Memorandum

Date: August 16, 2023

From: OFAS/Division of Food Ingredients, HFS-255
Toxicology Review Group 1

To: S. West-Barnette, Ph. D.
OFAS/Division of Food Ingredients
Regulatory Review Group 1

Subject: CAP 0C0317: Final memorandum on the safety evaluation of jagua (genipin-glycine) blue

I. Introduction

Jagua (genipin–glycine) blue is a water-soluble, dark blue colorant. It is obtained by reacting genipin, present in the juice of the unripe *Genipa americana* L. (Rubiaceae) fruit, with stoichiometric equivalents of glycine. This reaction forms the genipin-glycine polymer (CAS No. 1314879-21-4) that is the principal coloring component (hereafter referred to as "the polymer") (see Dr. N. Belai, Chemistry memorandum dated August 16, 2023[1] for the identity of the polymer, page 3, table 1).

The purpose of this memorandum is to review the toxicological studies in rats and dogs that were conducted with aqueous jagua (genipin–glycine) blue containing a specified percentage of the polymer (hereafter referred to as the "test material") (see Dr. N. Belai, Chemistry memorandum dated August 16, 2023[1] for the composition of the test material, page 6, table 2). To support the safety of oral consumption of jagua (genipin-glycine) blue, the Petitioner (Ecoflora S.A.S., c/o Exponent Inc.) submitted a total of 14 toxicological studies (Color Master File:000036), including in vitro mutagenicity and genotoxicity, acute, sub-chronic toxicity studies in rats and dogs, 12 months repeated dose toxicity study including in-utero exposure in rats, and allergenicity studies.[2] All these studies were conducted in accordance with the Organisation for Economic Cooperation and Development (OECD) guidelines of Good Laboratory Practice (GLP) and GLP Compliance Monitoring (as revised in 1997), except for the 28-day rat and Caco-2 permeability testing studies.

II. Chemistry

Identity

Chemical name: Genipin and glycine

CAS Registry Number: CAS 6902-77-8 (genipin) and CAS 56-40-6 (glycine)

[1] Dr. N. Belai, Chem memo
[2] The polymer content of the test material used for toxicological studies varied slightly from batch to batch; therefore, the toxicological evaluation of studies and the resulting safety conclusions were based on the polymer content of the batch used.

Alternate names: Genipin-glycine

Common name: ***Genipa americana***

Appearance: Deep blue to black water soluble

III. Dietary exposure from proposed use of jagua (genipin-glycine) blue

OFAS Chemistry estimated the dietary exposure to jagua (genipin-glycine) blue powder from the proposed uses (page 6, table 2). Ecoflora submitted exposure assessments for jagua (genipin-glycine) blue powder and OFAS Chemistry independently verified these values. OFAS Chemistry reported that the children aged 6-12 years as well as adolescents aged 13–18 years had the highest exposure (92 mg/p/d of jagua (genipin-glycine) blue powder at the 90th percentile; equivalent to 37 mg/p/d of the polymer) and adults 19 + years had the lowest exposure (74 mg/p/d of jagua (genipin-glycine) blue powder at the 90th percentile; equivalent to 30 mg/p/d of the polymer compared to the rest of the age groups, based on the exposure from the proposed uses (see Dr. R. Kolanos memo dated--2021[3]).

IV. Exposure to heavy metals and other impurities from the petitioned uses of jagua (genipin-glycine) blue

The petitioner performed the analysis for the heavy metals (arsenic, cadmium, lead, and mercury) and other impurities and provided in the certificate of analysis for different batches of jagua (genipin-glycine) blue. The Office of Cosmetics and Colors, Color Technology Branch (OCAC CTB) reported that the petitioner had provided all relevant information concerning the color additive's name, identity, and composition. OCAC Chemistry reviewed the data and indicated that the petitioner adequately identifies all possible impurities and the composition of jagua (genipin-glycine) blue for use in foods (see Dr. N. Belai, Chem memo dated August 16, 2023[1]). Furthermore, OCAC Chemistry set the specifications for lead, arsenic, mercury, and cadmium, not more than 1 mg/kg (1 ppm), genipin not more than 20 mg/kg (20 ppm), aflatoxins (B1, B2, G1, G2), not more than 0.01 mg/kg (0.01 ppm) and mycotoxins (fumonisins B1 and B2), not more than 0.5 mg/kg (0.5 ppm) in both powder and liquid forms **(see** Dr. N. Belai, Chem memo dated August 16, 2023[1]). The OCAC Chemistry reviewer concluded that the concentration of metals and other impurities would be within the specification limits. Therefore, OFAS Toxicology concludes that the presence of these metals and other impurities in jagua (genipin-glycine) blue would not pose any safety concern for the public health.

V. Toxicological evaluation of the polymer

Genotoxicity studies

The genotoxicity of the polymer has been examined in bacterial reverse mutation (OECD Test No. 471), mouse lymphoma (OECD Test No. 476), and micronucleus induction (OECD Test No. 474) assays. All these studies were certified for compliance with GLP and quality assurance (QA) and conducted in 2013. Test material CAS No.: 1314879-21-4 and Batch No.: 5313002; polymer content: 33.62%.

[3] Dr. R. Kolanos, Chem memo final draft

Bacterial reverse mutation assay

The test material containing 33.62% of the polymer was dissolved in Aqua destillata (distilled water) and diluted prior to treatment. The solvent was compatible with the survival of the bacteria and the S9 activity. Two separate experiments (plate incorporation and pre-incubation) were performed to investigate the potential mutagenic activity of the polymer in *Salmonella* strains (TA 98, TA 100, TA 102, TA 1535, and TA 1537) with 10.62, 33.62, 106.24, 336.20, 840.50 and 1681 µg/plate of the polymer without S9 or with S9 mix (containing metabolic activation enzymes and cofactors from phenobarbital and β-naphthoflavone induced male Wistar rat liver). Negative controls were Aqua destillata (BSL BIOSERVICE Lot No. 130510) and the positive controls were sodium azide, 4-nitro-o-phenylene-diamine, methyl methanesulfonate, and 2-aminoanthracene. For the test, each concentration was replicated three times. The number of colonies per plate was counted. The material was regarded mutagenic if a clear and dose-related increase in the number of revertants occurred and/or a biologically relevant positive response for at least one of the dose groups occurs in at least one tester strain with or without metabolic activation.

