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March 1, 2018

Paulette M. Gaynor, Ph.D.
Office of Food and Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740

re: GRAS Notification for the Use of Potassium Polyaspartate (A-5D K/SD) in Wine

Dear Dr. Gaynor:

Pursuant to 21 C.F.R. §170, Enartis USA, Inc., hereby provides notice of a claim that the food ingredient (potassium polyaspartate) described in the enclosed notification document is exempt from the premarket approval requirement of the Federal Food, Drug, and Cosmetic Act because it has been determined to be generally recognized as safe (GRAS), based on scientific procedures, for use in wine.

As specified in 21 C.F.R. §170.210, we are providing a copy of the notification on the enclosed CD-ROM. If you have any questions about this submission or require additional information, please contact me at (202) 393-3903, ext. 114 or eharrison@lewisharrison.com

Sincerely,

(b) (6)

Eliot Harrison,
Agent for Enartis USA, Inc.

GENERALLY RECOGNIZED AS SAFE (GRAS) NOTICE FOR THE USE OF POTASSIUM POLYASPARTATE (A-5D K/SD) IN WINE

Prepared for:
Enartis USA, Inc.
7795 Bell Road
Windsor CA 95492

Submitted by:
Lewis & Harrison
122 C Street, N.W.,
Suite 505
Washington, D.C. 20001

Date:
March 1, 2018

Table of Contents

Part 1. Signed Statements and Certification

Pursuant to 21 CFR, Part 170, subpart E, Enartis USA, Inc., ("Enartis") is submitting this Generally Recognized as Safe ("GRAS") Notice for the use of potassium polyaspartate (A-5D K/SD) as a stabilizer against tartrate crystal precipitation in wine. As described in Parts 2 through 7 of this GRAS Notice, Enartis is claiming that potassium polyaspartate (A-5D K/SD) is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act ("FFDCA") based on its conclusion that potassium polyaspartate (A-5D K/SD) is GRAS under the proposed conditions of use.

1.1 Name and Address of Notifier

Contact Person: Jose Santos
Company Name: Enartis USA, Inc.
Address: 7795 Bell Road, Windsor, CA 95492
Telephone Number: (707) 838-6312
E-mail Address: jose.santos@enartis.com

1.2 Common Name of Notified Substance

The common name of the notified substance is potassium polyaspartate (A-5D K/SD).

1.3 Conditions of Use

Potassium polyaspartate (A-5D K/SD) is intended for use as a stabilizer against tartrate crystal precipitation (anti-scaling additive) in wine (red, rosé and white wine), at a typical use level of 100-200 mg/L and a maximum level (ML) of 300 mg/L. The use level will depend on the degree of instability in the treated wine. The treatment level of 100 mg/L is usually sufficient to obtain complete inhibition of tartrate crystal formation during storage; however, wines with high levels of tartrate instability may require a maximum treatment of 300 mg/L.

The Codex Food Category (General Standard for Food Additives or "GFSA") that is applicable to this GRAS Notice is Category 14.2 - Alcoholic beverages, including alcohol-free and low-alcohol counterparts.

Oenological potassium polyaspartate (A-5D K/SD) is prepared exclusively from L-aspartic acid. The L-aspartic acid monomer used in the process is produced by fermentation. A thermal process converts the L-aspartic acid monomer into polysuccinimide, an insoluble compound. Polysuccinimide is then treated with potassium hydroxide under controlled conditions to obtain potassium polyaspartate.

1.4 Purpose for Which Substance is Added

Potassium polyaspartate is highly effective at inhibiting tartrate crystal precipitation due to a "colloid protector" effect.

1.5 Description of the Population

Since potassium polyaspartate will be used to treat wine, without geographical or other restrictions, dietary exposure will occur throughout the adult general population.

1.6 Basis for GRAS Determination

The basis for the GRAS determination regarding the use of potassium polyaspartate (A-5D K/SD) in wine is scientific procedures.

1.7 Availability of Information

The data and information that serve as the basis for this GRAS Notice will be made available to the FDA for review and copying upon request during business hours at the offices of Lewis & Harrison, LLC (L&H), at the following address:

122 C Street, N.W.,
Suite 505,
Washington, D.C. 20001

In addition, should the FDA have any questions or require additional information regarding this notification during or after the Agency's review of the notice, L&H will supply these data and information as requested.

1.8 Freedom of Information Act, 5 U.S.C. Section 552

None of the data and information presented in Parts 2 through 7 of this notice contain any trade secret, commercial, or financial information that is privileged or confidential; therefore, all data and information presented herein are not exempt from the Freedom of Information Act, 5 U.S.C. Section 552.

1.9 Certification

I certify that, to the best of my knowledge, this GRAS Notice is complete, representative and a balanced submission that includes unfavorable information, as well as favorable information, available and pertinent to the evaluation of the safety and GRAS status of the use of potassium polyaspartate (A-5D K/SD) for use in wine.

(b) (6)

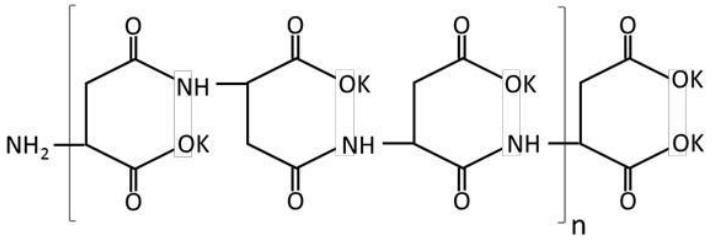
Eliot Harrison For Jose Santos

Name: Jose Santos

March 1, 2018
Date

Part 2. Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

Chemical Name:	L-aspartic acid, homopolymer, potassium salt
Other Names:	Potassium polyaspartate; A-5D K/SD; A-5D K / SD; A-5D K SD; A-5DK/SD; A-5DK; KPA
Trade Name:	Not yet assigned
Chemical Abstracts Service (CAS) Number:	64723-18-8
EINECS Number:	Not available
Number average molecular weight:	1100 g/mol
Weight average molecular weight:	5300 g/mol
Chemical Formula:	$[C_4H_4NO_3K]_n$
Chemical Structure:	

2.2 Method of Manufacture

2.2.1 Raw Materials and Processing Aids

Information regarding the raw or beginning materials, L-aspartic acid monomer and caustic potash liquid 45%, that are used in the manufacture of potassium polyaspartate (A-5D K/SD) is reported in Table 2.2.1-1.

Analysis of residual potassium hydroxide (KOH) cannot be performed directly because it is not possible to differentiate between potassium linked to potassium polyaspartate (A-5D K/SD) or KOH. Based on the proposed specification of 98% potassium polyaspartate (A-5D K/SD) in the dry material, residual KOH cannot be greater than 2%. As the pH of wine ranges between 3 and 3.8, any residual KOH would be neutralized during the production and storage of wine.

2.2.2 Manufacturing Process

Potassium polyaspartate (A-5DK SD) is produced from L-aspartic acid according to the reaction reported in Figure 2.2.2-1. The thermic process transforms aspartic acid to polysuccinimide, which is insoluble. Polysuccinimide is then treated with potassium hydroxide under controlled conditions, thereby allowing the opening of the ring and polymerization of the units. Through this process, a 40% solution of potassium polyaspartate at pH 8.3 is obtained. The last step in the preparation of potassium polyaspartate (A-5D K/SD) is the spray drying phase, which results in a light tan powder at 92–95% dry matter.

Table 2.2.1-1
Information on the two starting materials used in the manufacture of potassium polyaspartate (A-5D K/SD)

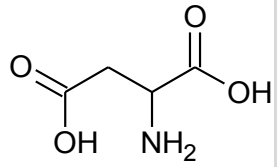
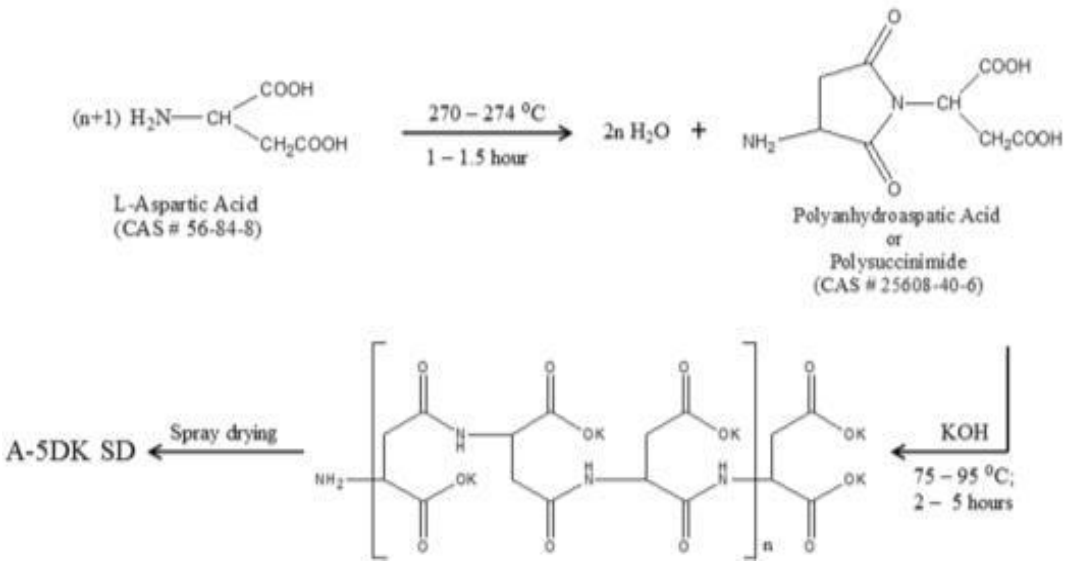
	Starting materials	
Name:	L-Aspartic acid monomer	Caustic potash liquid 45%
IUPAC name:	L-Aspartic acid	Potassium hydroxide
CAS No.:	56-84-8	1310-58-3
EC No.:	200-291-6	215-181-3
Chemical formula:	$C_4H_7NO_4$	KOH
Structural formula:		KOH
Molecular weight:	133.10 g/mol	56.11 g/mol
Purity	≥98.5%	45–47%

Figure 2.2.2-1: <u>Schematic overview of manufacturing process for potassium polyaspartate (A-5DK SD)</u>



2.3 Product Specifications and Batch Analyses

2.3.1 Product Specifications

Appropriate food-grade product specifications have been established for potassium polyaspartate (A-5DK SD) and are presented in Table 2.3.1-1 below.

