

Supporting document 1

Food technology, hazard and dietary assessment report - Application A1161

Potassium polyaspartate as a food additive in wine

Executive summary

FSANZ has assessed an application from Enartis Pacific Pty Ltd. to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of potassium polyaspartate as a food additive in wine at a maximum permitted level of 100 mg/L.

Potassium polyaspartate functions as a stabiliser by preventing the growth of potassium bitartrate crystals in wine.

Based on the food technology assessment, FSANZ has concluded that potassium polyaspartate, when used as a food additive to stabilise wine, is technologically justified in the quantity and form proposed. It is appropriately classified as a food additive since it provides a technological function as a stabiliser.

Results of *in vitro* studies indicate that gastrointestinal degradation and absorption of potassium polyaspartate is likely to be minimal. Potassium polyaspartate was not genotoxic *in vitro*, and no adverse effects were observed in 14-day and 90-day repeated dose oral toxicity studies in rats at doses up to 1000 mg/kg bw/day, the highest dose tested.

A dietary exposure assessment was conducted for Australian and New Zealand population groups based on the proposed draft variation. The estimated mean and 90th percentile dietary exposures range from 0.031 mg/kg bw/day to 0.35 mg/kg bw/day and from 0.072 mg/kg bw/day to 0.79 mg/kg bw/day, respectively, across the population groups assessed.

The no observed adverse effect level (NOAEL) in the 90-day repeated dose oral toxicity study in rats (1000 mg/kg bw/day) is more than 1200-fold higher than the highest 90th percentile exposure to potassium polyaspartate in the dietary exposure assessment.

Based on the reviewed data, it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' is appropriate for potassium polyaspartate.

There are no public health and safety concerns from the use of potassium polyaspartate as a food additive in wine at the proposed levels.

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1. Introduction

FSANZ received an application from Enartis Pacific Pty Ltd. to amend the Code to permit potassium polyaspartate as a food additive in wine at a maximum permitted level of 100 mg/L.

The technological purpose of potassium polyaspartate is as a stabiliser in wine to prevent the growth of potassium bitartrate crystals. Potassium bitartrate crystals, also known as “wine diamonds”, can develop during storage. The physical presence of these crystals in the base of a wine bottle or on the cork is unacceptable in terms of wine quality and can appear ‘glass-like’ which results in an unacceptable appearance for consumers.

Potassium polyaspartate is the potassium salt of polyaspartic acid, produced from L-aspartic acid, a naturally occurring amino acid in wine and other foods. Potassium polyaspartate is superior to other permitted food additives used as stabilisers in wine. It has no negative effects on the sensory properties of wine.

2. Food technology assessment

2.1 Objectives for the food technology assessment

To determine whether potassium polyaspartate, when used as a food additive in the form and quantity proposed, achieves its technological purpose as a stabiliser.

2.2 Chemical and physical properties

Enartis Pacific’s potassium polyaspartate is prepared from L-aspartic acid. A thermal process converts the L-aspartic acid into polysuccinimide. Polysuccinimide is then treated with potassium hydroxide to produce potassium polyaspartate.

Potassium polyaspartate is a light brown odourless powder that is soluble in water (>1000 g/L) and insoluble in organic solvents (<5 g/L). As shown in Figure 1 it is a polymer composed of aspartic acid units with the general chemical formula $[C_4H_5NO_3K]_n$ where n is the average degree of polymerisation and approximately 30.

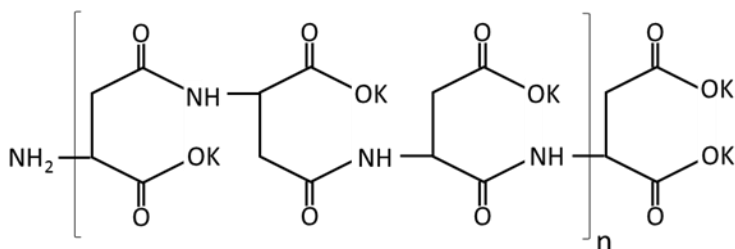


Figure 1 Chemical structure of potassium polyaspartate

The application includes information on the identity of potassium polyaspartate.