Study results and conclusions

The study authors observed cytotoxic effect in strain TA 1537 at ≥ 840.50 µg/plate of the polymer without metabolic activation in plate incorporation experiment, in strains TA 98 and TA 1535 at ≥ 840.50 µg/plate of the polymer without metabolic activation, in TA 100 at ≥106.24 µg/plate of the polymer without metabolic activation, in strain TA 1537 at ≥ 33.62 µg/plate of the polymer without metabolic activation, and at ≥ 840.50 µg/plate of the polymer with metabolic activation in the preincubation experiment.

The study authors did not observe any biologically relevant increases in revertant colony numbers of any of the five tester strains following treatment with the polymer at any concentration level with or without metabolic activation in plate incorporation and pre-incubation experiments (Table 2). Additionally, positive responses were obtained for appropriate positive controls (sodium azide, 4-nitro-o-phenylene-diamine, methyl methanesulfonate without S9 activation and 2-aminoanthracene with S9 activation).

The study authors stated that the polymer did not cause any mutagenic activity in tester strains used under the experimental conditions. Therefore, the study authors concluded that the polymer is non-mutagenic in the bacterial reverse mutation assay.

Reviewer's conclusion

This Reviewer agrees with the study authors' conclusion.

Mouse Lymphoma Assay

The polymer was investigated for its potential to induce mutations in the thymidine kinase gene using the cell line L5178Y. The test material containing 33.62% of the polymer was dissolved in RPMI (Roswell Park Memorial Institute medium) with HS (horse serum). The solvent was compatible with the survival of the cells and the S9 activity. Negative controls (treatment medium) and the positive controls (methyl methanesulfonate without S9 metabolic activation and Benzo[a]pyrene with S9 metabolic activation) were included in each experiment.

Two separate experiments were conducted to test the potential mutagenic activity in mouse lymphoma L5178Y cells.

Experiment-I: 168.1, 336.2, 672.4, 840.5, 1008.6, 1176.7, 1344.8 and 1428.85 µg/mL of the polymer without metabolic activation (4 h treatment), and 84.05, 168.1, 336.2, 672.4, 1008.6, 1344.8, 1512.9 and 1681 µg/mL of the polymer, with metabolic activation (4 h treatment).

Experiment II: 33.62, 84.05, 168.1, 336.2, 672.4, 840.5, 1008.6 and 1092.65 µg/mL of the polymer without metabolic activation (24 h treatment), and 201.72, 403.44, 1075.84, 1210.32, 1311.18, 1412.04, 1512.9 and 1681 µg/mL of the polymer with metabolic activation (4 h treatment).

The mutagenic potential of the polymer was determined based on two criteria: if the induced mutant frequency meets or exceeds the Global Evaluation factor (GEF) of 126 mutants per 10^6 cells, and if a dose-dependent increase in mutant frequency is detected. A test material is considered negative if the induced mutant frequency is below the GEF and the trend test is negative.

Study results and conclusions

The study authors observed growth inhibition in experiment I and II without and with metabolic activation at the highest concentration of the polymer. The study authors noted that there were no biologically relevant increases of mutants after treatment with different concentrations of the polymer with or without metabolic activation (Table 2). Additionally, the study authors found no evidence of a potential clastogenic effect on the experiments performed with and without metabolic activation related to the exposure of the different concentrations of the polymer.

Reviewer's conclusion

This Reviewer agrees with the study authors' conclusion.

Mouse micronucleus assay

The polymer's potential to produce genotoxicity was examined in vivo based on its ability to induce micronucleus formation in bone marrow cells of male and female NMRI mice (5 of each sex/dose group). The test material was intraperitoneally injected in a volume of 10 ml/kg bw in 0.9% NaCl solution to groups of 5 males and 5 females as single doses of 134.48, 336.2 and 672.4 mg/kg bw of the polymer. Mice in the negative control (0.9% NaCl) and the positive control (Cyclophosphamide) groups (5 of each sex) were intraperitoneally injected 0.9% NaCl solution in 10 ml/kg bw, and Cyclophosphamide (dissolved in 0.9% NaCl), respectively. To determine the maximum tolerated dose, 3 male and 3 female NMRI mice were treated at a dose of 672.4 mg/kg bw of the polymer in a volume of 10 ml/kg bw. Sampling of the peripheral blood was carried out on animals 44 and 68 h after the single application. For all dose groups, including positive and negative controls, at least 10,000 polychromatic erythrocytes per animal were scored for the incidence of micro nucleated immature erythrocytes.

Study Results and Conclusions

The study authors reported that the polymer did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse under the experimental condition (Table 2).

Reviewer's Conclusion
This Reviewer agrees with the conclusion of the study authors.

Table 2. Genotoxicity of the polymer in vitro and in vivo

Endpoint	Test system	Route of administration	Concentration/dose	Result	Reference
In vitro					
Reverse mutation	Salmonella typhimurium TA98, TA100, TA102, TA1535 and TA1537	-	10.62– 1681 µg/plate of the polymer test material± S9	Negative	Kraft (2013)[4]
Gene mutation	Mouse lymphoma L5178Y TK+/− cells	-	First experiment: 168.1– 1428.85 µg/mL of the polymer −S9: 84.05– 1681 µg/mL of the polymer +S9 Second experiment: 33.62–1092.65 µg/mL of the polymer −S9 201.72– 1681 µg/mL of the polymer +S9	Negative	Trenz (2013)[5]
In vivo					
Micronucleus induction	Mouse; male and female	Single Intraperitoneal	134.48, 336.2 and 672.4 mg/kg bw of the polymer	Negative	Wessels[6] (2013)

TK: thymidine kinase; S9: 9000 × g supernatant fraction from liver homogenate

Overall, OFAS Toxicology concludes that the polymer is not mutagenic or genotoxic under the experimental procedures and conditions applied.

[4] Kraft, M. 2013. Reverse mutation assay using bacteria (Salmonella typhimurium) with jagua extract. Report no. 132143. Unpublished report by BSL Bioservice, Planegg, Germany. Submitted to OFA/FDA by Ecoflora SAS, Colombia.
[5] Trenz, K. 2013. In vitro mammalian cell gene mutation assay (thymidine kinase locus/TK+/1) in mouse lymphoma L5178Y cells with jagua extract. Report no. 132041. Unpublished study. BSL Bioservice, Planegg, Germany. Submitted to OFA/FDA by Ecoflora SAS, Colombia.
[6] Wessels A (2013). Mammalian micronucleus test of murine peripheral blood cells with jagua extract. Report no. 132278. Unpublished report by BSL Bioservice, Planegg, Germany, submitted to OFA/FDA by Ecoflora SAS, Colombia.