Table 2.3.1-1
Specifications for potassium polyaspartate (A-5D K/SD)

Parameter	Product specification	
Description:	Light brown powder without odor	
Identification:		
pH:	7.5–8.5 (40% aqueous solution)	
Solubility:	Water	> 1,000 g/L
	Xylene	< 5.0 g/L
	Dichloromethane	< 5.0 g/L
	Methanol	< 5.0 g/L
	Acetone	< 5.0 g/L
	Ethylacetate	< 5.0 g/L
	n-Heptane	< 5.0 g/L
Purity:		
Assay:	Not less than 98.0% w/w on dry matter	
Degree of substitutions:	Not less than 91.5% w/w on dry matter	
KOH:	Not more than 2.0% w/w on dry matter	
Aspartic acid:	Not more than 1.0% w/w	
Other significant impurities:	Not more than 0.1% w/w	

2.3.2 Batch Analyses

Four representative production batches of potassium polyaspartate (A-5D K/SD) were analyzed for potassium polyaspartate concentration and impurities. The batches were selected in order to cover a manufacturing period ranging from 2012 to 2014.

The analytical data for the four batches tested is provided in Table 2.3.2-1 on page 11 of this notice.

The screening analysis of potassium polyaspartate batches was performed by the following steps:

1. Screening analysis of metals by ICP-OES of the dry matter sample.
   To calculate the substitution degree of potassium polyaspartate, the measured potassium concentration is compared with the theoretical content with a substitution degree of 100%. The linearity of the method was confirmed by analysis of five standard solutions in the range of 200 to 2000 mg/mL (correlation coefficient $r^2 = 0.9978$). The standard deviation among repetitions SDr was 8.0 for potassium and the Horwitz RSDr was 41.5% at a potassium concentration of 1117.7 mg/L. Since the relative standard deviation was lower than the Horwitz RSDr, the repeatability test for potassium was considered acceptable.
   For the accuracy, the SANCO/3030/99 rev. 4 guideline requires mean recovery values in the range 90 to 110 % for content higher than 0.01 % w/w and lower than 0.1 % w/w. Since all recovery values fulfilled these criteria, the accuracy of the analytical method was considered acceptable.

   As the method validation parameters, viz. specificity, linearity, accuracy, precision (repeatability), were found to be within the acceptance criteria it was concluded that the method was validated. Based on the results obtained in the study it was concluded that the ICP-OES spectroscopic method is suitable to quantify the content of potassium in the technical material.

   The results of the analysis of 4 batches of potassium polyaspartate dry matter are reported in the Table 2.3.2-2 on page 12 of this notice.

   Based on the values reported, the degree of substitutions of the dry matter material has minimum value of 91.5% w/w (based on mean minus three times the SD). The content of each other metals is not greater than the trigger value for significant impurity of 0.1% w/w, thus no inorganic fraction, except for the potassium salts, is present.

2. Determination of total nitrogen by Kjeldahl method in order to determine the purity of the polyaspartate.

   The determination of the total nitrogen contained in the potassium polyaspartate (KPA) was calculated by comparison between the percentage of nitrogen obtained with the analysis and the expected theoretical value, calculated on the basis of the molecular formula of the test item.

   The results of the analysis of 4 batches of potassium polyaspartate dry matter are reported in Table 2.3.2-2 on page 12 of this notice.

   The purity of the organic fraction was verified through the determination of total nitrogen of the powder and the comparison of this value with the theoretical content in nitrogen of potassium polyaspartate according to its molecular formula. It can be stated that 98.0% w/w (minimum value based on mean minus three times the SD) of the dry weight of the powder is composed of a polymer of aspartic acid.

3. Determination of free aspartic acid as a significant impurity in the potassium polyaspartate as manufactured.

   The presence of free aspartic acid - residual from the polymerization process - has been determined by HPLC-FLD (chromatography with a fluorimetric detection) analysis of the polyaspartate solution, through a derivatization reaction of aspartic acid with o-Phthalaldehyde (OPA). The concentration of free aspartic acid in the commercial preparation is very limited, below 1% dry weight.

   Based on the values reported, the proposed specifications (Table 2.3.2-3 of this notice) are as follow:
   - Potassium polyaspartate: minimum 88.0 % w/w (based on mean minus three times the SD).
   - Aspartic acid: maximum 1.0 % w/w (based on mean plus three times the SD).
   - Other significant impurities: No other impurities were found to be greater than 0.1% w/w.
   - Loss of drying / Water: maximum 11.0% w/w (based on mean plus three times the SD).

Table 2.3.2-1
Information of the tested batches

Batch no.	KHKS 040412	KHKS-072512-1	KHKS-070214-1	KHKS-060414-1
Manufacturing date	04/04/2012	07/25/2012	07/02/2014	06/04/2014
Expiry date	04/03/2016	07/24/2016	07/01/2018	06/03/2018
Number-Average Molecular Weight (Mn)	1037.8 g/mol	1103.9 g/mol	1037.8 g/mol	901.3 g/mol
Weight-Average molecular Weight (Mw)	5051.0 g/mol	5219 g/mol	5051.0 g/mol	5017.9 g/mol
Polydspersity Index (Mw/Mn) (P.I.)	4.867	4.728	4.867	5.567
Z-Average Molecular Weight (Mz)	11227.5 g/mol	11721.9 g/mol	11224.5 g/mol	11307.9 g/mol

Table 2.3.2-2
Batch analysis results on potassium polyaspartate dry matter

	Dry matter content analysis			
	Potassium content (% w/w)	**Degree of substitution (% w/w)**	**Nitrogen content (% w/w)**	**KPAA (% w/w)**
Analytical Method	ICP-OES		Kjeldahl method	
KHKS-040412	24.0	94	7.90	99
KHKS-072512-1	24.5	96	7.95	101
KHKS-070214-1	24.1	94	7.95	100
KHKS-060414-1	24.6	96	7.91	100
Mean	24.3	95	7.92	100
Max	24.6	96	7.95	101
Min	24.0	94	7.90	99
SD	0.29	1.15	0.03	0.82
Mean + 3sd	25.17	98.45	8.02	102.46
Mean - 3sd	23.43	91.55	7.84	97.54
Proposed specifications	-	Min 91.5	-	Min 98.0

Table 2.3.2-3
Batch analysis results on potassium polyaspartate as manufactured

	Aspartic acid (% w/w)	Loss of drying (% w/w)	KPAA (% w/w)
Analytical Method	HPLC-FLD	Gravimetric	By difference to 100%
KHKS-040412	0.4	8.4	91.2
KHKS-072512-1	0.4	8.3	91.3
KHKS-070214-1	0.5	5.5	94.0
KHKS-060414-1	0.5	5.4	94.1
Mean	0.45	6.90	92.65
Max	0.5	8.4	94.1
Min	0.4	5.4	91.2
SD	0.06	1.68	1.62
Mean + 3sd	0.63	11.94	97.51
Mean - 3sd	0.27	1.86	87.79
Proposed specifications	Max 1.0	Max 11.0	Min 88.0

2.3.3 Methods of analysis in food

The quantification of potassium polyaspartate (A-5D K/SD) in red or white wine was achieved by calculating the difference of the aspartic acid content before and after complete sample hydrolysis to aspartic acid monomer. The determination of aspartic acid liberated into wine after acid hydrolysis was performed by high-performance liquid chromatography (HPLC) using an external standard and fluorescence (FLD) detector. The recovery was between 73% and 119% for different samples of red and white wine, which was within the acceptance criteria of 70–120%. The limit of detection (LoD) and limit of quantification of the method were 0.7 and 2.1 mg/mL respectively. (Vassanelli, 2014[1])

The determination of potassium polyaspartate (A-5D K/SD) in water (e.g. the vehicle used for the toxicological studies) was performed by a UV spectrophotometric method based on the addition of a cationic polymer to a buffered solution of potassium polyaspartate (A-5D K/SD).

[1] Vassanelli G., 2014; Chemical Characterization of Potassium Polyaspartate, Laboratory Enocentro srl, Report no.01/2015

Turbidity was developed and absorbance of the solution was measured at 420 nm. The mean recovery was found to be 93.24%. (Marne S.K., 2014[2])

The results are shown in Table 2.3.3-1 below.

The analytical method was validated by determining its specificity, linearity, accuracy, precision (repeatability) and evaluating the same against the respective criteria of acceptance. The method of analysis was found to be specific as a blank solution did not showed any interference.

