, the Panel excluded genotoxicity concerns for the substance, for 2-aminobenzamide +1 formaldehyde and 2-aminobenzamide +1 AA, both with desaturation. In silico predictions, previous EFSA evaluations and the use of the threshold of toxicological concern (TTC) excluded genotoxicity concerns for 15 other impurities/reaction products. A tentatively identified by-product was predicted as possible DNA-reactive in vitro mutagen and clastogen, due to its aromatic hydroxylamine group. Its modelled migration would not exceed 0.14 μg/kg food, leading to a potential exposure below the TTC of 0.0025 μg/kg body weight per day. Non-identified reaction products are expected to be structurally related to the identified ones and, hence, not to raise concern for genotoxicity. The Panel concluded that the substance is not of safety concern for the consumer, if it is used as an additive at up to 650 mg/kg in PET intended for contact with foods simulated by simulants A, B and C, for storage above 6 months at room temperature and below, including hot-fill conditions and/or heating up to $70°C \leq T \leq 100°C$ for maximum $t = 120/2^{((T-70)/10)}$ minutes. The substance should not be used for infant formula (including water used for reconstitution) and human milk. The migration of the substance should not exceed 0.05 mg/kg food. The substance should not contain aromatic hydroxylamine derivatives at more than 0.15% w/w.

KEYWORDS

FCM substance No. 1095, food contact materials, N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide), NIAS, PET, reaction products, 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 an application from the Irish competent authority (Food Safety Authority of Ireland), requesting the safety evaluation of the substance N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide), FCM substance No 1095. The request has been registered in EFSA's register of received questions under the number EFSA-Q-2023-00721. The dossier was submitted by Avient Corporation, ColorMatrix Group.

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 CEF Panel, 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 5 February 2025 to 26 February 2025, 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 6 May 2024 (see Section 5).

Data submitted and used for the evaluation are:

Non-toxicological data and information

- Chemical identity
- Manufacturing process of the substance
- Physical and chemical properties
- Intended application
- Migration of the substance
- Identification, quantification and migration of impurities and reaction products

Toxicological data

Applied substance: N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide)

- 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, pp. 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, pp. 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-2023-19850.

Impurities and reaction products:

2-aminobenzamide +1 formaldehyde (FA) with desaturation

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

2-aminobenzamide +1 acetaldehyde (AA) with desaturation

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

12 other impurities and reaction products

- In silico data.

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 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 N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) is an additive intended to be used at up to 650 mg/kg polyethylene terephthalate (PET) to scavenge acetaldehyde (AA) produced during melt processing. The resulting PET is intended to be used for contact with aqueous, acidic and low-alcoholic beverages. Contact may be for long-term storage at room temperature and below.

The substance has not been evaluated in the past by the SCF or EFSA. The functional analogue 2-aminobenzamide (also an AA scavenger) is authorised according to Regulation (EU) No 10/2011 as FCM substance No 164, with a specific migration limit (SML) of 0.05 mg/kg and the following restriction: 'Only for use in PET for water and beverages'.

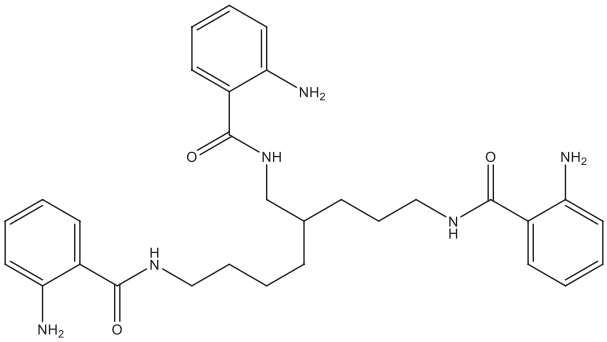
3.1 | Non-toxicological data

3.1.1 | Identity of the substance[5]

Chemical formula: $C_{30}H_{38}N_6O_3$; molecular mass: 530.7 Da. CAS number not available.[6]

[5]Technical dossier, section 'Identity of the substance' .
[6]EC number: 951-985-7.

Chemical structure:



N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) is manufactured by condensation of [redacted] with [redacted] using a mixture of acetone/water as solvent. The excess of [redacted] is quenched by aqueous [redacted] solution. The substance is then [redacted]. The purity of the substance is specified to be > 97%. For the 18 provided tested batches, it was between [redacted]% and [redacted]%, estimated as 100% area minus the other substances detected by liquid chromatography coupled with high-resolution mass spectrometry (LC–HRMS) not considering response factors.

According to the applicant, 14 impurities were detected in addition to [redacted] (< [redacted]%). They are listed in Table 1 below, while their related chemical structures and synonyms are available in Annex 1, Table A1.

TABLE 1 14 identified impurities. Note: 'substance' refers to the substance subject of the application, acetone (AC), acetaldehyde (AA), formaldehyde (FA), reaction products without desaturation unless stated otherwise.

Substance number (N: in the opinion)	Impurities	Note
1	Acetone	Residual solvent
2	[redacted]	Residual starting material
3	2-Aminobenzamide	Results from quenching of [redacted] with [redacted]
4	[redacted]	2-Aminobenzamide derivative
5	[redacted]	2-Aminobenzamide derivative
6	Aniline	2-Aminobenzamide derivative
7	2-Aminobenzamide +1 FA with desaturation (4-hydroxyquinazoline)	2-Aminobenzamide derivative; also found as reaction product
8	Disubstituted analogue of the substance	Sum of two isomers; with one unreacted amine
9	Over addition analogue of the substance	Sum of two isomers
10	Mono urea substance derivative	
11	Oxidised product	Generated by reaction of one end of the substance with 1 FA and the oxidation of a N atom to N-OH at another end. Its structure was inferred from the mass spectrum of LC–MS analysis without using an authentic standard. Hence, there is uncertainty on the correctness of the attribution
12	Substance +1 AC	AC-related reaction product
13	Substance +2 AC	AC-related reaction product
14	Substance +1 FA with desaturation	Sum of three isomers; also found as reaction product

3.1.2 | Physical and chemical properties[7]

The substance is an off-white powder. It has a melting point of 160°C and a boiling point above 375°C, both measured by differential scanning calorimetry (DSC). Decomposition starts at around 400°C under nitrogen and at 300°C under air, both determined by thermogravimetric analysis (TGA). Hence, no relevant thermal degradation of the substance is expected under PET processing conditions (at maximum 320°C).