Chemical names: Homopolymer of potassium L-aspartate or potassium polyaspartate

Common name: Potassium polyaspartate

Synonyms: A-5D K/SD, A-5D K SD, A-5DK/SD, A-5DK, KPA,

Molecular weight (average): 5300 g/mol

CAS Number: 64723-18-8

EC Number: E456

2.3 Technological purpose

The technological purpose of potassium polyaspartate is as a stabiliser in wine to prevent the growth of potassium bitartrate crystals. Potassium polyaspartate functions as a crystallisation inhibitor by interacting with the surface of potassium bitartrate crystals preventing their growth (Bosso et al, 2015b). The crystals do not present a health and safety risk, but their physical presence does affect wine quality and acceptability as they can appear 'glass like' to consumers. The crystals are more noticeable in white and sparkling wines.

Physical and chemical processes can be used to prevent the growth of and or remove tartrate crystals in wine.

2.3.1 Physical processes

Traditionally, a process known as cold stabilisation can be used to remove tartrate crystals before bottling. Refrigeration is used to reduce the temperature of the wine - potassium bitartrate precipitates and is then removed by filtration. Alternative technologies such as electrodialysis and cation exchangers are also available, but like the use of refrigeration for cold stabilisation, they are costly, especially for smaller wineries.

2.3.2 Chemical processes – use of food additives

Metatartaric acid, cellulose gums and yeast mannoproteins are permitted for use to stabilise wine and prevent tartrate crystallisation but all have disadvantages compared with potassium polyaspartate.

Potassium polyaspartate has significant advantages including being:

- more efficient than other food additives in preventing the growth of potassium bitartrate crystals
- cost effective, especially for smaller winemakers
- suitable for use in all wines – varieties, origin and varying levels of potassium bitartrate instability
- able to be used without effecting the taste or aroma of wines
- easy to solubilise in water and mix in before bottling
- not removed on filtering
- stable during storage
- able to be used without effecting colour stability in red wines
- safe for operators to use

2.4 Technological justification

The addition of up to 100 mg/L potassium polyaspartate to wine prevents the growth of potassium bitartrate crystals.

The International Organisation of Vine and Wine (OIV) recommend treatment of wine with potassium polyaspartate that does not exceed 100 mg/L. This is because there are no further improvements to stabilisation of wine with higher use levels and in some cases this may result in turbidity (OIV, 2017b).

Potassium polyaspartate is relatively easy to solubilise in water and wine. Potassium polyaspartate needs to be prepared in cold water or wine at ten times the weight for use in winemaking. The solution is then mixed into the wine by pumping-over or dosage in-line to ensure uniform distribution in the wine¹. The treated wine can then be filtered, bottled and packaged.

Potassium polyaspartate can stabilise both red and white wines during storage, including those with high levels of tartrate instability (up to 450 micro siemens/centimetre as determined by a mini-contact test²). Potassium polyaspartate is chemically stable in wine and stability can be maintained for at least a year of aging unlike metatartaric acid.

For red and white wines, colour stability is maintained during storage. In red wine, there are some instances of increased turbidity soon after addition due to interactions with proteins and polyphenols. In these situations, it is recommended that bentonite is used for clarification and the wine is filtered before bottling. OIV also recommends this treatment for red wines with high colloidal instability (OIV, 2017b).

2.5 The manufacturing process

Potassium polyaspartate is produced from two starting materials, L-aspartic acid and potassium hydroxide. L-aspartic acid is heated at 270-274°C for up to 90 minutes. Water is added and insoluble polysuccinimide is formed.

Polysuccinimide is treated with potassium hydroxide under controlled conditions, allowing for opening of the ring structure and polymerisation of the units. Throughout this process a 40% solution of potassium polyaspartate at pH 8.3 is obtained. Spray drying is then used to produce a light tan powder with 92-95% dry matter.