The linearity of the method was determined using 5 concentrations of test article in the range 5.04 to 25.19 mg/L. Correlation coefficient (r) obtained from linear regression line was 0.9968, which was well within the acceptance criteria. Hence it was concluded that the method of analysis was linear in the tested range of concentrations.

The precision of the method was performed by injecting five replicate samples of the same concentration solution of test article. The % RSD of % active ingredient in the five replicate samples was 0.57%, which was within the acceptance criteria of ± 1.35%. Hence it was concluded that method was precise for the purpose of quantification.

As the method validation parameters, viz. specificity, linearity, accuracy, precision (repeatability), were within the acceptance criteria it was concluded that the method was validated.

Based on the results obtained in the study it was concluded that the UV spectrophotometric method is suitable to quantify the content of A-5D K/SD in water.

Table 2.3.3-1
Accuracy data for A-5D K/SD in A-5D K/SD

Spike code	Recovery		Mean Recovery
	Added (mg/L)	(%)	(%)
Spike Low A	5.03	92.19	92.00
Spike Low B	5.03	91.80	
Spike High A	10.06	94.44	94.48
Spike High B	10.06	94.51	
	Total mean recovery (%)		93.24

[2] Marne S.K., 2014; Validation of Analytical Method for A-5D K SD, Study No. R/RA1384/AMV/14 (INTOX PVT. LTD.)

2.4 Stability of the substance and reaction and fate in food

The stability of the food additive potassium polyaspartate (A-5D K/SD), at different storage conditions, as produced and as well as in water and in wine were evaluated.

2.4.1 Stability of the food additive A-5D K/SD as produced

Dry matter was determined for two samples from the same batch (batch no. KHSH-070214-1) stored for 8 weeks at 40°C and 25°C, respectively. No significant variation in the content of dry matter occurred during storage in either of the two conditions (Table 2.4.1-1 below).

As shown by the analysis of the four batches and reported in the specifications, as the dry matter content correspond to almost 100% to potassium polyaspartate content, it can be stated that no degradation occurs during storage in accelerated conditions (40°C). Thus, a storage stability of at least 4 years is proposed for the potassium polyaspartate (A5DK/SD).

Moreover, the dry matter content of two batches of A-5D K/SD did not significantly change (3% difference) after storage at ambient temperature for 2 years (from 2012-2014). Thus, it can be concluded that potassium polyaspartate (A-5D K/SD) is stable when is stored also for long term at room temperature.

Table 2.4.1-1
Percentage dry matter at 40 °C and 25 °C before (T0) and after storage (8 weeks)

% Dry matter	T0	After 8 weeks	Difference (%)
Sample A (40°C)	94.573	94.572	-0.001
Sample B (25°C)	94.566	94.569	0.003

2.4.2 Stability of the food additive A-5D K/SD in water

A validated UV spectroscopic method was used for the determination of the A-5D K/SD (lot. No. KHKS-040412) in analytical grade water maintained at room temperature (27 ± 9°C) for 24 hr. The two concentrations tested, approximately 6 mg/mL (low dosage) and 100 mg/mL (high dosage), showed small percentage changes compared to their initial concentration (–1.43% and –2.42%, respectively). These results were within the acceptance criteria of ± 20% over their initial concentrations (before storage); therefore, it was concluded that A-5D K/SD was stable in analytical grade water up to 24 hours at room temperature (27 ± 9 0 C). (Marne S.K., 2014[3])

[3] Marne S.K., 2014, Stability Study of A-5D K SD in Water, Study No. R/RA1385/SHA/14 (INTOX PVT. LTD.)

2.4.3 Stability of the food additive A-5D K/SD in wine

Several experiments were conducted in different varieties of red and white wines mainly intended to assess the efficacy of the food additive in preventing tartaric crystallisation and maintaining colour stability in red wines.

The physico-chemical stability of the additive during storage of the wine was mainly assessed during the European research project Stabiwine (FP7-SME-2012-2, n. 314903).
The stability of the food additive A-5D K/SD in wine was assessed both directly, by measuring the concentration of aspartic acid during the wine storage and indirectly by the evaluation of the stability of the effectiveness of the additive over time.

For this purpose, in a first study different polyaspartate potassium salts (Potassium PAAs manufactured by a different producer) were added to different red and white wines, which were then bottled and analyzed during aging. The longest storage period considered so far is 1 year of bottle aging. The concentration of aspartic acid in wines was quantified after 5 and 12 months of storage. Only a slight increase in the aspartic acid concentration was observed in all the wines, after 12 months of bottle aging.

Results of the experiments conducted with the potassium salt of potassium polyaspartate (PAA4) are reported in the Table 2.4.3-1.

Table 2.4.3-1
Aspartic acid content in white wine and in red wine after 5 and 12 months storage

White wine [mg/L]				
Storage Time	**Sample**	**AA**	**Δ**	**% Δ**
After 5 months	control	6.1	-	-
	PAA4	7.4	1.3	1.3%
After 12 months	control	7.8	-	-
	PAA4	12.6	4.8	4.8%
Red wine CRA (red 2) [mg/L]				
Storage Time	**Sample**	**AA**	**Δ**	**% Δ**
After 5 months	control	7.9	-	-
	PAA4	14.2	6.3	6.3%
After 12 months	control	10.5	-	-
	PAA4	15.8	5.3	5.3%

Red wine CRA (red 3) [mg/L]				
Storage Time	**Sample**	**AA**	**Δ**	**% Δ**
After 5 months	control	4.5	-	-
	PAA4	8	3.5	3.5%
After 12 months	control	8.2	-	-
	PAA4	15.7	7.5	7.5%

Caption: AA = aspartic acid content expressed as mg/L; **Δ** = difference of aspartic acid content compared to control; % **Δ** = % of added potassium polyaspartate represented by **Δ**.

After 5 months, a maximum increase of 1.3 - 6.3 mg/L compared to the control was observed for the white and the red wines, respectively. These values correspond to 1.3% (white wine) and 6.3% (red wine) by weight of the added amount of 100mg/L of potassium polyaspartate (PAA4).

After 12 months, the PAA4 showed a maximum increase of 4.8 - 7.5 mg/L compared to the control, for the white and the red wine, respectively. These values correspond to 4.8% (white wine) and 7.5% (red wine) by weight of the added amount of potassium polyaspartate (PAA4). (100 mg/L).

A similar experiment was repeated on two wines, with added potassium polyaspartate (A-5D K/SD) and monitored over time by assessing the tartaric stability and by quantifying the release of aspartic acid monomers.

When 100 mg/L of potassium polyaspartate (A-5D K/SD) was added to a red wine from variety Dolcetto, the concentration of aspartic acid after 12 months of storage shows that the difference between the treated and the control wine is limited to 1.75% of the potassium polyaspartate added to the wine. This value is close to the impurity level of aspartic acid (about 1%) in the commercial preparation of potassium polyaspartate (A5-D K/SD).

The tartaric stability was also monitored through analysis of tartaric acid content before and after cold storage, showing that the wine treated with A-5D K/SD remained stable for 12 months of storage at room temperature, while the instability of control wine increased over time.

The same experiment has been repeated on a white wine. After 6 months of storage at room temperature, only the batch with 100 mg/L of potassium polyaspartate (A5D K/SD) remained stable and a non-significant analytical difference between the control and the added wine was observed, suggesting that also in white wine the degradation of polyaspartate into its monomer is negligible.

Assuming that the hydrolysis is the main reaction occurring in wine during storage, the aspartic acid is the only degradation product formed. The quantification of the aspartic acid after storage shows a non-significant increase compared to the control, thus it can be concluded that potassium polyaspartate (A-5D K/SD) is stable in wine for at least 12 months. This conclusion is also supported by the maintenance of the stabilization effect over time as observed in the experiment where the tartaric stability was measured.

Part 3: Dietary Exposure

An assessment of dietary exposures to potassium polyaspartate (A-5D K/SD) was conducted by the *European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources* (EFSA ANS Panel)[4]. The exposure values derived by this group can be considered appropriate for this notice since: 1) the exposure estimates are based on extensive survey data from several European countries; 2) on a per-capita basis, wine consumption in Europe is higher than the U.S. so the European exposure data can be considered worst-case. In this regard, it should be noted that the dietary estimates in the EFSA ANS Panel report are greater than corresponding estimates the notifier derived using the *Food Commodity Intake Database (FCID) Consumption Calculator*. The FCID Consumption Calculator is an application that uses National Health and Nutrition Examination Survey/What We Eat in America (NHANES/WWEIA) food intake and recipe data to estimate food commodity consumption (http://fcid.foodrisk.org).

Since potassium polyaspartate (A-5D K/SD) is limited to wine use, the population groups evaluated by the EFSA ANS Panel are adults (from 18-years and up to and including 64-years of age) and the elderly (from 65 years of age and older). The specific European countries included in the dietary assessment are as follows:

Adults: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK

Elderly: Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK.

The exposure assessment is based on the consumption of all wines and fortified wines, which includes: white wine, sparkling white wine, red wine, sparkling red wine, wine (undefined), fortified and liquor wines (undefined), vermouth and sherry.

The dietary exposure values for adults and the elderly from the EFSA ANS Panel dietary assessment are shown in Table 3.0 below.

