Appendix P - Genotoxicity studies submitted by Interested Business Operators

The evaluation of the studies was performed according the approach set in Appendix D

Test system/ Test object	**Exposure conditions (concentration/ duration /metabolic activation**	**Information on the characteristics of the test substance**	**Scoring for nanoscale considerations (dispersion and/or confirmation of internal exposure), assigned according to Appendix E**	**Result**	**Reliability/ Comments**	**Relevance of the result**	**Reference**
Bacterial reverse mutation assay (Ames test) in *S. typhimurium* TA98, TA100, TA1535, TA1537 and E. Coli WP2 *uvr*A	Plate incorporation methodology; Concentrations in the main test: 33.3, 100, 333, 1000, 3333 and 5000 µg/plate; ± S9 Vehicle: water A confirmatory test was not conducted.	The tested material corresponds to a commercial brand of E 171 for which data were submitted during the assessment of the amendment of the EU specifications for E171 (sample E) (EFSA FAF Panel, 2019), and consists of constituent particles with a median diameter in the order of 100 nm. Purity 99.5%.	NSC: 3 Dispersion was not considered and no information on agglomeration level provided.	**Negative** Precipitate observed > 1000 µg/plate No cytotoxicity observed. Reduction in revertant count in TA1537 from 3333 µg/plate, at the same concentrations there was an increase of interfering particulate.	Reliability: 5 Bacterial systems are not suitable for testing nanomaterials	Low	BioReliance, 2020a. Documentation provided to EFSA No. 14.
Mammalian Cell Micronucleus Assay	HPBL were treated for 4 hours in the absence and presence of S9, and for	The tested material corresponds to a commercial brand of E 171 for which data	NSC: 3 Protocol for poorly soluble substances not	**Inconclusive** (Negative without proof of internalisation)	Reliability: 3	Low	BioReliance, 2020b.

Human Peripheral Blood Lymphocytes (HPBL) from a healthy non-smoking female individual	24 hours in the absence of S9. 0.3, 1, 2, 3, 10, and 30 µg/mL for all three exposure groups. 0.3, 1, 10 and 30 µg/mL selected for the MN analysis Positive controls: -S9: Mitomycin C -S9: Vinblastine +S9:Cyclophosphamide CytoB at 6 µg/mL added after 4h in the 4h -S9 and +S9 tests and at the beginning in the 24h -S9 test	were submitted during the assessment of the amendment of the EU specifications for E171 (sample E) (EFSA FAF Panel, 2019), and consists of constituent particles with a median diameter in the order of 100 nm. Purity 99.5%.	accounting for the presence of nanoparticles. There is an indication that samples were collected for TEM analysis, but no data are reported.	Precipitation was observed at 2 µg/ml at the conclusion of the treatment period. No cytotoxicity was observed. CBPI levels for all concentrations at 4h -S9 exposure, are higher than CBPI for concurrent negative control.	Cellular uptake is not demonstrated. CytoB added at the beginning for the 24h -S9 protocol		Documentation provided to EFSA No. 15

CBPI: cytokinesis-block proliferation index, CytoB: Cytochalasin-B; NSC: Nanoscale considerations.