2.6 Product specifications

Potassium polyaspartate has an accepted specification in a secondary source, section S3-3(j) in the Code. The relevant specification is the OIV resolution OIV-OENO 572-2017 Monograph on potassium polyaspartate (OIV, 2017a).

The application contains analytical results for a batch sample of potassium polyaspartate. These results are shown in Table 1 and consistent with OIV resolution OIV-OENO 572-2017

¹ The term 'pumping-over' when used in winemaking refers to pumping wine up from the bottom of the tank and splashing it over the wine at the top of the tank. In this example, pumping-over is used as a way to uniformly distribute or mix in a food additive. The term 'dosage in-line' refers to a food additive being added to pipes and uniform distribution occurs as wine moves through pipes.

² A mini-contact test measures the drop in conductivity of 100 mL wine sample after seeding (addition of potassium bitartrate crystals at 10 g/L). The greater the change in conductivity the higher the tartaric instability (Bosso et al, 2015b).

Monograph on potassium polyaspartate (OIV, 2017a).

Table 1 Comparative potassium polyaspartate product specifications

Analysis	Monograph for potassium polyaspartate (OIV, 2017a)	Batch sample
Appearance/description	Light-brown odourless powder	Light brown odourless powder
Average degree of polymerisation	30	34.2
Solubility in water g/L	>1000	>1000
Solubility in organic solvents (xylene, dichloromethane, methanol, acetone, ethyl acetate, n-heptane) g/L	<5	<5
Average molecular mass g/mol	5000	5301
Degree of substitution %	≥91.5	≥91.5
Purity %	≥98	≥98
Free aspartic acid content %	≤2	≤1
Loss on drying	<10	≤6.90
Iron mg/Kg	<10	7.40
Arsenic mg/Kg	<3	0.00
Lead mg/Kg	<2	0.00
Mercury mg/Kg	<1	-
Cadmium mg/Kg	<1	0.00

2.7 Analytical method of detection

The application included information on the analytical method of determining the active substance (aspartic acid) produced during degradation of potassium polyaspartate in red and white wine. Based on relevant guidelines and criteria the applicant was able to conclude that the accuracy of the method was considered acceptable and met validation parameters.

In practice, winemakers may use the “cold test” to determine stability of wine when stored at -4°C for 6 days to allow potassium bitartrate crystallisation. To determine losses of potassium bitartrate as crystals during cold storage, the tartaric acid concentration is analysed before and after cold storage and the difference indicates tartaric instability (Bosso et al, 2015b).

2.8 Product stability

2.8.1 Shelf-life

The application stated that the shelf-life of potassium polyaspartate is 4 years. This was established from accelerated storage test results at ambient temperature (25°C) and 40°C, which did not show significant degradation in dry matter content. Also, storage at ambient temperature showed no significant reduction in potassium polyaspartate content.

2.8.2 Stability in wine

The stability of potassium polyaspartate in wine was assessed by measuring the aspartic acid concentration (as a degradation product of polyaspartate) in wine at 5 and 12 months. White and red wine samples were treated with 100mg/L potassium polyaspartate. There were only small increases in the amount of aspartic acid generated compared to controls (untreated wine). This was consistent with a study where wine treated with potassium polyaspartate showed a low increase in aspartic acid after 12 months (Bosso et al, 2015b).

Further studies on tartaric acid stability showed that the addition of 100 mg/L potassium polyaspartate significantly improved the stability in red and white wine compared to a control of untreated wine and also metatartaric acid, where stability of the wine was maintained only for a few months (Bosso, 2015a). In the red wine study, the amount of aspartic acid generated was close to the impurity content of the commercial potassium polyaspartate commercial, which is consistent with the stabilising effect shown by potassium polyaspartate over 12 months (Bosso, 2015a).