Table 3.0
Estimated exposure to potassium polyaspartate (A-5D K/SD) from its proposed use in wine at the typical and maximum use levels

Estimated exposure (mg/kg bw per day)	**Adults (18-64 years)**	**The elderly (≥65 years)**
Proposed typical use level: 200 mg/L		
Mean	0.01 – 0.2	0.04 – 0.4
High level	0 – 1.0	0.3 – 1.2
Proposed maximum level: 300 mg/L		
Mean	0.02 – 0.4	0.05 – 0.6
High level	0 – 1.4	0.4 – 1.8

[4]EFSA 2016, EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); "Safety of potassium polyaspartate (A-5D K/SD) for use as a stabiliser in wine". EFSA Journal 2016;14(3):4435. [25 pp.] doi:10.2903/j.efsa.2016.4435. Avalable online: www.efsa.europa.eu/efsajournal

The mean dietary exposure from the proposed typical use level of 200 mg/L ranged from 0.01 to 0.2 mg/kg-bw per day in adults up to 0.04 to 0.4 mg/kg-bw per day in the elderly. The high-level intake ranged from 0 to 1.0 mg/kg-bw per day in adults and from 0.3 to 1.2 mg/kg-bw per day in the elderly.

At the proposed maximum level (ML) of 300 mg/L, the mean dietary exposure ranged from 0.02 to 0.4 mg/kg-bw per day in adults up to 0.05 to 0.6 mg/kg-bw per day in the elderly. The high-level intake ranged from 0 to 1.4 in the adults and from 0.4 to 1.8 mg/kg-bw per day in the elderly.

The exposure contribution from each wine or fortified wine group are shown in Table 3.1 below.

Table 3.1

Main food categories contributing to exposure to potassium polyaspartate (A-5D K/SD) using both level of 200 mg/L and 300 mg/L for the proposed uses (% min–max) and the number of surveys ≥5% contribution (n) in which each food category contribute

FoodEx Level 3	Adults	The Elderly
	Range of % contribution to the total exposure (number of surveys)[a]	
Wine (undefined)	12.4-100 (10)	5.8-97.9 (8)
Wine, white	5.0-59.6 (17)	6.3-65.1 (13)
Wine, white, Sparkling	8.3-22.9 (2)	-
Fortified and liquor wines (undefined)	-	5.8 (1)
Vermouth	-	-
Sherry	-	5.7 (1)

-: no information available

(a): The total number of surveys may be greater than the total number of countries listed in the table because some countries submitted more than one survey for a specific population

Part 4. Self-Limiting Levels of Use

Potassium polyaspartate (A-5D K/SD) will only be added to wine at levels needed to achieve its technological function. The notifier is unaware of any self-limiting levels of use associated with potassium polyaspartate (A-5D K/SD).

Part 5. Experience Based on Common Use in Food Before 1958

The notifier is unaware of any common use of potassium polyaspartate (A-5D K/SD) in food prior to 1958.

Part 7: REFERENCES:

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2. Marne S.K., 2014; Validation of Analytical Method for A-5D K SD, Study No. R/RA1384/AMV/14 (INTOX PVT. LTD.)
3. Marne S.K., 2014, Stability Study of A-5D K SD in Water, Study No. R/RA1385/SHA/14 (INTOX PVT. LTD.)
4. EFSA Scientific Opinion: Safety of potassium polyaspartate (A-5D K/SD) for use as a stabilizer in wine. EFSA Journal Adopted 9 March 2016, Printed 17 March 2016; doi:10.2903/j.efsa.2016.4435.
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7. Itzhaki RF and Gill DM. 1964. A micro-biuret method for estimating proteins. Analytical Biochemistry 9: 401-410.
8. Moore S. and Stein WH. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. Journal of Biological Chemistry 211: 907-13.
9. Moore S. 1968. Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. Journal of Biological Chemistry 243: 6281-6283
10. EFSA 2011, EFSA Scientific opinion "Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment" EFSA Journal 2011; 9(9):2379. [69 pp.] doi:10.2903/j.efsa.2011.2379. Available online: www.efsa.europa.eu/efsajournal
11. OECD 471, 1997. OECD Guideline for the Testing of Chemicals: Bacterial Reverse Mutation Test, p. 471.
12. OECD 487, 2008a. OECD Guideline for the Testing of Chemicals: in Vitro Mammalian Cell Micronucleus Test, p. 487 OECD 408, 1998. OECD Guideline for the Testing of Chemicals: Repeated Dose 90-day Oral Toxicity Study in Rodents, Test, p. 408.
13. OECD 407, 2008b. OECD Guideline for the Testing of Chemicals: Repeated Dose 28-day Oral Toxicity Study in Rodents, Test, p. 407.
14. EFSA, 2012. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion Draft Guidance for submission for food additive evaluations.
15. EFSA Journal 2012; 10(7):2760. [65 pp.] doi:10.2903/j.efsa.2012.2760. Available online: www.efsa.europa.eur/efsajournal.htm
16. Restani P. 2015, EU project Stabiwine Final Report by Università degli Studi di Milano (UMIL) - DIPARTIMENTO DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI - DiSFeB., UNIVERSITY of MILANO No GLP, unpublished
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Kolanos, Renata

From: Eliot Harrison <eharrison@lewisharrison.com>
Sent: Tuesday, July 10, 2018 8:11 PM
To: Kolanos, Renata
Subject: RE: REGARDING: GRAS Notice No. GRN 000770
Attachments: Revised Section 6 FDA GRAS_Notice770.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Dear Dr. Kolanos,
Please find enclosed a revised Part 6 of GRA Notice No. 770.
Can you please give a call tomorrow to discuss.
Best regards,
Eliot Harrison
202-393-3903, ext. 114

Part 6. Safety Evaluation and Basis for Our Conclusion of GRAS Status

6.1 Information on existing authorizations and evaluations

Potassium polyaspartate (A-5D K/SD) has been authorized for experimental trials on tartaric stabilization in white wine, rosé wine and red wine, in accordance with Art 4 of EU Regulation 606/2009, in Italy (Ministry of Agricultural, Food and Forestry Policies - MIPAAF Protocol N. 0007017, 15 December 2014) and in Spain (Department of Agriculture, Food and Environment, Aragon Government, authorization issued on 4 September 2014). The same request has been submitted to France on 22nd December 2014 (General Directorate for Competition Policy, Consumer Affairs and Fraud Control – DGCCRF dossier 4C/2014/12/9108). These authorizations have been granted/requested after the submission of physico-chemical, toxicokinetic, immunological and mutagenicity data on potassium polyaspartate (A-5D K/SD).

Polyaspartic acid and its derivatives are environmentally friendly, biodegradable alternatives to traditional polyanionic materials, in particular as replacement for polyacrylic acid. Polyaspartic acid has the ability to inhibit deposition of various salts and can be used as an anti-scaling agent in cooling water systems, water desalination processes, and waste water treatment operations. It can be used in food packaging as a biodegradable detergent and there is a broad interest in this material from the biomedical and material research community.

Authorizations and evaluations are available for the sodium salt of polyaspartic acid (CAS 34345-47-6), the polyaminoacid containing sodium instead of potassium. The sodium salt of polyaspartic acid is authorized for use in US and Australia as:

1) a food contact substance (FCS) as a dispersant for fillers and an anti-scale additive in sugar processing;
2) a water treatment agent used as a scale inhibitor in cooling tower and boiler water applications, with properties of non-phosphor, non-nitrogen, non-pollution and complete biodegradation.

6.2 Biological and Toxicology data

A complete set of Tier 1 (EU base set of studies) have been performed with potassium polyaspartate (A-5D K/SD). These studies include: toxicokinetic *in vitro* ADME (absorption studies and *in vitro* gastrointestinal metabolism), genotoxicity (basic *in vitro* test battery) and subchronic toxicity studies (90 days oral toxicity study in rats).

Toxicological data are also available for sodium salts of polyaspartic acid (PAA) from the published literature. The findings observed in these studies were attributed to sodium ion; as a consequence, this GRAS Notification is specific for potassium polyaspartate (A-5D K/SD) in order to avoid the adverse effects observed in studies performed with sodium PAA that were attributed to the sodium ion.

Moreover, it should be considered that aspartic acid is a non-essential amino acid, so the human body produces its own supply. Furthermore, aspartic acid can also be found in such food sources as dairy, beef, poultry, sugar cane and molasses.

6.2.1 Absorption, distribution, metabolism and excretion

Administration of a chemical does not automatically mean that all of the dose will be systemically available (bioavailable). Therefore, data on systemic exposures to the chemical and its metabolites, as well as an assessment of the major processes involved in its absorption, distribution, metabolism and excretion (ADME), can assist in the interpretation of toxicity studies and the prediction of differences or similarities across animal species or from animal to man (Creton et al., 2009[1]).

To assess gastrointestinal digestibility and intestinal absorption of potassium polyaspartate (A-5D K/SD), the following Tier 1 ADME studies have been performed:

- *in vitro* assays using a sequential proteolytic attack with pepsin (porcine) and pancreatin (porcine) to simulate gastro-intestinal digestibility (proteolysis);
- *in vitro* absorption assay in human colon adenocarcinoma Caco-2 cells to simulate intestinal absorption (Sambuy et al, 2005[2]).

The proteolysis of potassium polyaspartate (A-5D K/SD) was quantified by measuring the undigested proteins by the microbiuret method (Itzhaki and Gill, 1964[3]) and the release of amino acids by ninhydrin method (Moore and Stein, 1954[4]; Moore, 1968[5]).

The potential effect of potassium polyaspartate (A-5D K/SD) on the integrity of the gut cells was also investigated. No significant adverse effects on the integrity of the cells were observed at all potassium polyaspartate (A-5D K/SD) tested concentrations in comparison with the control cells.