The solubility of the substance in water is 3.96 mg/L at 20°C and the log P_{ow} is 2.45.

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

N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) has three sites that can react with aldehydes or ketones. It is intended to scavenge acetaldehyde, but it also reacts with formaldehyde or acetone. The FCM Panel noted that in a first step, each aminobenzamide moiety can produce a two-ring structure (a 2,3-dihydro-1H-quinazolin-4-one) through an intermediary Schiff base and with water as a by-product. In a second step, desaturation may result in the formation of a quinazolinone (Kim & Cheon, 2014; Mrozinski et al., 2013; Figure 1). According to the data presented by the applicant, this second step remains minor. Unless stated otherwise, in the present opinion the reaction products are considered as not dehydrogenated derivatives.

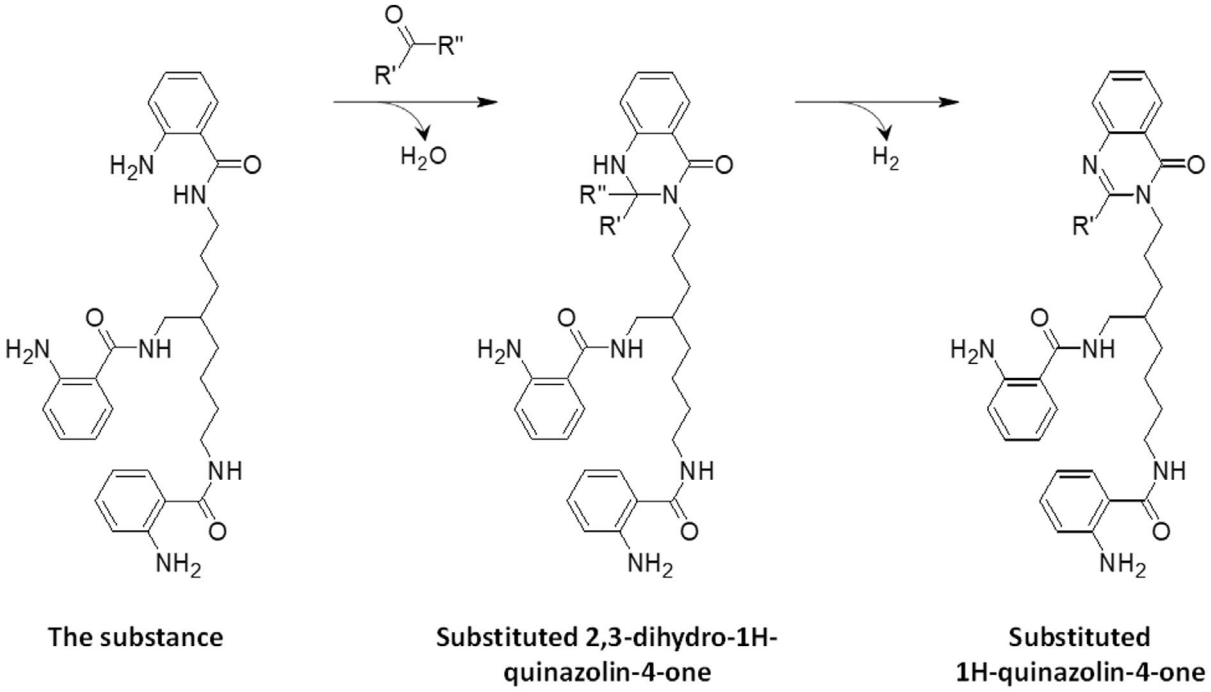


FIGURE 1 Reaction scheme according to the Panel of N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) with acetone (AC; R = R″ = CH_3), producing a 2,3-dihydro-1H-quinazolin-4-one (only step 1), formaldehyde (FA; R = R″ = H) or acetaldehyde (AA; R = CH_3, R = H), with possible subsequent desaturation in the case of FA and AA.

Scavenging reaction products have been investigated through an incomplete extraction with acetonitrile (ACN) from PET bottle preforms and characterised by headspace gas or liquid chromatography-(high-resolution) mass spectrometry (GC–MS, LC–MS and LC–HRMS). In addition to the substances reported in Section 3.1.1, seven reaction products were identified, all without desaturation. Two were side reaction products: (20) pyridine and (21) 2-aminobenzamide +1 AA with desaturation (2-methyl-4(3H)-quinazolinone), while the following five were scavenging reaction products (numeration follows from Table 1):

- (15) substance +1 FA
- (16) substance +3 FA
- (17) substance +1 AA
- (18) substance +2 AA
- (19) substance +3 AA

The FCM Panel pinpointed a remarkable gap in the mass balance presumably due to missed reaction products (with AA, FA, AC). For instance, the substance +2 FA and the substance with mixed FA/AA are to be expected, but they have not been identified. Based on the chemistry of the reaction, the FCM Panel considered those potential reaction products being largely composed by substances structurally related to the identified reaction products (14) to (19).