2.9 Food technology conclusions

FSANZ concludes that potassium polyaspartate, when used as a food additive to stabilise wine, is technologically justified in the quantity and form proposed. It appears to be more effective than other permitted food additives in preventing the growth of potassium bitartrate crystals.

Potassium polyaspartate has an acceptable international specification in a secondary source, section S3-3(j) in the Code.

3 Hazard assessment

3.1 Objectives for the hazard assessment

FSANZ has not previously assessed potassium polyaspartate. The objectives of this hazard assessment are to assess any health risks that may arise from the use of this stabiliser in wine. Specifically by considering the following:

- review all of the available data on the toxicology of potassium polyaspartate to determine its safety as a food additive
- if appropriate, establish a health-based guidance value (HBGV) for potassium polyaspartate.

3.1.1 Evaluation of the submitted data

FSANZ has assessed the submitted evidence on the safety of potassium polyaspartate, together with other relevant information identified in a literature review. Submitted studies included information on gastrointestinal digestibility and absorption *in vitro*, 14-day and 90-day toxicity studies in rats and *in vitro* genotoxicity studies. These studies were all conducted with a typical production batch of potassium polyaspartate and are considered suitable to evaluate the hazard of the substance.

3.2 Toxicological data

3.2.1 Toxicokinetics and metabolism

In vitro gastrointestinal digestibility and intestinal absorption studies (Restani 2015) ***Regulatory status: Non-GLP; non-guideline***

Gastrointestinal digestibility

The gastrointestinal digestibility of potassium polyaspartate was assessed *in vitro*, using sequential incubation with gastric and pancreatic enzymes. Aliquots of potassium polyaspartate (Batch no. KHK-S040412-1; also referred to as KHKS-040412; purity 99% w/w on dry matter) were suspended in a 0.06 N HCl solution containing pepsin from porcine gastric mucosa at a pH between 1.27 and 2.8. The final potassium polyaspartate concentration was 3 mg/mL and the enzyme:protein ratio was 1:60 (w/w). Samples were incubated for 5 minutes, 10 minutes or 2 hours at 37°C, and then either further digested with porcine pancreatin or evaluated for proteolysis. For samples that underwent pancreatic digestion, a solution of borate buffer containing pancreatin was added and the pH was adjusted to 6.8. The final pancreatin:potassium polyaspartate ratio was 1:21 (w/w) and samples were incubated for 4 or 24 hours. At the end of the incubation with pepsin or with pepsin followed by pancreatin, samples were heated to 100°C to terminate the enzyme activity. A 'time 0' sample was also prepared by mixing all reagents, substrates and enzymes and then terminating the enzyme activity immediately. Samples were then analysed for undigested protein using the microbiuret method or for the release of free amino acids (i.e. aspartic acid) using the ninhydrin method. All assays were performed in triplicate. Mean values of undigested protein and free amino acids following the various incubation conditions were similar to those of untreated potassium polyaspartate samples (Table 2), indicating that gastric or gastric and pancreatic digestion of potassium polyaspartate *in vitro* is minimal. Slight increases in the mean free aspartic acid content (less than 6%) were observed under some conditions. The authors stated that these differences were not statistically significant, although details of the statistical methods used were not reported.

Table 2 Mean undigested protein and free amino acid content following incubation with pepsin or with pepsin and pancreatin (Restani 2015)

Digestion time	Mean protein content (mg) \pm SD*	Mean free aspartic acid content (mg) \pm SD*
0	24.35 \pm 0.88	0.908 \pm 0.099
5 m pepsin	24.68 \pm 2.30	0.927 \pm 0.112
10 m pepsin	24.98 \pm 0.58	0.946 \pm 0.062
2 h pepsin	23.26 \pm 0.62	0.943 \pm 0.202
2h pepsin; 4h pancreatin	24.09 \pm 1.69	0.855 \pm 0.080
2h pepsin; 24h pancreatin	25.37 \pm 2.70	0.956 \pm 0.080

SD: Standard Deviation

* Mean of three samples

Intestinal absorption

Intestinal absorption of potassium polyaspartate (Batch no. KHK-S040412-1; also referred to as KHKS-040412; purity 99% w/w on dry matter) was investigated *in vitro* using human colon adenocarcinoma Caco-2 cells (Table 3).