Acute toxicity

This study was conducted according to the GLP guidelines of the OECD Test No. 420 (BSL BIOSERVICE Scientific Laboratories GmbH, Behringstraße 6/8, 82152 Planegg, Germany. BSL Munich Study No.: 136354; Test article Batch No.: 5313014; 33.05% polymer content; Sponsor:

EcoFlora S.A.S., Bodega, Columbia). A GLP compliance statement was included and signed by the study director on February 11, 2014. Study start date: December 2, 2013; Study completion date: December 26, 2013. A quality assurance (QA) unit statement was provided and signed by the QA unit (February 11, 2014). Final Report date: February 14, 2014. Study Director: Dr. Philip Allingham.

A group of female Wistar rats (n = 5; 9–10 weeks) were treated with the test material containing 33.05% jagua (genipin-glycine) blue by oral administration at a dosage of 661 mg/kg bw of the polymer and observed for 14 days. All animals used in the study were allowed to acclimatize for at least 5 days. All animals were examined for clinical signs several times on the day of dosing and once daily until the end of the observation period. Their body weights were recorded on day 1 (prior to the administration) and on days 8 and 15. All animals were necropsied and examined macroscopically. All five animals survived until the end of the study, and no signs of toxicity were observed. No treatment-related macroscopic findings were noted at necropsy. Under the conditions of this study, female rats administered a single oral dose of 661 mg/kg bw of the polymer was associated with no signs of toxicity or mortality over the period of the study. Based on the results, OFAS Toxicology concludes that the oral median lethal dose (LD_{50}) is greater than 661 mg/kg bw of the polymer for the female rats.

28-Day Dose Range-Finding Oral Toxicity Study in Rats

This study was performed at BSL BIOSERVICE, Scientific Laboratories GmbH, Behringstrabße 6/8, 82152 Planegg, Germany. BSL BIOSERVICE Study No.: 136364, Sponsor: Ecoflora S.A.S, Calle 80 Sur No. 47D-65 Urbanizacion Industrial La Holandas, Boclega 103 Sabaneta-Antioquia, Colombia. Study start date: December 9, 2013; Study completion date: December 26, 2013. Final Report date: February 11, 2014. Study Director: Dr. Philip Allingham.

This study was apparently not performed in accordance with the US FDA GLP guidelines, as evidenced by the absence of a signed and dated GLP statement. The purpose of the 28-day toxicity study was to select the doses for a 90-day study and to examine the systemic toxicity potential of the polymer Batch no. 5313014, 33.05%. The test material containing 33.05% polymer was administered daily by oral gavage for 28 consecutive days at doses of 0 (control), 3.31, 16.53, 33.05, 165.25 and 330.5 mg/kg bw/day of the polymer (n=3/sex/group; 9–10 weeks at study commencement). Rats in the control groups were handled in an identical manner to animals in the treated groups and received only aqua ad injectionem (vehicle used in this study). Observations included mortality, clinical signs, body weight changes, feed consumption, hematological and clinical chemistry parameters, organ weights and gross findings (macroscopic).

No treatment-related effects on mortality, feed intakes, body weight/body weight gains, and organ weights were observed at test doses up to 330.5 mg/kg bw/day of the polymer, the highest dose tested. No treatment-related changes were noted in the hematological or clinical chemistry parameters evaluated. The only treatment-related findings were incidences of dark discoloration of the kidneys and testes in the high dose polymer-treated rats. However, this discoloration effect was not associated with any observed histopathological consequence and was not considered to be biologically significant.

Based on the findings, the study authors selected a dose level of 330.5 mg/kg bw/day of the polymer as the highest dose level to be tested in the proposed 90-day rat study.

OFAS Toxicology considers this 28-day study only as a dose-range finding study. Because of the limited number of animals tested per group in this study (for example, this study tested significantly fewer animals per group compared to the Redbook guidelines, i.e., 3 vs. 10 animals per study group), and other study limitations included insufficient testing of hematological and clinical chemistry parameters, lack of urinalysis, and no assessment of immunotoxicity, OFAS Toxicology does not find this study to be appropriate to establish a no-observed-effect-level (NOEL) or no-observed-adverse-effect-level (NOAEL) (Dr. T. Tyler memo dated February 6, 2017[7]).

28-Day Dose Range-Finding Oral Toxicity Study in dogs

This study was performed at performed at Accelera S.r.l., V.le, Pasteur, 10, 20014 Nerviano (MI) – Italy. Accelera S.r.L. Study No.: 2015-0172, Sponsor: AnaPath GmbH, on behalf of Ecoflora, Buchsweg 56, 4525 Oberbuchsiten, Switzerland. Study start date: August 17, 2015 (males and females); Study completion date: September 29, 2015 (males) and October 02, 2015 (females). Final Report date: September 2, 2016. Study Director: Ferdinando Mancari, Ph.D.

This study was conducted according to the GLP guidelines of the OECD (Compliance Monitoring No. 13 Consensus Document) and the Italian Legislative Decree. A GLP compliance statement was included and signed by the study director on September 2, 2016. A signed QA unit statement (9/2/2016) was provided in the study report. Male and female beagle dogs (3/sex/group; approximately one year of age) were orally administered the 33.79% polymer-containing test material (Batch no: 5314034) by gavage at doses of 84.48, 168.95 and 337.9 mg/kg bw/day of the polymer; 0 (control) dogs received only sterile water (without the polymer) for 28 consecutive days.

The dogs were monitored twice daily for evidence of toxicity and each dog was weighed once weekly prior to the treatment and once weekly following the commencement of dosing. Feed consumption was monitored daily, but water consumption was not measured. Ophthalmoscopic and electrocardiographic evaluations were performed on all animals prior to the treatment and on day 25. Blood samples were collected prior to the initiation of dosing and on day 25 for hematology and clinical examinations. Urine samples were also collected prior to the initiation of dosing and on day 25 for analysis of urine parameters (e.g., total volume, pH, white blood cells, nitrites, proteins, glucose, ketone bodies etc). All surviving animals were euthanized at the end of the study and detailed gross necropsy was performed that included organ weight determinations and macroscopic examinations of the dogs. Necropsies were performed on all dogs at the end of the study and all the organs were collected for both gross and histopathological evaluation.