3.1.3 | Residual content and specific migration of the substance[8]

The residual amount of unreacted substance was not measured in PET bottles but in PET preforms made with the maximum amount of the substance, 650 mg/kg PET. The average residual content obtained by (presumably incomplete) THF extraction was [redacted] mg/kg PET preforms.

[8]Technical dossier, section 'Data on Migration of substance'.

The specific migration of the substance was determined from 1-L PET bottles made with 650 mg/kg of the substance. Bottles were filled with 3% acetic acid (food simulant B) or 20% ethanol (food simulant C), capped and kept for 10 days at 60°C. The exposed simulants were analysed by liquid chromatography–time-of-flight mass spectrometry (LC–QTOF-MS). The migration was 0.0022 and 0.0038 mg/kg food into 3% acetic acid and 20% ethanol, respectively. According to Reg. (EU) 10/2011, the applied test conditions cover also the use for hot-fill and/or heating up to 70°C ≤ T ≤ 100°C for maximum $t = 120/2^{((T-70)/10)}$ minutes.

3.1.4 | Migration of impurities and reaction products related to the substance[8]

The migration of the 21[9] identified impurities and reaction products was not tested. Instead, it was estimated using the recognised migration model (Hoekstra et al., 2015). Contact parameters were: 10 days at 60°C, PET wall thickness of 250 μm and a surface-to-volume ratio of 6 dm^2/L. The partition coefficient of all migrants between the polymer (p) and the food (f) was taken as $K_{p,f} = 1$ as a worst case. The initial concentration $C_{p,0}$ in the food contact material was either the maximum level permitted as part of the manufacturing specification of the substance or the level determined from the PET preform study in ACN mentioned in Section 3.1.2. Individual migration results were all below 0.05 mg/kg food (Annex 1, Table A2). The Panel considered the calculation of migration of the identified impurities/reaction products as overestimated due to the applied conservative migration model.

The FCM Panel calculated the possible migration of the sum of all (identified and not identified) reaction products, considering as a worst case the complete reaction of the substance. Neglecting the differences in molecular mass, their potential migration[10] does not exceed 0.02 mg/kg food. Therefore, their migration (if any) would be individually below the tier value of 0.05 mg/kg food, as defined in the 'EFSA Note for Guidance' (EFSA CEF Panel, 2008).

3.2 | Toxicological data[11]

In accordance with the 'EFSA Note for Guidance' (EFSA CEF Panel, 2008), the applicant assessed the genotoxic potential of the substance (data requirements in case of migration below 0.05 mg/kg food). A bacterial reverse mutation assay (Ames test) and an in vitro mammalian cell micronucleus test carried out on the substance were submitted, according to the EFSA Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011).

The migration of 21 impurities and reaction products was estimated using conservative migration modelling, and individual results were all below 0.05 mg/kg food. Two of them, acetone (N: 1) and 2-aminobenzamide (N: 3), are authorised as FCM substances No 119 and No 164, respectively, hence no data were submitted. For two others, 2-aminobenzamide +1 FA with desaturation (i.e. 4-hydroxyquinazoline; N: 7) and 2-aminobenzamide +1 AA with desaturation (i.e. 2-methyl-4(3H)-quinazolinone; N: 21), the same set of experimental data as for the substance was submitted. For the 17 other identified impurities or reaction products, no tests were carried out. They have been assessed as follows: (i) in silico data were used for 14 of those, (ii) one impurity was recently assessed by the EFSA Food Additives and Flavourings (FAF) Panel, (iii) the threshold of toxicological concern (TTC) for genotoxic carcinogens was used for one impurity and (iv) the remaining impurity, aniline, being a primary aromatic amine (PAA), is regulated as impurity in the Reg. (EU) 10/2011. The full list of data submitted for each substance is available in Annex 1, Table A2.

3.2.1 | N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide)

3.2.1.1 | *Bacterial reverse mutation assay*

A bacterial reverse mutation assay was conducted (2020) in Salmonella Typhimurium strains TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 *uvrA* to assess the mutagenicity of N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) (purity 97.64%). The study was performed in compliance with Good Laboratory Practice (GLP) principles, following the Organisation for Economic Co-operation and Development (OECD) TG 471 (OECD, 1997). The test material was dissolved in DMSO and evaluated in two separate experiments: 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 to 5000 μg/plate were applied. All concentrations, as well as negative (vehicle) and positive controls, were evaluated using triplicate plates. Precipitate of the test item was observed in both experiments at 5000 μg/plate, with and

[9]The 14 identified impurities (N: 1–14), the five identified reaction products (containing AA or FA) (N: 15–19) and the two other side reaction products (N: 20, 21).

[10]The Panel considered that the residual amount of 126 mg/kg of the substance -obtained from the THF extraction from PET preforms- may lead to the measured migration of the substance of 0.0038 mg/kg food. Proportionally, the potential migration of 650 mg/kg of reaction products would results in 0.0196 mg/kg food.

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

without S9-mix. No evidence of toxicity, as reduction of bacterial background lawn, 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 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.

3.2.1.2 | *In vitro mammalian cell micronucleus test*

N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) (purity 97.64%) was tested (2021) in the in vitro micronucleus assay in human peripheral blood lymphocytes. The test was carried out following GLP principles.

The test material was dissolved in DMSO and tested in duplicate lymphocyte cultures (quadruplicate for negative control). Cells were exposed to the test item applying both a short (4 h) treatment with or without metabolic activation by rat liver S9-mix, and with an extended (24 h) treatment without S9-mix. All treatments were followed by 24 h incubation with fresh medium supplemented with cytochalasin B to block cytokinesis.