Before assessing absorption, the effect of potassium polyaspartate on the integrity of the intestinal membrane barrier was assessed by measuring trans-epithelial electrical resistance (TEER). Caco-2 cells were seeded in Transwell cell culture inserts and cultured for 18-22 days to allow the cells to differentiate into enterocytes and establish a barrier between the apical and basolateral compartments. Non-cytotoxic concentrations of undigested potassium polyaspartate (0.5 and 1 mg/mL; determined on the basis of a prior cell viability assay using MTT) were added to the culture medium and TEER was measured at various time points up to 24 hours. Potassium polyaspartate samples obtained before and after incubation with pepsin for 2 hours and pancreatin for 24 hours, as described for the study on gastrointestinal digestibility, were also evaluated. No effect of undigested or digested potassium polyaspartate on intestinal membrane integrity was observed whereas the positive control, ethanol (20%), significantly reduced membrane integrity.

To assess absorption, samples of potassium polyaspartate before (time 0) and after digestion with pepsin (2 hours) and pancreatin (24 hours) were applied to differentiated Caco-2 cells in the apical compartment of the cell culture inserts. Control samples of peptic digestion and peptic plus pancreatic digestion (without potassium polyaspartate) were also included. Where necessary, the pH of the samples was adjusted to approximately 7 with 0.2 M NaOH to avoid cell toxicity. Absorption through the Caco-2 cell monolayer was measured in triplicate after incubation for 24 hours at 37 °C. Absorption of potassium polyaspartate was assessed by measuring protein/polypeptide concentrations in the apical and basolateral medium with the microbiuret test, as well as by measurement of aspartic acid concentrations using acid hydrolysis (to break down any potassium polyaspartate to aspartic acid) followed by high performance liquid chromatography with fluorescence detection (HPLC-FLD).

Levels of polypeptides and total aspartic acid in the apical and basolateral culture medium samples are shown in

Table 3. Only very low or no polypeptides as measured by the microbiuret test were detected in the basolateral samples, while potassium polyaspartate, measured as aspartic acid, was reported to be below the limit of detection of 0.0007 mg/mL in the basolateral samples, indicating that no significant absorption took place.

Table 3 Quantification of potassium polyaspartate absorption through the Caco-2 cell monolayer (Restani 2015)

Sample	Apical / Basolateral	Absorption measured by microBiuret method (peptides)			Absorption measured as aspartic acid (after acidic hydrolysis)
		Expected concentration (mg/mL)#	Determined concentration (mg/mL; mean ± SD)	Distribution (%; mean ± SD)	Distribution (%; mean ± SD)
Potassium polyaspartate (test solution)	Potassium polyaspartate suspended in medium	1	1.237 ± 0.29	Not reported	Not reported
Time 0	Apical	2.4	2.03 ± 0.63	100.0 ± 31.0	100 ± 13.7
Time 2 h (pepsin) + 24 h (pancreatin)	Apical	2.4	2.06 ± 1.23	101.5 ± 59.7	118 ± 54.8
Time 0	Basolateral	NA	0.004	NA	0*
Time 2 h (pepsin) + 24 h (pancreatin)	Basolateral	NA	0	NA	0*

Concentration added to apical cell

NA: Not Applicable

*0%: analytical values below limit of detection for aspartic acid (0.0007 mg/mL)

3.2.2 Acute toxicity studies in animals

No acute toxicity studies are available with potassium polyaspartate.

