OFI Technologie & Innovation GmbH
Firmensitz: 1030 Wien, Franz-Grill-Straße 5, Objekt 213
t: +43 1 798 16 01 - 0 | office@ofi.at | www.ofi.at

Test Report

Report No.: **2402523 / 25768** **Date:** 2024-12-17

Client: Plasgran Limited trading as Berry Circular Polymers
Unit B, Ashbourne Drive; Leamington Spa
CV 31 3SS Warwickshire

Subject: L80
L83
L86

Task: Analysis of migrates from sample materials for DNA-reactive, genotoxic effects using the Ames MPF assay

Order: Order of 2024-10-15

Date of sampling: —

Location of sampling: No samples taken by OFI staff
Samples provided by the client

Receipt of samples: 2024-11-06

DRAFT

1 SCOPE OF WORK

According to the order the samples provided were to be tested for DNA-reactive, genotoxic substances using *in-vitro* bioassays. DNA-reactive, genotoxicity was determined using the Ames MPF assay which detects substances that revert point or frame shift mutations in histidine auxotrophic *Salmonella* Typhimurium strains.

2 SCOPE OF APPLICATION

The results given in this Test Report have been obtained under the specific conditions of the individual tests. As a rule they are not the only criteria for assessing the product in question and its suitability for a specific purpose of application.

The test report may only be used by the client within the scope of the agreed right of use. Further rights, in particular the right to modify or edit, even in part, are not transferred to the client. This Test Report shall only be used for internal information of the client and shall not be used as a basis for decision-taking by third parties. OFI shall give its consent before this test report or its contents are passed on to third parties. Publication or reproduction, even in part, is prohibited in any case and always requires the prior written consent of the OFI.

3 SAMPLE MATERIAL

Our client submitted the following samples for the purpose of testing:

Table 1: Codes of the received sample materials.

Sample code	Description	OFI code
L80	PP granules	M676
L83	PP granules	M677
L86	PP granules	M678

Other documents submitted by our client:

No (other) documents submitted.

4 TESTS

Testing took place from 2024-11-06 to 2024-12-16.

The tests were carried out in the individual technical departments within the scope of competence of the authorised signatories according to the OFI QM manual.

4.1 Migration

The migration of the test items was carried out based on the regulations on plastic materials and articles intended to come into contact with food, regulation (EU) No. 10/2011, as amended, or EN 1186:2002. For each sample two or more independent migrations were prepared. For comparison a solvent blank was prepared to check for possible contaminations. The analysis and sample preparation of the blank was identical to the analysis of the samples.

Table 2: Migration conditions.

Sample code	OFI code	Simulant	Temperature	Storage time	Storage condition
L80	M676	95% EtOH	60°C	10 days	30 g/100 mL in glass bottle closed with PTFE-coated screw cap
L83	M677	95% EtOH	60°C	10 days	30 g/100 mL in glass bottle closed with PTFE-coated screw cap
L86	M678	95% EtOH	60°C	10 days	30 g/100 mL in glass bottle closed with PTFE-coated screw cap

4.2 Concentration step

After migration the migrates were concentrated by a factor of approximately 100 with the Syncore® Analyst parallel evaporator (BÜCHI). Concentrated migrates were transferred to the bioassay compatible solvent DMSO. Prior to analysis with bioassays, the samples were stored at 4°C.

4.3 Bioassays

4.3.1 Ames MPF assay

The Ames test (bacterial reverse mutation assay) is a bacteria-based bioassay to detect DNA-reactive, genotoxic activity.

Sample migrates were tested following instructions of OFI SOP 350.016.

Two different *Salmonella* Typhimurium strains, TA98 (for detection of frameshift mutations) and TA100 (for detection of point mutations), were used for testing. Each sample was analysed in the presence and absence of an external metabolic activation system (phenobarbital/β-naphthoflavone-induced rat S9-mix). Reversion indicator medium and exposure medium were prepared based on ISO 11350:2012. A sample migrate scored Ames positive if an at least 2-fold increase (including standard deviation) of the response was reported when compared to the negative control.

In order to assess inhibiting effects, each sample was tested with an external spike of a positive control substance (TA100 w/o S9: 4-nitroquinoline 1-oxide (4-NQO), TA98 w/o S9: 2-nitrofluorene (2-NF), TA100 +S9 and TA98 +S9: 2-aminoanthracene (2-AA)). Samples with a recovery of less than 60% had to be diluted to assess their DNA-reactive, genotoxicity.

Two or more migrates (DMSO concentrates) of each sample were each analysed in triplicates using the described method.

5 RESULTS

5.1 Determination of DNA-reactive genotoxicity with the Ames MPF assay

Two or more independent migrates of each sample were analysed in triplicates in a miniaturized format of the Ames assay (bacterial reverse mutation test). To check for possible inhibiting matrix effects the concentrated sample migrates were spiked with known

genotoxic substances. A sample migrate scored Ames positive if an at least 2-fold increase (including standard deviation) of the response was reported when compared to the negative control. The results and the detection limits for the respective positive controls referring to the migration solution are given in Table 3 and Table 4. An overview of the results is provided in Table 5.