The following three concentrations were applied with all treatments: 15.63, 31.25 and 62.5 μg/mL. 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 the OECD TGs 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 four cultures) for the vehicle controls. The cytokinesis-block proliferation index (CBPI), as an index of toxicity, was determined in 500 cells per culture.

Visible precipitation was observed at the highest concentration under all treatment conditions. Mild toxicity, with 22% cytostasis, was observed at the maximum concentration after the extended treatment. No increase in binucleated cells containing micronuclei was 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 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 2023 OECD TG 487 as the 24 h treatment of the cells was performed in the absence of cytochalasin B (CytB), which was added at the end of treatment in order to avoid any possible interaction between CytB and the test material. 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, 2021). The application of this modified protocol was considered acceptable for the testing of the substance, also in view of the highly significant positive response elicited under the study conditions by positive control substances covering both mechanisms implicated in micronucleus formation (clastogenic by mitomycin C and aneugenic by colchicine).

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

3.2.2 | 2-Aminobenzamide +1 FA with desaturation (i.e. 4-hydroxyquinazoline) (N: 7)

3.2.2.1 | *Bacterial reverse mutation assay*

A bacterial reverse mutation assay was conducted (2015) in *S.*Typhimurium strains TA98, TA100, TA1535, TA1537 and in *Escherichia coli* WP2 uvrA pKM101 to assess the mutagenicity of 4-hydroxyquinazoline (CAS 491-36-1, purity > 99.9%). The study was performed in compliance with GLP principles, following the OECD TG 471 (OECD, 1997). The test material was dissolved in DMSO and evaluated in two separate experiments: 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 seven concentrations ranging from 5 to 5000 μg/plate; in the second experiment, five concentrations in the range of 50 to 5000 μg/plate were applied. All concentrations, as well as negative (vehicle) and positive controls, were evaluated using triplicate plates. Toxicity (reduction in revertant numbers) was observed at 5000 μg/plate in both experiments in some strains: in the first experiment in strains TA1535 (without S9-mix) and WP2 uvrA pKM101 (with S9-mix); in the repeated experiment, in strain TA1537 (with S9-mix). No increase in revertant colonies was observed at any concentration in any bacterial tester strain, with or without metabolic activation. The test item did not induce gene mutations under the conditions of the study. The Panel considered the study reliable without restrictions and the results of high relevance.

3.2.2.2 | *In vitro mammalian cell micronucleus test*

4-Hydroxyquinazoline (CAS 491-36-1, purity > 99.9%) was tested (2023) in the in vitro micronucleus assay in human peripheral blood lymphocytes. The test was carried out following GLP principles and the OECD TG 487 (OECD, 2016). The test material was dissolved in DMSO and tested in duplicate lymphocyte cultures (quadruplicate for negative control). Cells were exposed to the test item applying both a short (3 h + 17 h of recovery) treatment with or without metabolic activation by

rat liver S9-mix, and with an extended (20 h) treatment without S9-mix. The following three concentrations were applied: 400, 800 and 1461.5 μg/mL (10 mM, maximum recommended concentration) with short (3/17 h with and without S9-mix) treatment; 40, 700 and 800 μg/mL with continuous treatment (20 h without S9-mix); 2000 (1000/culture) BN cells scored/dose (4000 in controls). The CBPI, as an index of toxicity, was determined in 500 cells per culture.

No cytotoxicity was observed after short treatments; dose-related cytotoxicity (up to 51% at the top concentration) was seen with continuous treatment. No increase in the incidence of micronucleated cells was observed at any concentration, with or without S9-mix.

The test item did not induce the formation of micronuclei under the conditions of the study. The Panel considered the study reliable without restrictions and the results of high relevance.

3.2.3 | 2-Aminobenzamide +1 AA with desaturation (i.e. 2-methyl-4(3H)-quinazolinone) (N: 21)

3.2.3.1 | *Bacterial reverse mutation assay*

A bacterial reverse mutation assay was conducted (2015) in *S.*Typhimurium strains TA98, TA100, TA1535, TA1537 and in Escherichia coli WP2 uvrA pKM101 to assess the mutagenicity of 2-Methyl-4(3H)-quinazolinone (CAS 1769-24-0, purity 99.8%). The study was performed in compliance with GLP principles, following the OECD TG 471 (OECD, 1997). The test material was dissolved in DMSO and evaluated in two separate experiments: 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 seven concentrations ranging from 5 to 5000 μg/plate; in the second experiment, five concentrations in the range of 50 to 5000 μg/plate were applied. All concentrations, as well as negative (vehicle) and positive controls, were evaluated using triplicate plates. Toxicity (reduction in revertant numbers) was observed at 5000 μg/plate in strain WP2 uvrA pKM101 in the first experiment and in the repeat experiment (with and without S9-mix, respectively). No increase in revertant colonies was observed at any concentration in any bacterial tester strain, with or without metabolic activation.

The test item did not induce gene mutations under the conditions of the study. the Panel considered the study reliable without restrictions and the results of high relevance.

3.2.3.2 | *In vitro mammalian cell micronucleus test*

2-Methyl-4(3H)-quinazolinone (CAS 1769-24-0, purity 99.6%) was tested (2023) in the in vitro micronucleus assay in human peripheral blood lymphocytes. The test was carried out following GLP principles and the OECD TG 487 (OECD, 2016). The test material was dissolved in DMSO and tested in duplicate lymphocyte cultures (quadruplicate for negative control). Cells were exposed to the test item applying both a short (3 h + 17 h of recovery) treatment with or without metabolic activation by rat liver S9-mix, and with an extended (20 h) treatment without S9-mix. The following three concentrations were applied: 400, 800 and 1601.7 μg/mL (10 mM, maximum recommended concentration) with short (3/17 h with and without S9-mix) treatment; 20, 400 and 550 μg/mL with continuous treatment (20 h without S9-mix); 2000 (1000/culture) BN cells scored/dose (4000 in controls). The CBPI, as an index of toxicity, was determined in 500 cells per culture. No cytotoxicity was observed after short treatments; dose-related cytotoxicity (up to 51% at the top concentration) was seen with continuous treatment. No increase in the incidence of micronucleated cells was observed at any concentration, with or without S9-mix.