Acute toxicity studies with sodium polyaspartate have been conducted on behalf of Bayer Australia Ltd and LANXESS Deutschland GmbH and submitted to NICNAS and the US FDA, respectively. Sodium polyaspartate has the same structure as potassium polyaspartate, except the potassium is replaced by sodium. NICNAS (2001) reported that in an acute oral study in rats (3/sex) conducted in accordance with OECD Test Guideline (TG) 423 (Acute Toxic Class method) no adverse effects were observed at a limit dose of 2000 mg/kg bw/day. LANXESS (2007) also reported an LD₅₀ > 2000 mg/kg bw in rats.

3.2.3 Short-term toxicity studies in animals

The LANXESS submission to the US FDA reports that no adverse effects were observed in a study in which Wistar rats were administered sodium polyaspartate at doses up to 1000 mg/kg bw/day by oral gavage for four weeks, followed by a recovery period of two weeks (LANXESS 2007). The original study report was not provided.

14-day repeated dose oral toxicity study in rats (Gumaste 2014a) Regulatory status: Non-GLP; Protocol based on OECD TG 407

This study was conducted as a dose range finding study to inform the design of a 90 day oral toxicity study. The test article was potassium polyaspartate (Batch no. KHKS-040412; 99% w/w on dry matter) and the vehicle control was water. Groups of five male and five female Wistar rats (aged 8 weeks) were administered potassium polyaspartate by oral gavage for 14

days at doses of 0, 60, 125, 250, 500 and 1000 mg/kg bw/day. Rats were examined daily for signs of toxicity, morbidity and mortality, with detailed clinical examination performed before initiation of the study and weekly thereafter including at termination. Body weight and food consumption were recorded weekly, and at termination blood samples were collected for haematology and clinical chemistry analyses. At termination all rats were subjected to a detailed necropsy and selected organ weights were recorded.

No mortality or treatment-related clinical signs were observed during the course of the study in any dose group. Body weight gain and food consumption were similar in all groups. No treatment-related adverse changes in haematology or clinical chemistry parameters were observed. No gross pathological changes were found in treated rats at the end of the study. The no observed adverse effect level (NOAEL) in this study was 1000 mg/kg bw/day, the highest dose tested.

90-day repeated dose oral toxicity study in rats (Gumaste 2014b) Regulatory status: GLP; Conducted according to OECD TG 408

The test article in this study was potassium polyaspartate (Batch no. KHKS-040412; 99% w/w on dry matter) and the vehicle control was water. The study protocol included a modification to include additional parameters recommended in the OECD Test Guideline for repeated dose 28 day oral toxicity studies in rodents (TG 407). These additional parameters are intended to place more emphasis on endocrine-related endpoints, and include assessment of thyroid hormones, oestrus cycles, and histopathology of tissues that may indicate endocrine activity of test substances.

Groups of ten male and ten female Wistar rats (aged 8 weeks) were administered potassium polyaspartate by oral gavage for 90 days at doses of 0, 250, 500 and 1000 mg/kg bw/day. Additional recovery groups (5/sex/group) of rats were administered the vehicle or the high dose for 90 days and then observed for a further 28 days following the end of treatment. Rats were examined daily for signs of toxicity, morbidity and mortality, with detailed clinical examinations before the start of the study and weekly thereafter during the treatment period, recovery period and at termination. Ophthalmoscopy examinations were conducted on control and high dose group animals prior to initiation of the study and at termination of treatment. Body weight and food consumption were recorded weekly. In the final week of treatment all animals underwent a neurological examination in which they were assessed for sensory reactivity, grip strength and motor activity, including a functional observational battery. Blood samples were collected from main study animals at the end of the treatment period (Day 91) and from recovery group animals at the end of the recovery period (Day 119). Urine samples were collected from animals a few days before the end of treatment (Day 86) and just before the end of the recovery period (Days 116 and 117). At termination of treatment and at the end of the recovery period the oestrus cycle stage for all females was determined by taking vaginal smears. All animals were subjected to a detailed necropsy on study termination, and histopathological evaluation was performed on all rats from the main study control and high dose groups.