A discoloration was observed in the urine (green) and feces (blue) in all polymer-treated male and female dogs. Discoloration was also noted in a few mesenteric lymph nodes. However, this discoloration effect was not associated with any observed histopathological consequences and was not considered to be biologically significant. There were no toxicologically relevant effects of treatment on body weight gains or on feed consumption. No effects of the polymer treatment were revealed on ophthalmoscopic and electrocardiographic examinations.

[7] Tyler, T. 2017. Review of a 28-day dose range-finding oral (gavage) toxicity study in Wistar rats with (BSL BIOSERVICE Study # 136027). DPR Toxicology review group, CMF 000036, Toxicology Review memo, February 6, 2017.

Based on the data generated from this 28-day dog study, the study authors selected a dose level of 337.9 mg/kg bw/day of the polymer as the highest dose level to be tested in the proposed 90-day dog study. OFAS Toxicology considers this 28-day study to be a dose-range finding study only. Because of the study inadequacies (for example, this study tested fewer animals per group compared to the Redbook guidelines, i.e., 3 vs. 4 animals per study group; 1 vs 4-6 years) and insufficient testing of hematological and clinical chemistry parameters (hematology and clinical chemistry parameters at end of the study vs first two weeks), OFAS concludes that a no-observed-adverse-effect-level (NOAEL) for the polymer cannot be established in this study (Dr. T. Tyler memo dated February 27, 2018[8]).

90-day repeated dose oral toxicity study in Wistar rats

The study was performed at BSL BIOSERVICE, Scientific Laboratories GmbH, Behringstraße 6/8, 82152 Planegg, Germany. BSL BIOSERVICE Study No.: 140667, test material Batch No.: 5313014, 33.05 % polymer content, Sponsor: Ecoflora S.A.S, Calle 80 Sur No. 47D-65 Urbanizacion Industrial La Holandas, Boclega 103 Sabaneta-Antioquia, Colombia. Study start date: April 11, 2014; Study completion date: August 21, 2014. Final Report date: November 27, 2014. Study Director: Dr. Philip Allingham. A GLP compliance statement was included and signed by the study director on November 27, 2014. A QA unit statement signed and dated November 27, 2014, was also provided in the study report.

This 90-day repeated dose oral (gavage) toxicity study of the polymer in Wistar rats included a 28-Day Recovery period. The study was conducted in accordance with the GLP guidelines of the OECD Test no. 408. The goal of the study was to evaluate and characterize the potential toxic effects that could arise from repeated oral exposure to the polymer.

Male and female rats (10/sex/group; approximately 7-8 weeks old) were orally administered the test material containing 33.05% polymer by gavage at doses of 33.05, 99.15 and 330.5 mg/kg bw/day of the polymer; control groups received only aqua ad iniectabilia for 90 consecutive days. Additional groups of 5 male and 5 female rats were assigned to the control (0) and 330.5 mg/kg bw/day of the polymer groups for 90 days of treatment, followed by a 28-day recovery period. All animals were observed for mortality, clinical signs, body weight changes, feed consumption, functional observational battery, motor activity, and ophthalmoscopy. Standard hematology, clinical chemistry, and urine analysis were measured during the study. All animals surviving until the end of treatment were killed and subjected to macroscopic pathological evaluations. The weights of several organs were recorded, and tissues preserved for histopathological examination. Any abnormal tissues were also retained for histopathological examination. Preserved tissues were evaluated for all animals in both the main study groups and in those in the recovery groups.

There were no treatment-related effects on mortality, feed intakes, body weight/body weight gains, ophthalmology, organ weights, and urinalysis. Additionally, no treatment-related changes were observed in the hematological or clinical chemistry parameters evaluated in this study. The discolored feces observed in all male and female rats and the dark discoloration of the kidneys observed in mid dose males and high dose male and female rats after treatment with the polymer was considered to be compound-related, i.e., because of the coloring agent. Therefore, this effect was not considered to be biologically significant or to have toxicological consequences. No other treatment-related clinical symptoms were noted. The study authors concluded that the NOAEL was 330.5 mg/kg bw/day of the

[8] Tyler, T. 2018. Review of a 28-day oral (Gavage) toxicity study in the dog (Accelera S.r.l. Study # 2015-0172). DPR Toxicology review group, Toxicology review memo, CMF 000036 Toxicology Review memo, February 27, 2018.

polymer (the highest dose tested) for both males and females. OFAS Toxicology concurs with the study authors that the NOAEL for the Wistar rat under the conditions of this study was 330.5 mg/kg bw/day of the polymer (Dr. T. Tyler memo dated February 7, 2017[9])

90-day repeated dose oral toxicity study in Beagle dogs

This study was performed at Accelera S.r.l., V.le, Pasteur, 10, 20014 Nerviano (MI) – Italy. Accelera S.r.L. Study No.: 2015-0175, Sponsor: AnaPath GmbH, on behalf of Ecoflora, Buchsweg 56, 4525 Oberbuchsiten, Switzerland. Study start date: October 06, 2015 (males and females); Study completion date: January 13, 2016 (males) and January 15, 2016 (females). Final Report date: September 2, 2016. Study Director: Ferdinando Mancari, Ph.D. A signed QA unit statement (September 2, 2016) was provided with the study report.

This study was conducted to evaluate and characterize the potential toxic effects which could arise from repeated exposure to the polymer via oral administration (gavage). The study was performed in accordance with the GLP guidelines of the OECD Test No. 409, and the Italian Legislative Decree. A GLP Compliance statement was included in the study report and signed by the study director on September 2, 2016.

Male and female Beagle dogs (3/sex/group; 1 year old at study initiation) were orally administered the test material (Batch no. 5314034, 33.79 % polymer content) by gavage at doses of 0, 84.48, 168.95 and 337.9 mg/kg bw/day of the polymer in water. Control groups received only sterile water for 90 consecutive days.

All dogs were observed daily for mortality and clinical signs and weekly for body weight changes and feed consumption. Ophthalmoscopic and electrocardiography examinations were performed prior to treatment and towards the end of treatment period (Day 85). Hematology and clinical chemistry parameters were examined prior to the treatment and at the end of the treatment period (day 88 and 90, males and females, respectively). Furthermore, urine was collected from all dogs prior to the treatment and at the end of the treatment period (day 88 and 90, males and females, respectively) and examined for total volume, pH, white blood cells, nitrites, proteins, glucose, ketone bodies, urobilinogen, bilirubin, hemoglobin/red blood cells, and specific gravity, macroscopic appearance, and microscopic examination of the sediment. Additionally, blood samples for toxicokinetic analyses were collected from all dogs prior to treatment and 0.5, 2, 4, 8 and 24 hours after dosing on day 1 and day 91. Samples were analyzed for the presence of the polymer in the plasma with a lower limit of detection of 1 mg/ml. All animals surviving until the end of treatment were killed and subjected to macroscopic pathological evaluations. The weights of several organs (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and thymus) were recorded at the end of the treatment period and a standard list of tissues fixed for histopathological examination. The tissues subjected to histopathological examination were from all animals in the study.