The test item did not induce the formation of micronuclei under the conditions of the study. The Panel considered the study reliable without restrictions and the results of high relevance.

3.2.4 | Other 19 identified impurities and reaction products

Acetone (N: 1) and 2-aminobenzamide (N: 3) are authorised as FCM substance No 119 with no restrictions, and as FCM substance No 164 with an SML of 0.05 mg/kg food and with the following restriction: 'only for use in PET for water and beverages', respectively. Both substances migrate below 0.05 mg/kg food, with modelled values reported to be 1.5 and 3.9 μg/kg food, respectively. Acetone did not raise concern for genotoxicity based on the available in vitro, in vivo and in silico data (EFSA CEP Panel, 2020; EFSA CONTAM Panel, 2013).[12] 2-Aminobenzamide was assessed with no concern for genotoxicity (SCF, 2003).

An in silico assessment of the genotoxicity of the following 12 reaction products was performed using the Derek Nexus 6.1.0 (Derek KB 2020 1.0 and 2023 1.0) and Leadscope Model Applier (Version 3.0.2.) software (numeration refers to Table 1):

- (8) disubstituted analogue of the substance
- (9) over addition analogue of the substance

[12]The EFSA CONTAM Panel established for acetone a health-based guidance value of 0.9 mg/kg bw per day (EFSA CONTAM Panel, 2013). This would currently result in an acceptable daily intake (ADI) of 4.5 mg/kg bw per day (EFSA CEP Panel, 2020).

- (10) mono urea substance derivative
- (11) oxidised product
- (12) substance +1 AC
- (13) substance +2 AC
- (14) substance +1 FA with desaturation
- (15) substance +1 FA
- (16) substance +3 FA
- (17) substance +1 AA
- (18) substance +2 AA
- (19) substance +3 AA

No reliable indication of potential genotoxicity, mutagenicity or chromosome damage was provided by the QSAR analysis by the rule-based Nexus models and the suite of Leadscope Genetox statistical models, except for the oxidised product (N: 11). This substance was predicted as a possible DNA-reactive in vitro mutagen and clastogen due to the presence of an aromatic hydroxylamine group. However, the migration of this by-product, estimated by conservative modelling, would not exceed 0.14 μg/kg food and thus leads to a potential exposure below the TTC of 0.0025 μg/kg body weight (bw) per day under the SCF food consumption scenario (EFSA Scientific Committee, 2012).

For [redacted] (N: 4) and [redacted] (N: 2), no structural alerts for mutagenicity and DNA binding were identified from the OECD QSAR Toolbox 4.4.

[redacted] (N: 5) was evaluated as not genotoxic in the re-evaluation of saccharin and its sodium, potassium and calcium salts (E 954) as food additives (EFSA FAF Panel, 2024) and also in the 'Flavouring Group Evaluation 84, (FGE.84) […]' (EFSA AFC Panel, 2008).

According to the conservative model, pyridine (N: 20) migrates, if at all, below 0.15 μg/kg food (0.002 μg/kg food), hence leading to a potential exposure below the TTC of 0.0025 μg/kg body weight per day (EFSA Scientific Committee, 2012).

Aniline (N: 6) is a primary aromatic amine (PAA), and it is classified as Muta.2 according to the Classification, labelling and packaging of substances and mixtures (CLP) Regulation. Being an impurity of the substance under assessment, it would be subject to point 2 of Annex II of Reg. (EU) 10/2011.[13] The Panel noted that for aniline the migration estimated through modelling was 0.022 μg/kg food.

3.3 | Discussion

The measured and the modelled migrations of the substance and of its identified impurities and reaction products are all individually below 0.05 mg/kg food.

The substance N,N′-(2-(4-(2-aminobenzamido) butyl)pentane-1,5-diyl)bis(2-aminobenzamide), as well as two impurities/reaction products (2-aminobenzamide +1 FA with desaturation, N: 7 and 2-aminobenzamide +1 AA with desaturation, N: 21) were evaluated in the recommended battery of in vitro genotoxicity tests (bacterial reverse mutation and in vitro micronucleus), with highly relevant negative results. Acetone (N: 1) and 2-aminobenzamide (N: 3) are authorised in accordance with Reg. (EU) 10/2011 (FCM substance No 119 and No 164, respectively). Based on in silico data, previous EFSA evaluations and the use of the TTC, the Panel concluded that another 15 identified impurities/reaction products formed under the reported conditions do not raise a concern for genotoxicity.

An alert for genotoxicity was only identified for the oxidised product (N: 11) due to the hypothesised presence of an aromatic hydroxylamine group. According to the specifications provided by the applicant, the oxidised product may amount to up to 0.15% w/w of the substance. The migration modelling estimated a possible migration of 0.14 μg/kg food, leading to a potential exposure below the TTC of 0.0025 μg/kg body weight (bw) per day, under the SCF food consumption scenario (EFSA Scientific Committee, 2012). The Panel, however, could not rule out the potential formation of toxicologically related aromatic hydroxylamine groups arising from other impurities. For this reason, the amount of the sum of the aromatic hydroxylamine derivatives should not exceed 0.15% w/w of the substance[14] to avoid a safety concern.