All animals survived to the end of the study and no clinical signs of toxicity were observed. Food consumption and body weight gain were not affected by treatment and body weight gain in all treated groups was similar to that of control rats throughout the treatment period and the recovery period. Ophthalmological examination did not find any treatment-related ocular abnormalities. The functional observations conducted in the thirteenth week of the study did not show any indications of neurotoxicity. No treatment-related changes in haematology, clinical chemistry and urinalysis parameters were observed. Females from all control and treated groups exhibited a normal pattern of oestrus cycling. No significant alterations in absolute and relative organ weights were observed, and no treatment-related gross pathological changes were identified. Histopathological examination of tissues from the

control and high dose group animals found no adverse changes in the high dose group, and in the absence of any histological findings the investigations were not extended to the lower dose groups or the recovery groups.

The NOAEL of potassium polyaspartate in this study was 1000 mg/kg bw/day, the highest dose tested.

3.2.4 Chronic toxicity and carcinogenicity

No chronic toxicity or carcinogenicity studies of potassium polyaspartate were submitted in the application or located from other sources. Such studies are not considered to be necessary because negligible gastrointestinal digestion and absorption was found *in vitro*, the results of genotoxicity assays are negative and there is no evidence from subchronic studies of lesions that could lead to neoplasia through non-genotoxic mechanisms.

3.2.5 Genotoxicity

In vitro bacterial reverse mutation assay (Mane 2014a) Regulatory status: GLP; Conducted according to OECD TG 471

Potassium polyaspartate (Batch no. KHKS-040412; 99% w/w on dry matter) was evaluated for mutagenicity in *Salmonella typhimurium* tester strains TA1535, TA97a, TA98, TA100 and TA102, using the pre-incubation method. The vehicle and negative control was water. Based on findings of a dose range finding study, concentrations ranging from 50 – 5000 µg/plate were used in the test, which was performed in the presence and absence of metabolic activation (S9 mix). Positive control groups were also included. Triplicate plating was performed at each dose level, and the entire study was conducted twice to confirm the reproducibility of the results.

No increase in the number of revertant colonies was observed at any concentration of potassium polyaspartate compared with the vehicle controls, in the presence or absence of metabolic activation. Revertant counts were within the historical negative control range observed at the test facility. The concurrent positive controls all produced the expected increases in the number of revertant colonies, confirming the validity of the test system. It was concluded that potassium polyaspartate was not mutagenic in *S. typhimurium* strains TA1535, TA97a, TA98, TA100 and TA102.

In vitro micronucleus assay in human lymphocytes (Mane 2014b) Regulatory status: GLP; Conducted according to OECD TG 487

The test material for this study was potassium polyaspartate (Batch no. KHKS-040412; 99% w/w on dry matter) and the vehicle was water. Based on preliminary solubility/precipitation and cytotoxicity studies, duplicate cultures of human peripheral blood lymphocytes were exposed to potassium polyaspartate at concentrations of 500, 1500 and 5000 µg/mL. Three experiments were conducted. In experiments 1 and 2, proliferating cells were exposed to the test article in the absence of a metabolic activation system for 3 hours and 20 hours, respectively. In experiment 3, cells were exposed to the test article in the presence of metabolic activation (S9 mix) for 3 hours. In all three experiments, cells were exposed to the test article 48 hours after stimulation of mitosis by the addition of phytohaemagglutinin-M. Cytochalasin B was added to the cultures at 68 hours after the start of the culture, and cells were harvested 96 hours after culture initiation. In addition to the test article and the vehicle control, negative and positive controls were also tested. The negative control was sodium chloride (0.9% w/v), while the positive controls for the experiments without metabolic activation were mitomycin C (directly acting clastogen) and vinblastine (directly acting aneugen). In the experiment conducted with metabolic activation the positive control was

cyclophosphamide (indirectly acting clastogen). For each exposure group, 2000 binucleated cells were evaluated microscopically for the presence of micronuclei.