There were treatment-related findings, such as dark discoloration of the feces (blue) and urine (green) in all male and female dogs in the treatment groups. The study authors indicated that the change in color of urine and serum bilirubin was due to the coloring component of the test substance that was absorbed. However, this effect was not associated with any observed histopathological consequence and therefore was not considered to be biologically significant. There were other incidences of findings noted in some

[9] Tyler, T. 2017. Review of a 90-day repeated dose oral (Gavage)toxicity study in Wistar rats including a 28-day recovery period (BSL BIOSERVICE Study # 140667). DPR Toxicology review group, CMF 000036 Toxicology review memo, February 7, 2017.

of the parameters, i.e., decrease in red blood cells, hemoglobin, and hematocrit; increase in total cholesterol and serum bilirubin, in dogs of the treatment groups, but a thorough evaluation of those effects could not be performed because of the inadequacies and limitations in this study's experimental design. For example, this study tested fewer animals per group compared to the Redbook guidelines i.e., 3 vs. 4-6 animals per study group; hematology and blood chemistry examination at study termination instead of prior to initiation of treatment, during the first two weeks of treatment, monthly or midway through treatment, and at study termination. Furthermore, the study also deviated from the OECD Test No. 409, which recommends using a minimum of four animals per sex per dose. Clinical chemistry and urine analysis were performed at the beginning and end of the study rather than monthly or at the midway point, as recommended; the dogs were around 1 year old at study initiation, when the guideline recommends starting by 9 months of age. Additionally, no comparable historical control clinical chemistry data were submitted by the petitioner for evaluation of clinical chemistry findings in this 90-day dog study. OFAS concludes that a no-observed-adverse-effect-level (NOAEL) for the polymer cannot be established in this study due to the above-stated inadequacies and limitations (Dr. T. Tyler memo dated March 19, 2018[10]).

Caco-2 permeability testing of jagua (genipin-glycine) blue

During a September 6, 2012, meeting with the FDA, Ecoflora gave a slide presentation regarding the chemical characteristics of the test material. In the presentation, Ecoflora stated that the polymer has a molecular weight of 6,000 Daltons and is expected to be stable and not absorbed from the gastrointestinal tract. No data were provided to support this statement. Moreover, EcoFlora also stated in a November 22, 2014, safety narrative submission that no structure in the test material is expected to be potentially toxic. Furthermore, the most important substance could be absorbed only in small quantities, after fracturing. However, no data were provided in support of these statements as well.

> *Concern with low molecular weight fraction of the total polymer in the test material*: The absorption, distribution, metabolism, and excretion (ADME) data are lacking. No study was conducted to address the biotransformation of the polymer. Additionally, there were no long-term studies on the polymer. As a result, OFAS Toxicology recommended that EcoFlora conducts a Caco-2 permeability assay to investigate potential intestinal permeability of the polymer (Dr. T. Tyler memo dated February 22, 2016[11]). This assay may serve as a screening test to determine potential absorptive characteristics of the polymer. OFAS noted that if EcoFlora could provide data/evidence to support their above statement that the polymer is not absorbed from the gastrointestinal tract, then there may not be a need for chronic toxicity testing of the polymer. Based on the recommendation EcoFlora conducted the recommended Caco-2 permeability assay and submitted to FDA on October 28, 2015 for toxicology review and comments (see Dr. B. Roth memo dated January 27, 2016[12]). The review concluded … that the fraction of an oral dose absorbed in humans or experimental animals would be in the range of 3 - 30% based on representative data sets (Artursson and Karlsson,

[10] Tyler, T. 2018. Review of a 90-day oral (gavage) toxicity study in the dog (Accelera S.r.l. Study # 2015-0175). DPR Toxicology review group, CMF 000036, Interim toxicology review memo, March 19, 2018.
[11] Tyler, T. 2018. Review of the Caco-2 permeability testing ((Study# CYP1291-Rl). DPR Toxicology review team, CMF 000036 , Toxicology cover memorandum for the review of the Caco-2 permeability testing, February 22, 2016.
[12] Roth, W. 2016. Review of Caco-2 permeability testing (Study# CYP1291-Rl). FCN Toxicology review group, CMF 000036 Toxicology review memo, January 27, 2016.

1991[13]; Grass, 1997[14]; Stenberg et. al., 2001[15]) and 4 - 10% (average) based on standard modeling extrapolations for whole animal outcomes (see Dr. B. Roth memo dated January 27, 2016[12]).

Based on the review of data from this in vitro Caco-2 permeability assay, OFAS Toxicology concluded that there is a potential for in vivo intestinal absorption of the polymer. Additionally, the data from these studies do not corroborate the previous statements made by Ecoflora regarding the polymer not being absorbed from the gastrointestinal tract. Therefore, OFAS Toxicology recommended that chronic oral toxicity testing of the polymer in a rodent species should be conducted, specifically, a study with a minimum duration of one year.

12 Months repeated dose toxicity study including ln-utero exposure in Wistar rats

This study was conducted in response to the OFAS/FDA recommendation for the evaluation of potential toxic effects of chronic oral exposure to the polymer in a rodent species. EcoFlora performed the 12 months repeated dose toxicity study including in-utero exposure in Wistar rats according to the GLP guidelines of the OECD and US FDA Redbook 2000: IV.C.8 guideline (BSL BIOSERVICE Scientific Laboratories GmbH, Behringstr. 6/8, D-82152 Planegg/Munich, Germany; Eurofins Munich / BSL Munich Study No.: 171414; Sponsor: EcoFlora S.A.S., Bodega, Columbia). A GLP compliance statement was included and signed by the study director on September 26, 2019. Two different batches of the test material were used for the study– Batch No.: 5317002 (Diet batch 1-10) with 36.4% polymer content, and Batch No.: 5317011 (Diet batch 11-12) with 34.22% polymer content. Study Director: Dr. Pradeep Takawale and a signed QA unit statement (September 25, 2019) was provided.