For the primary aromatic amine aniline, the migration modelling estimated a possible migration, if any, of 0.022 μg/kg food. The Panel noted that this is below 0.01 mg/kg in food or food simulant set for the sum of PAAs in point 2 of Annex II of Reg. (EU) 10/2011.

The Panel identified an important gap in the mass balance of the residual contents in the PET and could not exclude the presence of non-identified reaction products of the substance, when reacting with AA, FA or AC. The Panel concluded that: (i) they are structurally related to the identified reaction products, (ii) their total possible migration -including also the identified ones- does not exceed 0.02 mg/kg food and (iii) they do not raise genotoxicity concern based on the predicted structural similarity with the other substance-related reaction products for which studies and in silico data have been

[13]For PAAs not listed into entry 43 to Appendix 8 of Annex XVII to Regulation (EC) No 1907/2006, but for which no specific migration limit is specified in Annex I of Reg. (EU) 10/2011, compliance with Article 3 of Regulation (EC) No 1935/2004 shall be verified in accordance with Article 19. The sum of those PAAs shall however not exceed 0.01 mg/kg in food or food simulant.

[14]This implies that, as a group, they will not migrate above 0.14 μg/kg food, leading to a potential exposure below the TTC of 0.0025 μg/kg body weight per day.

provided. The proposed restrictions in uses and a specific migration limit of 0.05 mg/kg food aim to cover the possible migration of identified and not-identified impurities and reaction products.

The Panel noted that an alternative synthesis route of the substance may involve N-methyl-2-pyrrolidone (NMP) as solvent. In that case, the possible formation of N-nitrosamines should be assessed.

The contact with infant formula and human milk was not specified as intended application of the substance. However, PET is largely used to pack water, which in turn is also used to reconstitute infant formula. In this case, additional data should be provided in accordance with the Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA Scientific Committee, 2017). In the absence of such data, the FCM Panel concluded that the contact with water used to reconstitute infant formula, human milk or liquid infant formula is not covered by the current assessment.

4 | CONCLUSIONS

The FCM Panel concluded that the substance N,N′-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide) is not of safety concern for the consumer if:

- it is used as acetaldehyde scavenger at a maximum concentration of 650 mg/kg PET; and
- the PET articles are used in contact with foods simulated by simulants A, B and C for storage up to and above 6 months at room temperature and below, including hot-fill conditions and/or heating up to $70°C \leq T \leq 100°C$ for maximum $t = 120/2^{((T-70)/10)}$ minutes; and
- the PET articles are not used in contact with human milk, liquid infant formula and the water that could be used to reconstitute powdered infant formula[15]; and
- the migration of the substance does not exceed 0.05 mg/kg food; and
- the substance does not contain aromatic hydroxylamine derivatives at more than 0.15% w/w of the substance.

The substance contains aniline as an impurity; therefore, compliance with the restrictions set for primary aromatic amines in point 2 of Annex II of Reg. (EU) 10/2011 should be verified.

5 | DOCUMENTATION AS PROVIDED TO EFSA

Dossier 'N,N'-(2-(4-(2-aminobenzamido)butyl)pentane-1,5-diyl)bis(2-aminobenzamide)'. November 2023. Submitted by Avient Corporation, ColorMatrix Group.

Additional information, October 2024. Submitted by Avient Corporation, ColorMatrix Group.

ABBREVIATIONS

AA	acetaldehyde
AC	acetone
ACN	acetonitrile
Bw	body weight
CAS	Chemical Abstracts Service
CBPI	cytokinesis-block proliferation index
CEF	Panel EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	Panel EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CLP	Classification, labelling and packaging
DSC	differential scanning calorimetry
FA	formaldehyde
FCM	food contact materials
GC–MS	gas chromatography–mass spectrometry
GLP	Good laboratory practice
LC–HRMS	liquid chromatography coupled with high-resolution mass spectrometry
LC–MS	liquid chromatography–mass spectrometry
LC–QTOF-MS	liquid chromatography–time-of-flight mass spectrometry
NMP	N-methyl-2-pyrrolidone
OECD	Organisation for Economic Co-operation and Development
PAA	primary aromatic amine
PET	polyethylene terephthalate
SCF	Scientific Committee on Food

[15]Sparkling water is not considered to be used for reconstituting infant formula.

SML	specific migration limit
TGA	thermogravimetric analysis
THF	tetrahydrofuran
TTC	threshold of toxicological concern

REQUESTOR

Irish competent authority (Food Safety Authority of Ireland)

QUESTION NUMBER

EFSA-Q-2023-00721

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ANNEX 1

TABLE A1 Names, CAS numbers and chemical structures of the 21 identified impurities and reaction products.