These ADME *in vitro* studies showed that potassium polyaspartate (A-5D K/SD) is scarcely digested both at gastric (by pepsin) and at intestinal level (by pancreatin). There was very limited breakdown to aspartic acid (less than 4%) in the simulated gastric digestion following incubation with porcine pepsin and pancreatin. However, any aspartic acid released and subsequently absorbed would go into the amino acid pool. The majority of the material remained as polyaspartate following simulated gastric digestion *in vitro.*

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[1] Creton S, Billington R, Davies W, Dent MP, Hawksworth GM, Parry S, Travis KZ, 2009. Application of toxicokinetics to improve chemical risk assessment: implications for the use of animals. Regulatory Toxicology and Pharmacology 55, 291-9.

[2] Sambuy Y, De Angelis I, Ranaldi G, Scarino ML, Stammati A, Zucco F. 2005. The Caco-2 cell line as a model of the intestinal barrier: influence of cell and culture-related factors on Caco-2 cell functional characteristics. Cell Biol Toxicol. 21: 1-26

[3] Itzhaki RF and Gill DM. 1964. A micro-biuret method for estimating proteins. Analytical Biochemistry 9: 401-410.

[4] Moore S. and Stein WH. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. Journal of Biological Chemistry 211: 907-13.

[5] Moore S. 1968. Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. Journal of Biological Chemistry 243: 6281-6283.

The proteolysis was always close to zero and no significant statistical difference between data from the beginning to the end of enzymatic attack was observed.

The absorption, and therefore the bioavailability of potassium polyaspartate (A-5D K/SD), was determined not to be significant before and after the simulated gastro-intestinal digestion. No adverse effects on the gut cells were observed following oral administration of potassium polyaspartate (A-5D K/SD).

Therefore, the scarce digestion and the insignificant absorption suggests that potassium polyaspartate (A-5D K/SD) is negligibly absorbed in the human gastrointestinal tract following oral administration and it is excreted with the feces. This conclusion is reinforced by physicochemical characteristics of A-5D K/SD (relatively high molecular weight and high hydrophilicity) and other relevant data on A-5D K/SD, showing a low chemical reactivity and the lack of specific target organ toxicity (no genotoxicity in *in vitro* genotoxicity studies, lack of adverse effects in the 90-day study).

Regarding the results from the gastrointestinal digestion study, the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]) stated: "Based on 4% breakdown of aspartic acid, the EFSA ANS Panel estimated that the maximal amount of aspartic acid released would be 0.04 mg/kg-bw per day at the typical use level and 0.07 mg/kg-bw per day at the proposed maximum level (ML). This intake could be compared with the estimates of dietary intake of aspartic acid from estimates of mean and high-level exposure to aspartate ions from the diet (9.1 and 13 g/day, respectively, which is equivalent to approximately 130 and 186 mg/kg-bw per day) (EFSA, 2008). The EFSA ANS Panel considered that this additive use would increase dietary exposures by less than 0.05% at the proposed ML, which it considered negligible. Based on a hypothetical 100% breakdown of potassium polyaspartate (A-5D K/SD), the corresponding intake of aspartic acid would correspond to the maximal intake levels of 1.2 and 1.8 mg/kg-bw per day at the proposed typical and ML, respectively. Under this assumption, the intake of aspartic acid from the proposed use of potassium polyaspartate (A-5D K/SD) would correspond to less than 1.5% at the proposed ML".

In addition, the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]) made the following observation regarding intestinal absorption: "There was very limited (less than 0.07% based on the LoD), if any, absorption of intact polyaspartate into either the cell layer or the receiving fluid (below the LoD of 0.7 mg/L in the basolateral fluid) in the Caco-2 cell absorption model at concentrations of 1 mg/mL *in vitro*. There was good correlation between the duplicate samples at each of the time points studied. All the administered material remained in the apical fluid. The EFSA ANS Panel considered that there was negligible absorption of polyaspartate in this model".

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[6]EFSA, 2016. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS): Safety of potassium polyaspartate (A-5D K/SD) for use as a stabilizer in wine. EFSA Journal 2016;14(3):4435. Available online: www.efsa.europa.eur/efsajournal.htm

Tier 1 absorption studies and *in vitro* gastrointestinal metabolism show that neither A-5D K/SD nor its breakdown products are digested in gastro-intestinal tract (proteolysis of A-5D K/SD close to zero) or absorbed from human colon adenocarcinoma Caco-2 cells.

6.2.2 Genotoxicity studies

Two *in vitro* genotoxicity studies on potassium polyaspartate (A-5D K/SD) have been performed in accordance with the recommendations of the EFSA Scientific Committee on genotoxicity testing (EFSA 2011[7]).

The basic test battery performed consisted of:

- a bacterial reverse mutation assay (OECD TG 471[8]).
- an *in vitro* mammalian cell micronucleus test (OECD TG 487[9]).

All genotoxicity studies were conducted in accordance with OECD guidelines and GLP compliance (Good Laboratory Practice ENV/MC/CHEM (98)17).

Bacterial reverse mutation assay

In this study (Ames/Salmonella pre-incubation assay), the ability of potassium polyaspartate (A-5D K/SD) to cause gene mutations in five tester strains of histidine dependent *Salmonella typhimurium* (TA1535, TA97a, TA98, TA100 and TA102) was examined. The study was performed with and without the S9 metabolic activation system (Liver S9, induced in Wistar rats by phenobarbitone with β - naphthoflavone, was used for this purpose). The procedures used in this study were in accordance with OECD Guideline 471 (OECD, 1997[7]).

Based upon the preliminary tests conducted to assess the solubility/precipitation and cytotoxicity of potassium polyaspartate (A-5D K/SD), the tester strains were exposed to the test substance in triplicate cultures at the doses of 5000 µg, 1500 µg, 500 µg, 150 µg and 50 µg/plate both in the presence and absence of a metabolic activation system (S9). Analytical grade water was used as a vehicle. The exposed bacteria were plated onto minimal glucose agar medium supplemented with L-histidine. The plates were incubated at 37ºC for 68–69 hours after which the histidine revertant colonies were counted and their frequency was compared with that in the vehicle control group. Strain specific positive controls also tested without metabolic activation were sodium azide (TA1535; 2 µg/plate), ICR 191 (TA97a; 1 µg/plate), 4-nitroquinoline-N-oxide (TA98; 0.5 µg/plate), 3-methylmethane sulfonate (TA100 and TA102; 1 µg/plate). For the experiment with the metabolic activation system, 2-aminoanthracene (TA1535; 10 µg/plate), 2-aminofluorene (TA97a, TA98 and TA100; 20 µg/plate), Danthron (TA102; 30 µg/plate) were used as positive controls. In order to confirm the reproducibility of the results, the entire study was carried out twice as experiment No.1 and experiment No.2.

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[7] EFSA 2011, EFSA Scientific opinion "Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment" EFSA Journal 2011;9(9):2379. [69 pp.] doi:10.2903/j.efsa.2011.2379. Available online: www.efsa.europa.eu/efsajournal

[8] OECD 471, 1997. OECD Guideline for the Testing of Chemicals: Bacterial Reverse Mutation Test, p. 471.

[9] OECD 487, 2008a. OECD Guideline for the Testing of Chemicals: in Vitro Mammalian Cell Micronucleus Test, p. 487

As shown in the table below, the frequency of revertant colonies in the potassium polyaspartate (A-5D K/SD) and vehicle control groups was comparable. In addition, the concurrent positive controls and vehicle control groups were considered acceptable. Under the conditions of the study, potassium polyaspartate (A-5D K/SD) was not mutagenic in *Salmonella typhimurium* strains TA 1535, TA97a, TA98, TA 100 and TA 102.

Table: Summary data on Histidine Revertant Colonies

Treatment	Concentration		TA1535		TA97a		TA98		TA100		TA102	
	(µg/plate)	S9	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
A-5D K SD	5000	_	14.67	2.31	115.33	12.22	30.33	7.57	119.33	5.03	249.33	6.11
		+	13.33	2.52	132.00	2.00	24.67	3.51	120.00	12.00	242.67	8.33
	1500	_	14.00	4.00	122.67	3.06	26.33	4.73	140.00	17.09	224.00	8.00
		+	13.67	4.51	108.00	29.05	31.00	7.00	127.33	13.61	256.00	10.58
	500	_	21.00	3.61	125.33	5.03	21.00	4.00	130.00	19.08	237.33	4.62
		+	17.67	3.51	111.33	22.03	29.67	6.11	121.33	11.02	248.00	4.00
	150	_	13.33	3.06	94.67	18.58	19.33	1.53	129.33	20.13	261.33	12.86
		+	14.33	1.53	92.67	9.24	23.00	6.08	118.00	24.58	265.33	24.44
	50	_	15.33	4.51	124.67	4.62	23.00	2.00	134.67	7.02	250.67	6.11
		+	17.00	2.65	124.00	2.00	19.33	3.21	158.67	18.58	261.33	10.07
Vehicle Control												
Analytical grade water	100 µl	_	14.00	2.65	138.67	17.01	28.33	6.43	127.33	20.13	266.67	32.58
		+	21.00	7.00	115.33	8.33	23.33	4.93	123.33	14.19	252.00	8.00
Positive Controls												
Sodium azide	2	_	840.00	32.74	-	-	-	-	-	-	-	-
ICR 191	1	_	-	-	1008.00	141.53	-	-	-	-	-	-
4-Nitroquinoline-N-oxide	0,5	_	-	-	-	-	490.67	18.90	-	-	-	-

In vitro mammalian cell micronucleus assay

The methodology used in this study was based on draft OECD Test Guideline 487 (OECD, 2008a[9]). The objective of the *in vitro* micronucleus assay was to evaluate the test substance, potassium polyaspartate (A-5D K/SD), for its ability to induce the formation of small membrane-bound DNA fragments such as micronuclei in the cytoplasm of the interphase cell. An appropriate volume of whole blood from one healthy young-adult, non-smoking male volunteer was collected from the peripheral circulation into heparinized tubes.