The study objectives were: 1) to assess the effects of oral (dietary) administration of the polymer on the reproductive performance of the male and female rats, including gonadal function, mating behavior, conception, gestation, parturition, lactation, and weaning, and the growth and development of the offspring; 2) to investigate the effects of the polymer on neonatal morbidity, mortality, and on prenatal and postnatal developmental toxicity; 3) to provide information on the possible health hazards that may arise from repeated exposure to the polymer through diet over 12 months including in utero exposure; and 4) to estimate the no-observed-adverse-effect-level (NOAEL) value for maternal and offspring that can be used for establishing safety criteria for human exposure.

Combined reproductive and chronic study by dietary administration of the test material to Wistar rats for 12 months with an in utero exposure phase (Eurofins Munich / BSL Munich Study No.: 171414): This study included 2 phases: 1) an in utero reproduction phase, and 2) 52 weeks chronic toxicity phase. In each of the study phases, the test material was administered in the diet at levels of 0, 2,500, 12,500, or 50,000 ppm. The control groups received diet without the test material. Dietary concentration of the test material was transformed into the polymer dose based on the food consumption, as discussed below.

In the in utero reproduction phase of this study Wistar rats were exposed to the test material prior to mating during mating, throughout gestation, lactation and up to weaning with the start of the main

[13] Artursson and Karlsson.1991. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (CACO-2) cells. Biochem. Biophys. Res. Comm. 175(3):880-885.
[14] Grass, G.M. 1997. Simulation models to predict oral drug absorption from in vitro data. Adv. Drug Delivery Reviews 23: 199-219.
[15] Stenberg et. al. , 2001. Experimental and computational screening models for the prediction of intestinal drug absorption. J. Med. Chem. 44:1927-1937

chronic study at 4 weeks of age. Offspring produced during the F_0 mating were used to produce the F_1 generation that was subsequently used in the 52 week chronic toxicity study in rat.

Body weight, feed consumption and survival were evaluated in both the F_0 and F_1 rats. Mating and parturition abilities were also evaluated in the F_0 rats. The numbers of offspring, sex ratios, and litter weights were recorded for the F_1 offspring.

Hematological parameters, clinical chemistry and urinalysis were performed in selected male and female animals per group at PND 21 from non-selected pups at week 5 (2 weeks after weaning from selected pups but not used for F_1 generation), at 3, 6, 12 month of the study and at terminal sacrifice from animals selected for post weaning F1 phase.

By the end of the treatment period, all F_0 and F_1 generations animals were sacrificed and subjected to necropsy. The wet weight of a subset of organs/tissues was preserved. Animals that died or were sacrificed in a moribund condition were also macroscopically examined and histopathologically confirmed. Additionally, a full histopathological evaluation of the collected tissues from the F_0 and F_1 generations was performed on high dose and control animals. All gross lesions macroscopically identified were examined microscopically in all F_0 and F_1 generation animals selected for post weaning phase.

The study authors reported that there was no treatment-related effect on body weight gain, food consumption, mortalities, or clinical signs in F_0 parental rats. Additionally, there were no treatment-related, adverse effects on pre-coital interval**,** mating, gestation length, corpora lutea, implantation sites, litter sizes, sex ratios, or on the growth and survival of pups. Furthermore, no test material associated gross external abnormalities of toxicological significance were observed on PND 0-2 in the F_1 pups.

There was no treatment-related effect on body weight gain, food consumption, mortalities, or clinical signs in F_1 animals. However, the mean body weight gain was marginally but-statistically significantly lower on various days in both mid dose and high dose F_1 males and females during the study period. Nevertheless, the body weight gain in all treatment groups was comparable with the controls over the study period (day 1-344). Moreover, the hematology, blood coagulation, clinical chemistry, and urine parameters of F_1 male and female rats did not show any test material related and toxicologically relevant effects.

There were no statistically significant differences in absolute or body weight related organ weights of any dose groups of F_0 parental rats as well as F_1 phase rats compared to the controls.

The test article-associated blue discoloration was seen only in kidneys from the high dose F_0 males and females, and this blue discoloration was not seen in the kidneys of any dosed F_1 males and females. Additionally, the blue discoloration was also observed in gastrointestinal segments in one male at mid dose and four males at high dose groups. However, there was no histopathological correlation associated to these macroscopic findings in F_0 and F_1 animals. As a result, these macroscopic findings are not considered to be adverse. The study authors concluded that the NOAEL under the conditions of the chronic study was 50,000 ppm of the test material in the diet (for both F_0 and F_1 male and female rats. OFAS Toxicology concurs with the study authors conclusion of the NOAEL under the conditions of this study.

Based on the dietary level of 50,000 ppm of the test material and food consumption by rats, the NOAEL of the test material was determined to be 3094.7 mg/kg bw/day in males and 5633.5 mg/kg bw/day in females in the F_0 parental generation, and 3384.7 mg/kg bw/day in males and 3750.3 mg/kg bw/day in females during post-weaning F_1 generation. Thus, the lowest of these NOAELs is 3094.7 mg/kg bw/day of the test material. Taking into account that the test material had 36.4% polymer content, the lowest adjusted NOAEL of F_0 male rats is 1127 mg/kg bw/day of the polymer (see Dr. A. Khan memo dated June 15, 2022[16]).

VI. Allergenicity studies

Observations in humans

The repeated insult patch test was conducted at Essex Testing Clinic, Inc., 799 Bloomfield Avenue Verona , NJ 07044. Sponsor: Institut Dermatologique d'Aquitaine Technopole Montesquieu 5, rue Jacques Monod CS 60077, 33652 MARTILLAC CEDEX, France; Study start date: May 1 , 2013; Final evaluation: June 7, 2013; This study (ETC Panel No.: 13213; ETC Entry No .: 23901) was conducted in accordance with the intent and purpose of GLP guidelines described in 21 CFR Part 50 (Protection of Human Subjects - Informed Consent) and the standard operating procedures of Essex Testing Clinic, Inc., Study Director: Annemarie E. Hollenback, BA

The objective of this study was to determine the irritation and/or sensitization potential of the polymer (prepared by dissolving the test material in 1.5% distilled water) after repeated application under occlusive patch test conditions to the skin of 55 human subjects. Subjects (11 males and 44 females) aged from 18 to 79 years with self-perceived sensitive skin were enrolled in the repeated insult (occlusive) patch test, which consisted of an induction phase (~0.05 ml/cm^2 of test material placed onto an occlusive patch) and challenge phase. There was no skin reactivity observed at any time during the study.