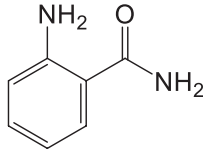
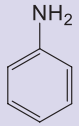
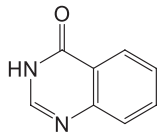
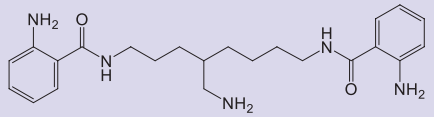
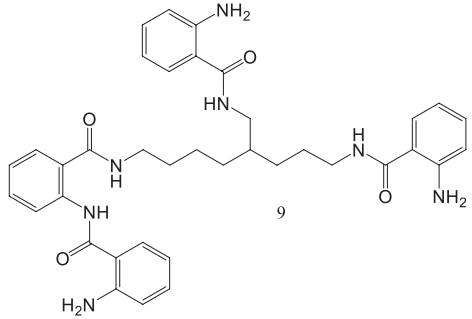
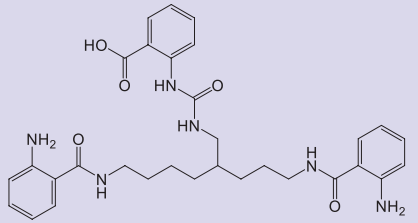
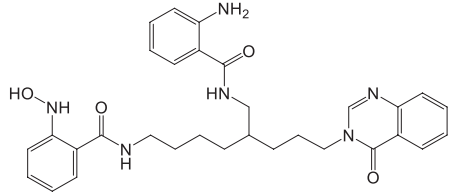
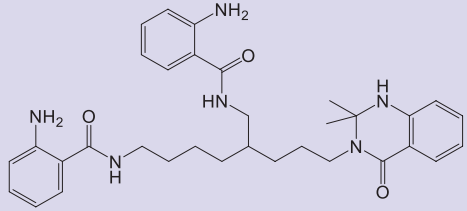
N	Name used in the opinion	Synonym	CAS number	Classification	Specification	Chemical structure
1	Acetone	2-Propanone	67-64-1	Listed in the substance specification	< 1000 ppm	
2	[redacted]	[redacted]	[redacted]	Listed in the substance specification	< [redacted]%	
3	2-Aminobenzamide	Anthranilamide	88-68-6	Listed in the substance specification	< [redacted]%	
4	[redacted]	[redacted]	[redacted]	Listed in the substance specification	< [redacted] ppm	
5	[redacted]	[redacted]	[redacted]	Listed in the substance specification	< [redacted] ppm	
6	Aniline	Aminobenzene	62-53-3	Listed in the substance specification	< 20 ppm	
7	2-Aminobenzamide +1 FA with desaturation	Anthranilamide +1 FA with desaturation or quinazolin-4(1H)-one or 4-hydroxyquinazoline or IUPAC: 3H-quinazolin-4-one	491-36-1	Listed in the substance specification	< 1000 ppm	

TABLE A1 (Continued)

N	Name used in the opinion	Synonym	CAS number	Classification	Specification	Chemical structure
8	Disubstituted analogue of the substance*	Disubstituted 4-(aminomethyl) octane-1,8-diamine		Listed in the substance specification	< [redacted]%	
9	Over addition analogue of the substance*	Tetrasubstituted 4-(aminomethyl) octane-1,8-diamine		Listed in the substance specification	< [redacted]%	
10	Mono urea substance derivative*			Listed in the substance specification	< [redacted]%	
11	Oxidised product*	Oxidised substance +1 FA with desaturation or oxidised product of (14)		Listed in the substance specification	< 0.15%	
12	Substance +1 AC*			Listed in the substance specification	< 1%	

(Continues)

TABLE A1 (Continued)

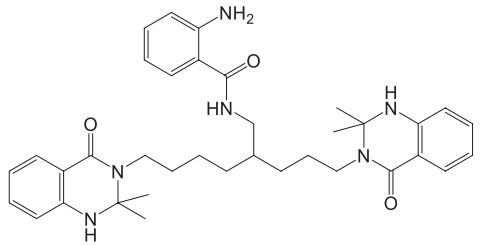
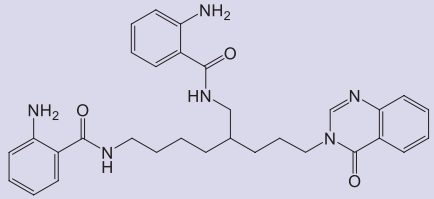
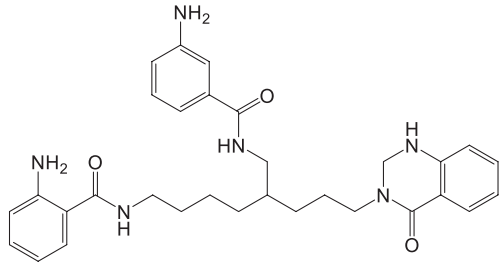
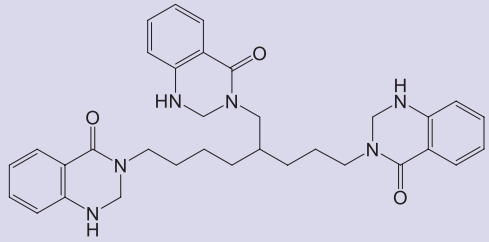
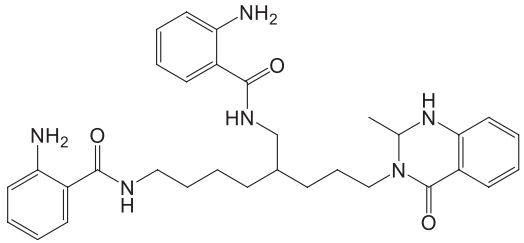
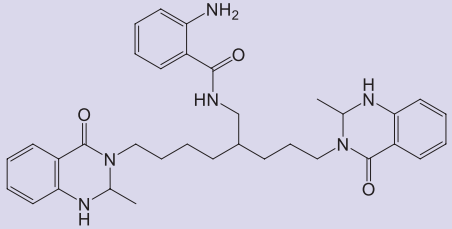
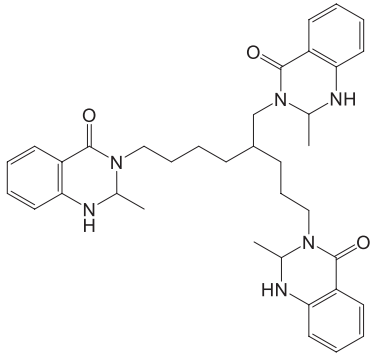
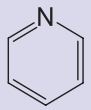
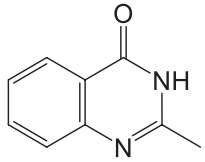
N	Name used in the opinion	Synonym	CAS number	Classification	Specification	Chemical structure
13	Substance +2 AC*			Listed in the substance specification	< 1%	
14	Substance +1 FA with desaturation*			Other identified reaction product		
15	Substance +1 FA*			Other identified reaction product		
16	Substance +3 FA			Other identified reaction product		