According to the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), "Cultures of human peripheral blood lymphocytes were exposed to potassium polyaspartate (A-5D K/SD) dissolved in analytical grade water at concentrations of 5000, 1500 and 500 µg/mL (concentrations based upon preliminary solubility/precipitation and cytotoxicity studies) with and without the metabolic activation system (i.e. rat liver S9). Duplicate cultures were used at each concentration. In experiments No. 1 and No. 2, cells were exposed to the test substance for 3 and 20 hours, respectively, without the supplementary metabolic activation system. In experiment No. 3, conducted with the supplementary metabolic activation system, the cells were exposed for 3 hours to the test substance, 48 hours after culture initiation. Cytochalasin B (actin polymerization inhibitor) was added at 68 hours, whereas cell harvesting was performed 96 hours after culture initiation. Positive, negative and vehicle controls, both with and without metabolic activation, were tested concurrently with the test substance. Analytical grade water was used as a vehicle. Mitomycin C (MMC) and vinblastine (VBC), known micronucleus forming agents, were employed as positive controls, at concentrations of 0.8 and 0.08 µg/mL respectively, for the experiments without the metabolic activation system, whereas cyclophosphamide (CPM) was employed at a concentration of 6.25 µg/mL for the experiment with the metabolic activation system. Each culture was harvested and slide preparations were made and stained with 5% Giemsa (OECD 487, 2008a[8])". In addition, the cytotoxicity was determined from the number of cell cycles per cell during the period of exposure to cytochalasin B (CBPI, Cytokinesis-Block Proliferation Index) derived from at least 500 cells per culture (1000 cells per test concentration). Two thousand binucleated cells with well spread cytoplasm were evaluated microscopically for the presence of micronuclei, if any.

The EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]) stated the following regarding the study findings: "A comparison of the percentage incidence of micronucleated binucleated cells (BNCs) for each of the three experiments conducted with potassium polyaspartate (A-5D K/SD), either with or without the metabolic activation system, did not reveal any biologically significant or dose-related increase. Also, there was no incidence of a biologically significant increase in the percentage incidence of micronucleated BNCs at any of the concentration levels in the cultures treated with potassium polyaspartate (A-5D K/SD). Sensitivity of the test system and activity of S9 mix were demonstrated in the positive control group. The positive controls (i.e. directly acting clastogen mitomycin C, directly acting aneugen vinblastine and indirectly acting clastogen cyclophosphamide) induced significant increase in frequencies of micronucleated binucleated cells over the concurrent controls, which validated the test method".

In addition, assessment of the cytokinesis-block proliferation index (CPBI) made during the preliminary cytotoxicity test and the main study indicated that potassium polyaspartate (A-5D K/SD) exerted no cytotoxic effects on the cultured human lymphocytes in any of the three experiments conducted, both with and without metabolic activation system. Under the conditions of the assay, potassium polyaspartate (A-5D K/SD) did not induce any biologically significant and concentration related increase in the incidence of micronucleated (BNCs) over the tested range. Therefore, these negative results indicate that potassium polyaspartate (A-5D K/SD) did not induce chromosome breaks and/or gain or loss (i.e. it is not clastogenic and aneugenic) in cultured mammalian cells (i.e. human peripheral blood lymphocytes).

All *in vitro* genotoxicity endpoints are clearly negative; thus, it can be concluded with reasonable certainty that potassium polyaspartate A-5D K/SD is not a genotoxic hazard.

6.2.3 Subchronic toxicity

Two subchronic toxicity studies on potassium polyaspartate (A-5D K/SD) have been performed in rats:

- a 14-day range-finding study performed to collect information of target organs and to select appropriate doses for a 90-day study;
- a 90-day subchronic toxicity study (OECD TG 408, 1998[10]), modified to include assessment of additional parameters described in the more recent guideline on repeated-dose 28-day oral toxicity study in rodents (OECD TG 407, 2008b[11]). This approach allows the identification of chemicals with the potential to cause neurotoxic, immunological or reproductive organ effects or endocrine-mediated effects, as recommended in EFSA Guidance for submission for food additive evaluations (EFSA ANS Panel 2012[12]).

In the 14-day range-finding study, groups of five male and five female Wistar rats were administered potassium polyaspartate (A-5D K/SD) by oral gavage daily at the doses of 60, 125, 250, 500 and 1000 mg/kg-bw for 14 days and were sacrificed on day 15 to evaluate its toxicity. A concurrent vehicle control group receiving analytical grade water at the dose of 10 mL/kg was also maintained.

The rats were examined daily for signs of toxicity, morbidity and mortality. Animals were subjected to detailed clinical examination before initiation of the study and weekly thereafter during the treatment period and at termination. Body weight and food consumption were recorded weekly. Laboratory investigations were performed on blood at termination of the study. All animals sacrificed terminally were subjected to a detailed necropsy and weights of kidneys, liver, adrenals, testes, spleen, brain and heart were recorded.

The test article did not induce any mortality and treatment related clinical abnormalities in rats treated at and up to the dose of 1000 mg/kg-bw. No mortality or abnormal clinical signs were observed in vehicle control group animals.

Body weight gain and food consumption was not affected at and up to the dose of 1000 mg/kg-bw. The hematological parameters of hemoglobin, packed cell volume, total RBC count, total and differential WBC counts, RBC indices and platelet count of male and female rats, treated with the test article at and up to the level of 1000 mg/kg-bw were found to be comparable to those of the control animals at termination.

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[10] OECD 408, 1998. OECD Guideline for the Testing of Chemicals: Repeated Dose 90-day Oral Toxicity Study in Rodents, Test, p. 408.

[11] OECD 407, 2008b. OECD Guideline for the Testing of Chemicals: Repeated Dose 28-day Oral Toxicity Study in Rodents, Test, p. 407.

[12] EFSA, 2012. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion Draft Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760. [65 pp.] doi:10.2903/j.efsa.2012.2760. Available online: www.efsa.europa.eur/efsajournal.htm

The test article, at and up to the level of 1000 mg/kg-bw, did not alter the plasma levels of total protein, albumin, globulin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, glucose, urea nitrogen, urea, creatinine and total cholesterol in male and female rats.

The values of absolute and relative organ weights of male and female rats treated with the test article, at and up to 1000 mg/kg-bw were found to be comparable with those of the control rats at termination.

No gross pathological alterations were encountered in the rats sacrificed at termination of the study. Based on these findings, the doses selected for the 'Repeated Dose 90 Days Oral Toxicity Study of A-5D K/SD in Wistar Rat' were 250, 500 and 1000 mg/kg-bw.

In the 90-day study, groups of ten male and ten female Wistar rats were administered potassium polyaspartate (A-5D K/SD) oral gavage daily at the doses of 250, 500 and 1000 mg/kg-bw for 90 days and were sacrificed on day 91 to evaluate its toxicity. A concurrent control group of ten males and ten females receiving the vehicle, i.e. analytical grade water, at 5 ml/kg was also maintained for 90 days. Additionally, groups of five rats per sex which had received the vehicle at 5 ml/kg and the test article at the high dose level, i.e. 1000 mg/kg-bw, were further observed for a period of 28 days following the 90 days treatment, for assessment of reversibility, persistence or delayed occurrence of toxicity.

The rats were examined daily for signs of toxicity, morbidity and mortality. They were subjected to detailed clinical examination before initiation of the study and weekly thereafter during the treatment period, reversal period, and at termination. Ophthalmoscopic examination was conducted on control and high-dose group animals before initiation of the study and at termination of treatment. In the thirteenth week of treatment, animals were additionally examined for assessment of sensory reactivity, assessment of grip strength and motor activity. Body weight and food consumption were recorded weekly.

Laboratory investigations were performed on blood and urine at termination of the treatment and at the end of recovery period. All animals sacrificed terminally were subjected to a detailed necropsy and weights of kidneys, liver, adrenals, testes, epididymides, uterus, thymus, spleen, brain, ovaries and heart were recorded. Histopathological evaluation was performed on all tissues [(brain, spinal cord, eye, pituitary, thyroid, parathyroid, spleen, thymus, adrenals, pancreas, trachea, lungs, heart, aorta, esophagus, stomach, duodenum, Jejunum, terminal ileum, colon, rectum, liver, kidneys, urinary bladder, prostate, seminal vesicle, epididymides, testes, ovaries, uterus, skin. Sciatic nerve, bone marrow (smear), mammary gland (females), mesenteric lymph node, axillary lymph node and salivary glands)] in all rats from the control and high dose groups.