The study author indicated that under the conditions of a repeated insult (occlusive) patch test procedure did not produce skin irritation or allergic contact dermatitis in human subjects after 72-96 hours. OFAS Toxicology indicated that the study author used an unequal number of sexes in this study. Additionally, they did not provide the actual concentration of geniposide and genipin in the test solution as well as information of the ethnic groups of the human subjects.

VII. Teratology/developmental toxicity

No teratology/developmental toxicity studies were identified in the scientific literature for genipin or related compounds.

VIII. Metabolism and pharmacokinetics

No metabolism and pharmacokinetics studies were identified in the scientific literature for the blue.

IX. Human studies

No human studies were identified in the scientific literature for genipin or related compounds.

[16] Khan, A. 2022. Toxicological evaluation of 12 months repeated dose toxicity study including in-utero exposure in rats. Toxicology review memo dated June 15, 2022.

X. Evaluation of jagua (genipin–glycine) blue by Joint FAO/WHO Expert Committee on Food Additives

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the genotoxicity, sub-chronic toxicity studies in rats and dogs, and 12 months repeated dose toxicity study in rats including in-utero exposure for the jagua (genipin-glycine) blue (JECFA, 2019[17]; JECFA, 2020[18]).

JECFA and FDA used the same approach of safety evaluation and arrived at the same safety conclusion in terms of NOAEL. Both JECFA and FDA used the 12 Months repeated dose toxicity study including in-utero exposure in Wistar rats and identified a NOAEL of 1127 mg/kg bw/day of jagua (genipin–glycine) blue based on no effects observed in F_0 parental male rats.

XI. Publication

Latest literature search indicated that the polymer caused allergic contact dermatitis and significantly inhibited the human trophoblast derived BeWo cells in BeWo cell growth.

Bircher et. al. 2017[19]. Allergic contact dermatitis caused by a new temporary blue–black tattoo dye – sensitization to genipin from jagua (*Genipa americana* L.) fruit extract. Contact Dermatitis. 77:374–378.

The study authors reported the presence of the allergenic substance genipin in a self-administered (repeated application) temporary tattoo dye made from the fruit juice of jagua (*Genipa americana*). In this case, a 39-year-old female developed allergic contact dermatitis within 6 weeks after repeated application of completely natural and 100% safe' Earth jagua® tattoo (obtained via the internet) on her left hand. Patch testing with Earth jagua® dye kit {xanthan gum (10% aqueous), sorbic acid (2% pet), EDTA-sodium (1% aqueous), isopropyl alcohol (10% aqueous) and eucalyptus oil (2% pet)} were performed. Due to the poor solubility and stability of Earth jagua in water, concentrations of 0.5%, 1% and 2% genipin in dimethyl sulfoxide (DMSO)/water 1:1 (vol/vol) as vehicle were freshly prepared and renewed after 1 week. The patient had positive reactions on day 2 and day 5 to all three concentrations of genipin, whereas the vehicle DMSO/water gave negative results. Tests had the characteristic bluish hue and stayed positive for >7 days. Analysis of the dye showed the presence of geniposide and genipin. The authors indicated that stimulating allergen appears to be genipin, most likely because of its high affinity for proteins or its radical-scavenging antioxidant activity, making it a potential contact allergen. Additionally, the authors also stated that the patients did not display any allergic contact dermatitis and tolerated the p-phenylenediamine-containing hair dyes for >30 years without any allergic symptoms. The study authors reported that jagua extract may cause allergic contact dermatitis of the skin, but this does not predict oral allergenicity potential. OFAS Toxicology specifies that these data plus the lack of evidence of any adverse reactions to jagua fruit in the literature, or in adverse event databases, the relatively low allergenicity potential of jagua fruits, not to mention non-protein derivatives, and the likely low oral exposures from the proposed use as a color additive, all point to a low level of concern

[17] JECFA, 2019. Evaluation of certain food additives (eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives). WHO food additives series: 75: 131-141.
[18] JECFA, 2020. Evaluation of certain food additives (eighty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives). WHO food additives series: 80: 29-44.
[19] Bircher et. al. 2017. Allergic contact dermatitis caused by a new temporary blue–black tattoo dye – sensitization to genipin from jagua (genipa americana L.) fruit extract. Contact Dermatitis. 77:374–378.

for allergenicity from oral exposure (e-mail to Dr. A. Khan from Dr. S. Choudhuri and Dr. S. Luccioli[20]). OFAS Toxicology notes that the study authors did not provide the actual concentration of geniposide and genipin in the jagua extract as well as the stability. Therefore, based on the facts discussed above, there is reasonable certainty that the polymer does not pose significant allergenic risk when consumed orally at the level(s) specified in the petition.

Conceicao et. al . 2011[21].*Genipa americana* (Rubiaceae) fruit extract affects mitogen-activated protein kinase cell pathways in human trophoblast–derived BeWo **cells: implications for placental development. J Med Food 14 (5): 483–494.**

The study authors investigated the effect of *Genipa americana* fruit ethanolic extract on the mechanism for proliferation and differentiation of the BeWo cells, which are trophoblast-like cells, (a well-established placental choriocarcinoma cell line that can undergo differentiation). These cells were treated with different concentrations of *G. americana* plant extract (50–500 µg/mL). The endpoints measured were cell viability, detection of release of human chorionic gonadotrophins, cell fusion, and evaluation of cell-signaling pathways (production of cAMP and phosphorylation of mitogen-activated protein kinases). The authors reported that the *G. americana* fruit extract did not cause any cytotoxicity and did not interfere with BeWo cell differentiation at concentrations up to 1000 µg/mL. However, the *G. americana* fruit extract significantly inhibited BeWo cell growth under physiologic doses (≤ 100 µg/mL). The authors concluded that the steroids from *G. americana* may affect placental cell regulation. The establishment and function of the human placenta depend on the proliferation, migration, and invasion of trophoblasts into the maternal decidua and myometrium. The study authors concluded that the interference on cell-signaling pathways may lead to aberrant or adaptive placental cell turnover that could cause early termination of pregnancy, gestational abnormalities, or fetal growth defects due to the presence of steroids in the *G. americana*. OFAS Toxicology notes that the study authors did neither provide the level of steroid nor identify the steroid in the *G. americana*.

Imazawa et. al. 2000[22]. Lack of carcinogenicity of gardenia blue colour given chronically in the diet to F344 rats. Food Chem Toxicol. 38(4):313–318.