Table 3: Results of the Ames MPF genotoxicity screening in strain TA98.

Sample	OFI code	Simulant	- S9		+ S9	
			Result [µg/L]	LOD (2-NF) [µg/L]	Result [µg/L]	LOD (2-AA) [µg/L]
L80	M676	95% EtOH	<LOD [1]	143 [1]	<LOD	2.9
L83	M677	95% EtOH	<LOD [1]	149 [1]	<LOD	3.0
L86	M678	95% EtOH	<LOD [1]	146 [1]	<LOD	2.9
Solvent blank	BW	95% EtOH	<LOD	14	<LOD	2.8

< LOD… not detectable, below limit of detection

1……. undiluted migrates not analysable due to insufficient recovery when spiked with known mutagen, higher limit of detection

Table 4: Results of the Ames MPF genotoxicity screening in strain TA100.

Sample	OFI code	Simulant	- S9		+ S9	
			Result [µg/L]	LOD (4-NQO) [µg/L]	Result [µg/L]	LOD (2-AA) [µg/L]
L80	M676	95% EtOH	<LOD	5.5	<LOD	12
L83	M677	95% EtOH	<LOD	5.7	<LOD	12
L86	M678	95% EtOH	<LOD	5.6	<LOD	12
Solvent blank	BW	95% EtOH	<LOD	5.3	<LOD	11

< LOD… not detectable, below limit of detection

Table 5: Summary of the Ames MPF genotoxicity screening results.

Sample	OFI code	Simulant	Results
L80	M676	95% EtOH	Negative, no genotoxic activity
L83	M677	95% EtOH	Negative, no genotoxic activity
L86	M678	95% EtOH	Negative, no genotoxic activity
Solvent blank	BW	95% EtOH	Negative, no genotoxic activity

6 SUPPLEMENTARY STATEMENT ON THE TEST RESULTS

All provided samples were analysed for DNA-reactive, genotoxic effects.

To simulate a possible leaching of packaging compounds into food during storage, sample materials were stored with food simulants based on the regulations on articles intended to come into contact with food regulation (EU) No. 10/2011, as amended, or EN 1186:2002.

All sample treatment procedures and test methods were validated for sufficiency using genotoxic reference standards. In order to check for potential contaminations additional solvent blanks were analysed to compare sample results.

6.1 Testing for DNA-reactive, genotoxic activity

To assess the genotoxicity of the sample migrates the bacterial reverse mutation test (Ames test) was used. The Ames test is a widely accepted genotoxicity test, based on different strains of the bacterium *Salmonella* Typhimurium. It is able to detect substances that cause DNA mutations and is recommended by an ILSI expert group (Schilter et al. 2019) as it is considered to be the most sensitive test for the detection of DNA-reactive substances. A miniaturized version of the Ames assay was used for testing, to reduce the required amount of sample migrate. To evaluate a possible metabolic activation, metabolism was simulated by addition of S9 liver extract. Spiking the sample migrates with known genotoxic substances demonstrates whether or not the Ames MPF test is able to detect genotoxic substances in the presence of the sample matrix.

The Ames MPF assay did not detect DNA-reactive, genotoxic effects for any of the tested samples. However, inhibiting effects of the sample matrix of the samples “L80” (M676), “L83” (M677) and “L86” (M678) caused higher limits of detection in the strain TA98 without metabolic activation.

Even though the *in-vitro* tests used in this study show whether or not substances migrating from the sample show DNA-reactive, genotoxic potential, no direct conclusions on human health are possible based on these *in-vitro* results alone.

7 LITERATURE

Schilter, B. et al. (2019) 'Value and limitation of in vitro bioassays to support the application of the threshold of toxicological concern to prioritise unidentified chemicals in food contact materials', Food Additives & Contaminants: Part A, 36(12), pp. 1903–1936.

European Commission (2011) 'Commission Regulation (EU) No 10/2011 (as amended) of 14 January 2011 on plastic materials and articles intended to come into contact with food', Official Journal of the European Union, L 12, pp. 1–89.

European Committee for Standardization (2002) EN 1186:2002: Materials and articles in contact with foodstuffs - Plastics.

International Organisation for Standardisation (2012) ISO 11350:2012: Water quality — Determination of the genotoxicity of water and waste water — Salmonella/microsome fluctuation test (Ames fluctuation test).

Xenometrix (2015) Ames MPF™ 98/100 Microplate Format Mutagenicity Assay. S. typhimurium TA98 and TA100. Instructions for use. 5.01.

DRAFT

This Test Report No. **2402523 / 25768** comprises
9 sheets with 5 table(s), 0 figure(s) and 0 appendix(es).

Any test results relate only to the samples tested. All tests applied are subject to a quality assurance program according to EN ISO/IEC 17025:2017. The Test Report may only be used by the client within the scope of the agreed right of use. Publication or reproduction, even in part, always requires the prior written consent of the OFI.

The General Terms and Conditions of OFI Technologie & Innovation GmbH shall apply as amended. They are available for download on our website (www.ofi.at).

Posch Markus	Washuettl Michael
Testing engineer	Director in charge

DRAFT