There was no incidence of treatment related mortality in rats treated with the test article at any of the dose levels. The test article did not induce any remarkable or treatment related clinical abnormalities in rats treated at and up to the dose of 1000 mg/kg-bw. No mortality or abnormal clinical signs were observed in the vehicle control animals. Ophthalmological examination did not reveal any treatment related ocular abnormalities. Also, the observations on sensory reactivity, grip strength and motor activity conducted in the thirteenth week of treatment did not reveal any neurotoxic potential of the test article. Body weight gain was not affected in male and female rats treated at and up to the dose of 1000 mg/kg-bw and were found to be comparable to that by the control rats throughout the treatment period and also during the recovery period. The test article did not have any adverse effect on the average daily food consumption by the male and female rats treated at any of the dose levels.

The hematological parameters of hemoglobin, packed cell volume, total RBC count, total and differential WBC counts, RBC indices, platelet count, activated partial thromboplastin time and prothrombin time of male and female rats treated with the test article were found to be comparable to those of the vehicle control animals at termination of the treatment and also at the end of the recovery period.

The test article did not alter the plasma levels of total protein, albumin, globulin, alanine aminotransferase, alkaline phosphatase, glucose, creatinine, calcium, total cholesterol, phosphorous, total bilirubin, urea nitrogen, urea, sodium, potassium, thyroid hormones and triglycerides in male and female rats. The data on urinalysis indicated no adverse effect due to the treatment.

The values of absolute and relative weights of kidneys, liver, adrenals, testes, epididymides, uterus, thymus, spleen, brain, ovaries and heart of male and female rats treated with test article, were found to be comparable with those of the control rats at the end of treatment period and also at the end of the recovery period.

The test article did not have any adverse effect on the estrus cycle of female rats treated at any of the dose levels.

No treatment related gross pathological changes were noted. Histopathological examination was performed on tissues of the control and high dose group animals, where the changes in the high dose group were incidental or comparable to the control group or unrelated to treatment.
Based on the findings of this study, the No-Observed-Adverse-Effect-Level (NOAEL) of potassium polyaspartate (A-5D K/SD) in Wistar rats, following oral administration for 90 days was found to be equal to or greater than 1000 mg/kg body weight.

6.2.4 Chronic toxicity and carcinogenicity

No long-term bioassays or carcinogenicity studies have been performed with potassium polyaspartate (A-5D K/SD). Based on the results from the toxicokinetic (negligible absorption found in *in vitro* absorption studies) genotoxicity (negative in the *in vitro* test battery) and 90-day oral toxicity (no treatment related effects at dosing levels up to 1000 mg/kg-bw) studies conducted with potassium polyaspartate (A-5D K/SD), chronic toxicity and carcinogenicity studies are not necessary.

This position is consistent with the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), that stated: "the data from the repeated dose 90-day toxicity study in rats conducted with potassium polyaspartate (A-5D K/SD) and the Tier 1 toxicokinetics did not trigger additional testing for chronic toxicity and carcinogenicity".

6.2.5 Reproductive and developmental toxicity

No reproductive or developmental toxicity studies have been performed with potassium polyaspartate (A-5D K/SD). Based on the results from the toxicokinetic (negligible absorption found in *in vitro* absorption studies) and the 90-day oral toxicity (no evidence of effects on reproductive organs and the estrous cycle) studies conducted with potassium polyaspartate (A-5D K/SD) these studies are not necessary. This position is consistent with EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), that stated: "the data from the repeated dose 90-day toxicity study in rats conducted with potassium polyasparatate (A-5D K/SD) and the Tier 1 toxicokinetics did not trigger additional testing for reproductive and developmental toxicity".

6.2.6 Hypersensitivity, allergenicity and food intolerance

In exposed individuals, substances added to food may interact with the immune system in several ways and induce changes in the immune response resulting in either immunosuppression or immunostimulation. Immunostimulation may lead to hypersensitivity reactions, including autoimmunity and allergy. An allergic response to a substance can be induced by the presence of allergenic components or residues, in particular proteins, or alternatively because the substance itself is an allergen (e.g. a protein or a peptide) or capable of acting as a hapten.

Therefore, other studies which generally examine specific biological processes have been considered to be relevant and useful for assessing the risk and establishing the safety of a substance. These additional studies include immunotoxicity, hypersensitivity and food intolerance.

Thus, additional studies on immunotoxicity, hypersensitivity and food intolerance have been performed on potassium polyaspartate (A-5D K/SD).

Immunotoxicity

In the 90-day oral toxicity study performed with potassium polyaspartate (A-5D K/SD), no effects were observed in any of the parameters that may be indicative of an immunotoxic or immunomodulatory effects. In addition, an *in vitro* assay was performed to further evaluate the potential immunotoxicity of potassium polyaspartate (A-5D K/SD). As noted in the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), "the potential stimulation of immune cells has been assessed *in vitro* on pro-myelocytic human cell line THP-1, used as a surrogate of monocytes (Restani P., 2015[13]).

[13] Restani P. 2015, EU project Stabiwine Final Report by Università degli Studi di Milano (UMIL) - DIPARTIMENTO DI SCIENZE FARMACOLOGICHE E BIOMOLECOLARI - DiSFeB., UNIVERSITY of MILANO No GLP, unpublished

Prior to the study, the cytotoxicity of potassium polyasparate (A-5D K/SD) was assessed through the MTT test. The THP-1 cells were diluted to 10^6 cells/mL in RPMI 1640, supplemented with 10% heated-inactivated fetal calf serum (media) and cultured in 37°C in a 5% CO_2 incubator. Cytokine IL-8 release (with an enzyme-linked immunosorbent assay) and CD86 expression (with flow cytometric analysis) were then assessed. For IL-8 release, 1.0 x 10^6 cells were seeded in a 24-well plate. Cells were incubated for 24 hr in the presence or absence of potassium polyaspartate (A-5D K/SD) at 2 mg/mL. Lipopolysaccharide from Escherichia coli serotype 0127:B8 10 ng/mL was used as a positive control. The results showed that potassium polyaspartate (A-5D K/SD) did not induce any activation of the immune system (monitored parameters: up-regulation of CD86 and release of IL-8). Thus, it can be concluded that potassium polyaspartate (A-5D K/SD) has no immunotoxicity and no Tier 2 or 3 studies on immunotoxicity were necessary. In the positive control, a statistical significant increase in both CD86 expression and IL-8 release was observed".

Allergy

According to the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), a literature search that included PubMed, Medline, CAB Abstracts, Web of Sciences did not find any reports of allergenic reactions associated with the correspondent polymer of sodium salt.

The results from the literature search are also consistent with the results from the evaluation of potassium polyaspartate (A-5D K/SD) in the *in vitro* human promyelocytic THP-1 cell line assay since there was no stimulation of the immune system. The lack of stimulation strongly implies that potassium polyaspartate (A-5D K/SD) would not induce any allergic reactions.

Intolerance Reactions

According to the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), "no inborn errors of metabolism have been reported for aspartic acid. Moreover, no intolerance reactions to the correspondent polymer of sodium salt have been observed. Thus, potassium polyaspartate (A-5D K/SD) is not expected to cause intolerance reactions".

6.2.7 Other studies

Neurotoxicity

As part of the 90-day oral toxicity study in rats conducted with potassium polyaspartate (A-5D K/SD), a neurological battery was performed in order to assess the potential neurotoxicity of the substance.

The neurological examination (functional observations) occurred during the 13th week of treatment of the 90-day oral toxicity study. The neurological examination included:

- Examinations in home-cage and open field: Posture / Movement, Respiration, Palpebral closure, Lacrimation, Salivation, Skin and hair coat, Urination, Defecation, Locomotor activity, Rearing and Gait
- Manipulative examination / Responses to stimuli: Tactile (touch) response, Response to nociceptive stimuli (tail pinch), Pupil response to light, Proprioception – Righting reflex, Auditory response, Head shaking, Landing foot splay

According to the EFSA ANS Panel report on potassium polyaspartate (A-5D K/SD) (EFSA, 2016[6]), "the neurological examinations (functional observations) conducted in the thirteenth week of the 90-day study did not reveal any remarkable and treatment related incidence of neurological abnormalities. Also, no findings indicative of a neurotoxic potential of potassium polyaspartate (A-5D K/SD) were encountered during these examinations. Thus, it can be concluded that potassium polyaspartate (A-5D K/SD) has no neurotoxicity and no Tier 2 or 3 studies were necessary".

Interaction with minerals

Potassium polyaspartate (A-5D K/SD) is rich in negative charges, which are essential for the enological properties. As a result, it was deemed appropriate to assess the potential of this substance to sequester nutrients in order to exclude nutrient depletion effects for consumers drinking wine treated with this additive. Consequently, a study was conducted to evaluate the interaction between potassium polyaspartate (A-5D K/SD) and minerals (Restani & Colombo, 2017[14]). This study has not been published but is summarized in detail below.

The aim of the study was to assess the binding properties of potassium polyaspartate with three minerals (calcium, iron, magnesium), which were considered the most suitable nutrients to develop a good model of the phenomenon and with a significant relevance concerning their nutritional characteristics (frequent deficiency and/or low bioavailability).

In the study, a solution of potassium polyaspartate (A-5D K/SD) was firstly incubated with a defined amount of mineral and afterwards the solution was loaded onto the column for Size-Exclusion Chromatography (SEC). Selected aliquots of the eluted solution were then dosed to quantify the amount of polyaspartate (PAA by microbiuret method) and the amount of bind mineral (by ICP-OES method). The Size-Exclusion Chromatography (SEC) was selected as suitable method to assess the possible binding between potassium polyaspartate (A-5D K/SD) and minerals. SEC is a chromatographic method in which molecules in solution are separated by their molecular weight (MW) during the elution through a column packed with a gel media. The gel is a heterogeneous phase system characterized by pores with controlled range of size. Small molecules can diffuse into the gel from the surrounding solution while sufficiently large molecules are completely excluded from the pores.