TABLE A1 (Continued)

N	Name used in the opinion	Synonym	CAS number	Classification	Specification	Chemical structure
17	Substance +1 AA*			Other identified reaction product		
18	Substance +2 AA*			Other identified reaction product		
19	Substance +3 AA			Other identified reaction product		
20	Pyridine	Azabenzene	110-86-1	Other identified reaction product		
21	2-Aminobenzamide +1 AA with desaturation	2-methyl-4(3H)-quinazolinone	1769-24-0	Other identified reaction product		

Note: Acetone (AC), acetaldehyde (AA), formaldehyde (FA), reaction products without desaturation unless stated otherwise.

*The chemical structure shown is one of several possible isomers that differ in the position of the reactive site.

TABLE A2 Calculated migration (presented as rounded values) using the conservative migration model (for 10 days at 60°C) and provided genotoxicity data with related conclusions and assessments for the 21 identified impurities and reaction products.

N	Impurities and reaction products	Calculated migration (μg/kg food)	Data provided and used	In silico results: Derek Nexus *(1st line)* Leadscope models *(2nd, 3rd line)*	Results and reliability and relevance (*where applicable*)	Assessment
1	Acetone	1.5			EFSA CONTAM Panel (2013) and EFSA CEP Panel (2020)	FCM substance No 119 with no restrictions No concern for genotoxicity
2	[redacted]	0.7			No structural alerts for mutagenicity and DNA binding found in OECD QSAR Toolbox	No concern for genotoxicity
3	2-Aminobenzamide	3.9			SCF (2003)	FCM substance No 164 with an SML: 0.05 mg/kg food and restriction: 'only for use in PET for water and beverages' No concern for genotoxicity
4	[redacted]	0.8			No structural alerts for mutagenicity and DNA binding found in OECD QSAR Toolbox	No concern for genotoxicity
5	[redacted]	0.07			EFSA FAF Panel (2024) and EFSA AFC Panel (2008)	No concern for genotoxicity
6	Aniline	0.02				Verification of compliance with the restriction set for primary aromatic amines in Annex II (2) of Reg. (EU) 10/2011 is necessary
7	2-Aminobenzamide +1 FA with desaturation (quinazolin-4(1H)-one)	1.4	– Bacterial reverse mutation assay – In vitro mammalian cell micronucleus test		- Negative - Negative - Both studies: reliable without restrictions and results of high relevance	No concern for genotoxicity
8	Disubstituted analogue of the substance	0.8	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	No alert Pos 1/17[16] Neg 10/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
9	Over addition analogue of the substance	0.6	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	No alert Pos 0/17 Neg 4/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity

[16]The positive prediction for in vitro SCE in CHO cells is not considered valid as the training set of the Leadscope model included structures with reactive groups (aromatic amines and aromatic nitro) not found in the test compound.

TABLE A2 (Continued)

N	Impurities and reaction products	Calculated migration (μg/kg food)	Data provided and used	In silico results: Derek Nexus *(1st line)* Leadscope models *(2nd, 3rd line)*	Results and reliability and relevance (*where applicable*)	Assessment
10	Mono urea substance derivative	0.8	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 8/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
11	Oxidised product	0.1	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 In vitro chromosome damage and mutagenicity Pos 2/17 Neg 3/17	Predicted as possible DNA-reactive in vitro mutagen and clastogen due to the presence of an aromatic hydroxylamine group	Migration (if any) lower than 0.15 μg/kg food → potential exposure below the TTC of 0.0025 μg/kg body weight per day, under the SCF food consumption scenario
12	Substance +1 AC	0.9	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 5/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
13	Substance +2 AC	0.7	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 1/17[17] Neg 2/17	No reliable indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
14	Substance +1 FA with desaturation	3.9	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 5/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
15	Substance +1 FA	1	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 5/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
16	Substance +3 FA	0.9	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 4/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
17	Substance +1 AA	5.5	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 5/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity

[17]The positive prediction for bacterial mutagenicity by one Leadscope model is not considered valid as the training set included substances with alerting structures (aromatic nitro and quinoline groups) is not present in the test substance.

(Continues)

TABLE A2 (Continued)

N	Impurities and reaction products	Calculated migration (μg/kg food)	Data provided and used	In silico results: Derek Nexus *(1st line)* Leadscope models *(2nd, 3rd line)*	Results and reliability and relevance (*where applicable*)	Assessment
18	Substance +2 AA	3.3	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 5/17	No indication of potential genotoxicity, mutagenicity or chromosome damage	No concern for genotoxicity
19	Substance +3 AA	0.2	QSAR: Derek Nexus 6.1.0 Leadscope Model Applier	 No alert Pos 0/17 Neg 3/17	No indication of potential genotoxicity, mutagenicity or chromosome damage and migration	No concern for genotoxicity
20	Pyridine	0.002				Migration (if any) lower than 0.15 μg/kg food → potential exposure below the TTC of 0.0025 μg/kg body weight (bw) per day, under the SCF food consumption scenario
21	2-aminobenzamide +1 AA with desaturation (2-methyl-4(3H)-quinazolinone)	0.07	- Bacterial reverse mutation assay - In vitro mammalian cell micronucleus test		- Negative - Negative - Both studies: reliable without restrictions and results of high relevance	No concern for genotoxicity

Note: Acetone (AC), acetaldehyde (AA), formaldehyde (FA); reaction products without desaturation unless stated otherwise.