Ecoflora did not conduct any long-term carcinogenicity study. However, to address the data gap, EcoFlora submitted a non-GLP carcinogenicity study in Fischer 344 (F344) rats on genipin-based blue from *Gardenia jasminoides* (gardenia blue), which is also a genipin–amino acid/peptide and is expected to have a structure similar to jagua blue (Imazawa et. al. 2000[22]).

Imazawa et. al. (2000[22]) conducted a carcinogenicity study of gardenia blue derived from geniposide extracted from *Gardenia jasminoides* in Fischer 344 (F344) rats. Groups of 50 males and 50 females were given the material at dietary doses of 0 (control), 2.5 or 5% for 104 weeks and by the end of the experiment all surviving rats were sacrificed. The doses were selected based on results from a 13-week subchronic toxicity study. The study authors reported a slight increase in relative weights of the left lung in male rats of the 5% group. However, no significant differences between the control and treated groups were noted regarding clinical signs, mortality, and hematological parameters. A variety of tumors developed in all groups including the controls; histological examination revealed that

[20] e-mail from Choudhuri and SLuccioli to AKhan regarding allergenic potential of jagua blue color additive.
[21] Conceicao et. al . 2011. Genipa americana (Rubiaceae) fruit extract affects mitogen-activated protein kinase cell pathways in human trophoblast–derived BeWo cells: implications for placental development. J Med Food 14 (5) 2011, 483–494.
[22] Imazawa et. al. 2000. Lack of carcinogenicity of gardenia blue colour given chronically in the diet to F344 rats. Food Chem Toxicol. 38(4):313–318.

these tumors were similar to those known to occur spontaneously in F344 rats. Additionally, no statistically significant increase in the incidence of any type of neoplastic lesion was found for either sex in the treated groups. Based on the results, the study authors concluded that the gardenia blue color is not carcinogenic in F344 rats under the experimental conditions.

Geniposide constitutes the principal glucoside out of nine iridoid forms extracted with methanol. The mixtures of these glucosides are blended with powdered defatted soybean protein, and further treated with β-glycosidase and protease in buffer solution, resulting in a blue (Inouye et al., 1969[23]) with a melting-point of 118–120°C and a mean molecular weight of 15,600 ± 400 (Touyama et al., 1994[24]). OFAS Toxicology indicates that the test material was not described in terms of its composition, polymer structure, content, or impurities, which give rise to a blue color. Additionally, Gardenia blue used in this study was formed from a mixture of genipin and a protease digest of soy proteins, resulting in different amino acids connected to genipin.

XII. Summary and conclusions: Selection of pivotal studies and estimation of an acceptable daily intake (ADI) for the proposed uses of the polymer

Ecoflora summarized the toxicological evaluation of the polymer based on the JECFA (2020**Error! Bookmark not defined.**) evaluation of the 12 months toxicity study including in-utero exposure, the estimated daily intake (EDI) and acceptable daily intake (ADI) in terms of the polymer. Ecoflora requested FDA to evaluate the test material based on only the polymer (e-mail dated July 15, 2020[25])**.** Therefore, OFAS toxicology calculates the acceptable daily intake (ADI) based on the polymer content of the test material.

To summarize, OFAS toxicology evaluates several genotoxicity and toxicological studies in rats and dogs for regulatory decision. The polymer is non-genotoxic based on a battery of genotoxicity studies (a bacterial reverse mutation assay, an in vitro mouse lymphoma, and an in vivo mammalian micronucleus induction assay). OFAS toxicology considers data from the 12 months repeated dose toxicity study including in-utero exposure and 90-day toxicity studies in Wistar rats as pivotal in the determination of an acceptable daily intake (ADI) for the polymer. As discussed in this memorandum, OFAS Toxicology identifies NOAELs for the polymer in these two studies.

In determining an ADI for a new food ingredient, OFAS/CFSAN uses data from studies with the most sensitive species or most sensitive endpoint, unless there are compelling reasons to use a different approach. The study chosen to establish an ADI for jagua blue was the 12 months repeated dose toxicity study including in-utero exposure to rats over the 90-day study in rats. This study combined the in utero phase and a one-year chronic toxicity phase of sufficient length and overall complexity to produce information on chronic exposure to the polymer. Therefore, OFAS Toxicology chose to use the data from this study for the ADI calculation. Furthermore, OFAS/CFSAN Toxicology always applies the highest EDI (90th percentile) to a highly susceptible subpopulation to estimate the acceptable margin of safety.

As discussed above, using the concentration of the test material in food and food consumption by rats, the NOAEL from the 12 months repeated dose toxicity study in rats including in-utero exposure was

[23] Inouye et. al. 1969. Zweineue iridoidglucoside aus Gardenia jasminoides: Gardenosis und Geniposid. Tetrahedron Letters 28:2347-2350.
[24] Touyama et. al. 1994. Average molecular weight and gastro-intestinal absorption of a natural food color gardenia blue. Japanese J. Toxicol. and Environ Health 40: 259-265.
[25] E-mail from Nga Tran to Richard Bonnette, dated July 15, 2020.

determined to be 3094.7 mg/kg bw/day of the test material. Based on the polymer content (36.4%) of the test material, the NOAEL of the polymer is calculated to be 1127 mg/kg bw/day (3094.7 mg/kg bw/day x 0.364 = 1127 mg/kg bw/day). Applying a safety factor of 500 (10 to account for possible increased sensitivity of humans compared to test animals, 10 to account for sensitive individuals in determining safe intake for humans, and another 5 for the lack of metabolism and pharmacokinetics and long term chronic study), the AD1 for the polymer is calculated as follows: 1127 mg/kg bw/day (NOAEL)/500 = 2.3 mg/kg bw/day or 138 mg/person/day (based upon 60 kg bw/person).

OFAS Chemistry estimated the highest exposure of 37 mg/person/day of the polymer at the 90th percentile for the children aged 6-12 years as well as adolescents aged 13–18 years (see Tables 2 and 3, OFAS Chemistry, Dr. R. Kolanos memo dated August 7, 2023[3]). This estimated highest exposure values of the polymer (37 mg/person/day at the 90th percentile) is lower than the ADI value (138 mg/person/day of the polymer). Therefore, OFAS Toxicology concludes that foods containing the polymer at the proposed highest exposure level is considered to have reasonable certainty of no harm upon exposure through oral route.

Abu T. Khan -S

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