The gel filtration medium Sephadex G-15 (Pharmacia, Sweden) was selected to separate potassium polyaspartate (a polymer with MW close to 5 kDa) from free minerals. This medium is a gel containing dextran crosslinked with epichlorohydrin; its separation range is 500-1500 Da and it is commonly used to separate peptides and small biomolecules. Potassium polyaspartate (without or with bound minerals) has a MW of about 5 kDa therefore it is expected to be totally excluded from the gel pores and to elute with the void volume (V0). On the contrary, having a MW lower the 500 Da, all free minerals are expected to elute with the total volume (Vt).

[14] Restani & Colombo, 2017. Report on the Results Obtained from the Trials Aimed to Assess the Exchange Between KPAA and Metals Performed at The Università Degli Studi Di Milano Dept. Pharmacological And Biomolecular Sciences.

The analysis of selected aliquots of the eluted solution dosed to quantify the amount of polyaspartate (PAA by microbiuret method) and the amount of bind mineral (by ICP-OES method) confirm the capability of potassium polyaspartate to bind cations. Specifically, potassium polyaspartate showed the potential to bind:

1. Calcium: potential to bind an amount of calcium corresponding to 8.2% of potassium polyaspartate weight (at saturation of free charges).
2. Magnesium: potential to bind an amount of magnesium corresponding to 4.89% of potassium polyaspartate weight (at saturation of free charges).
3. Iron: potential to bind an amount of iron corresponding to 7.94% of potassium polyaspartate weight (at saturation of free charges).

The maximum permitted dose of potassium polyaspartate (A-5D K/SD) into wine is 300 mg/L; therefore, the maximum amount of minerals that can be sequestrated by potassium polyaspartate added into wine are:

- Calcium: 24.6 mg /L wine (8.2% of 300 mg)
- Magnesium: 14.67 mg /L wine (4.89% of 300 mg)
- Iron: 23.82 mg/L wine (7.94% of 300 mg)

Wine contains several minerals of natural origin coming from soil where vines are grown and reach wine through grapes. The concentration of these minerals is characteristic and comprises the largest part of the total minerals content in wine. It is connected with the maturity of the grapes, their variety, the type of soil in the vineyard, and the climatic conditions during their growth (Powl P., 2007[15]).

According to literature data, the amount of calcium, magnesium and iron naturally present into wine ranges between (Aceto et al., 2002[16]):

- Calcium: 50-150 ppm (mg/L wine)
- Magnesium: 50-150 ppm (mg/L wine)
- Iron: 1-5 ppm (mg/L wine)

In addition to these 3 minerals, wine contains also significant amounts of:

- Potassium: 300-1500 ppm (mg/L wine)
- Sodium: 5-60 ppm (mg/L wine)

It can be reasonably expected that when potassium polyaspartate is added into wine, the free charges of potassium polyaspartate are saturated by the minerals naturally present in wine, with the result being no residual sequestration potential.

[15] Pawel Pohl, 2007. What do metals tell us about wine? Trends in Analytical Chemistry, Vol. 26, No. 9, 2007.
[16] Maurizio Aceto, Ornella Abollino, Maria Concetta Bruzzoniti, Edoardo entasti, Corrado Sarzanini and Mery Malandrino, 2002. Determination of metals in wine with atomic spectroscopy (flame-AAS, GF-AAS and ICP-AES); a review. Food Additives and Contaminants , 2002, Vol. 19, No. 2, 126±133.

Therefore, no nutrient depletion effects for consumers drinking wine treated with potassium polyaspartate (A-5D K/SD) at maximum dose level of 300 mg/L wine are expected.

6.3 Safety Evaluations by Authoritative Bodies

6.3.1 European Food Safety Authority (EFSA)

In March 2016, EFSA published a scientific opinion on "Safety of potassium polyaspartate (A-5D K/SD) for use as a stabilizer in wine" (EFSA 2016[6]). Based on the available scientific information, the EFSA ANS Panel concluded that there was no safety concern from the proposed use and use levels of potassium polyaspartate (A-5D K/SD).

The EFSA ANS Panel considered all data as fulfilling the requirements for the evaluation of the new food substance and did not request additional testing for chronic toxicity and carcinogenicity, nor for reproductive and developmental toxicity.

As such, potassium polyaspartate (A-5D K/SD) is permitted for use as stabilizer in wine, with a maximum use level of 300 mg/L and typical levels in the range of 100-200 mg/L.

6.4 Overall Conclusions Related to Safety

The results of toxicological studies clearly demonstrate that potassium polyaspartate (A-5D K/SD) is negligibly absorbed, it does not affect gut cells integrity and it does not induce any activation of the immune system. Two *in vitro* tests (a sequential proteolytic attack with pepsin and pancreatin, and an absorption study in human colon adenocarcinoma Caco-2 cells) aimed at assessing gastrointestinal digestibility and intestinal absorption of potassium polyaspartate (A-5D K/SD) showed that proteolytic digestion of potassium polyaspartate (A-5D K/SD) was minimal and that no absorption of intact A-5D K/SD was observed *in vitro*.

The genotoxic potential of potassium polyaspartate (A-5D K/SD) was investigated in a bacterial reverse mutation assay and using an *in vitro* mammalian cell micronucleus test. No genotoxic effect was observed in either of these two standard regulatory studies carried out in recognized testing facilities according to the relevant guideline and Good Laboratory Practice (GLP) compliance and reported in accordance with the relevant guideline.

Data from two toxicity studies performed in rats were submitted, a 14-day range-finding study performed to collect information of target organs and to set appropriate dosing, and a 90-day subchronic toxicity study. The 90-day study was performed in accordance with the OECD Test Guideline 408, modified to include assessment of additional parameters to allow for the identification of chemicals with the potential to cause neurotoxic, immunological or reproductive organ effects or endocrine-mediated effects. In this study, potassium polyaspartate (A-5D K/SD) was administered daily via gavage to groups of Wistar rats (10 animals per sex per dose), at doses of 250, 500 and 1000 mg/kg-bw per day.

Based on the findings of this study, the No-Observed-Adverse-Effect-Level (NOAEL) of potassium polyaspartate (A-5D K/SD) has been set at 1000 mg/kg-bw per day, the highest dose tested, and also that there were no triggers for additional toxicological testing.

Exposure estimates to potassium polyaspartate (A-5D K/SD) from its proposed use were calculated for both typical and maximum use levels (200 mg/L and 300 mg/L). In the worst-case scenario of high-level intake (300 mg/L) of potassium polyaspartate (A-5D K/SD), the maximum estimated intake would be 1.8 mg/kg-bw per day in the elderly and 1.4 mg/kg bw per day in adults, resulting in a margin of safety (MOS) of at least 550. Therefore, it can be concluded that there are no safety concerns that result from the proposed use and use levels of potassium polyaspartate (A-5D K/SD) as a stabilizer in wine.

6.5 Expert Opinion

An outside expert opinion that considered whether the intended use of potassium polyaspartate (A-5D K/SD) in wine is GRAS was performed by Dr. Nicholas Skoulis. Based on the manufacturing, use, exposure and safety information and data presented in this notice, Dr. Skoulis has concluded that the intended use of potassium polyaspartate (A-5D K/SD) in wine, as specified in Part 1.3 of this notice, is GRAS. Dr. Skoulis is an expert in assessing the safety of food ingredients and is highly qualified to issue a determination regarding the GRAS status of potassium polyaspartate (A-5D K/SD). Furthermore, it is the opinion of Dr. Skoulis that other qualified experts would concur with this conclusion.

(b) (6)

Kolanos, Renata

From: Eliot Harrison <eharrison@lewisharrison.com>
Sent: Monday, July 30, 2018 3:28 PM
To: Kolanos, Renata
Subject: RE: REGARDING: GRAS Notice No. GRN 000770
Attachments: kolanos.ltr_20180730151849.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Dear Dr. Kolanos:
Please refer to the attachment.
Best regards,
Eliot Harrison

LEWIS &
HARRISON
Consultants in Government Affairs

2461 South Clark Street
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Arlington, VA 22202
telephone 202.393.3903
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July 30, 2018

Renata Kolanos, Ph.D.
Consumer Safety Officer
U.S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice Review
5001 Campus Drive
College Park, MD 20740

re: **GRAS Notice No. 000770**
Substance: Potassium Polyaspartate (A-5D K/SD)
Notifier: Enartis USA, Inc.
Your e-mail Dated July 25, 2018

Dear Dr. Kolanos:

On behalf of Enartis USA, Inc. ("Enartis"), I am responding to your e-mail of July 25, 2018. Please note the following:

- Enartis confirms that the appropriate reference for the genotoxicity studies described in Section 6.2.2 and the subchronic toxicity studies described in Section 6.2.3 is the publication by Galbusera *et. al.* (2017).

- Enartis confirms that its conclusions are based on the results of the genotoxicity and subchronic toxicity studies published by Galbusera *et. al.*(2017), even though EFSA's conclusions are quoted in Sections 6.2.2 and 6.2.3 of the revised Part 6.

If you have any questions about this response, please contact me at (202) 393-3903, ext. 114 or by e-mail at eharrison@lewisharrison.com.

Sincerely,

(b) (6)

Eliot Harrison
Agent for Enartis USA, Inc.