Potassium polyaspartate did not induce cytotoxicity at concentrations up to 5000 µg/mL. No significant increases in the number of binucleated cells with micronuclei were observed in cultures treated with potassium polyaspartate compared with the vehicle and negative controls. The positive controls induced significant increases in the frequencies of micronucleated binucleated cells, confirming the validity of the test system. It was concluded that potassium polyaspartate was not clastogenic or aneugenic in human peripheral blood lymphocytes.

3.2.6 Developmental and reproductive toxicity

No reproductive or developmental toxicity studies of potassium polyaspartate were submitted in the application or located from other sources. Such studies are not considered to be necessary based on the *in vitro* findings indicating that gastrointestinal digestion and absorption of potassium polyaspartate is likely to be minimal, the absence of genotoxicity and no evidence of adverse effects on reproductive tissues or the oestrus cycle in the 90 day oral toxicity study.

3.2.7 Special studies

Immunotoxicity in vitro (Restani 2015) Regulatory status: Non-GLP; Non-guideline

The potential stimulation of immune cells was assessed *in vitro* using the human monocytic cell line THP-1. After an initial investigation of cytotoxicity, THP-1 cells were incubated for 24 hours in the presence or absence of potassium polyaspartate at 2 mg/mL. Expression of CD86 and release of the cytokine interleukin-8 (IL-8) were measured using flow cytometry and ELISA methods, respectively. Lipopolysaccharide from *Escherichia coli* serotype 0127:B8 (LPS) was used as a positive control.

Potassium polyaspartate did not induce up-regulation of CD86 expression or release of IL-8. In contrast, the positive control LPS induced a significant increase in both CD86 expression and IL-8 release. The authors concluded that potassium polyaspartate did not induce any activation of immune cells under the conditions of this study.

3.3 Assessments by other agencies

The European Food Safety Authority (EFSA) reviewed the safety of potassium polyaspartate as a food additive and published its Opinion in March 2016 (EFSA, 2016).

EFSA concluded that the available data fulfilled European requirements for evaluation of a new food additive and did not request additional testing for chronic toxicity and carcinogenicity or reproductive and developmental toxicity. Maximum estimated dietary exposure estimates to potassium polyaspartate from its proposed use were calculated to be 1.8 mg/kg bw/day in the elderly and 1.4 mg/kg bw/day in adults, resulting in a margin of safety of approximately 550 in comparison with the NOAEL of 1000 mg/kg bw/day identified in the 90-day oral toxicity study. It was concluded that there was no safety concern from the proposed use of potassium polyaspartate at levels from 100-200 mg/L and at a maximum limit of 300mg/L.

Potassium polyaspartate has now been approved by the European Commission as a food additive for tartaric stabilisation in wine at a maximum concentration of 100 mg/L (European Commission, 2017).

Potassium polyaspartate as a stabiliser in wine at a maximum use level of 100 mg/L was added to the priority list of substances for evaluation by JECFA at CCFA's 50th session in 2018 (Codex, 2018).

JECFA has evaluated aspartic acid as a flavouring agent (WHO 2006). The Committee concluded that there was no safety concern at current levels of intake when used as a flavouring agent. Aspartic acid was not evaluated using JECFA's Procedure for the Safety Evaluation of Flavouring Agents, as it is a macronutrient and a normal component of protein. As such, human exposure through food is orders of magnitude higher than the anticipated level of exposure from use as a flavouring agent.

Sodium polyaspartate has been assessed by NICNAS (2001) with respect to use as an ingredient in liquid formulations used within the water treatment industry. The products were to be used within cooling and heating systems where they act as scale inhibitors. This evaluation noted that sodium polyaspartate is of very low acute toxicity and mutagenicity is not expected to occur. It was concluded that releases from industrial use are unlikely to pose a significant hazard to human health.

4 Dietary exposure assessment

4.1 Objective for the dietary exposure assessment

The objective of this dietary exposure assessment is to estimate the dietary exposure to potassium polyaspartate when used in class 14.2.2 Wine, sparkling wine and fortified wine at a proposed maximum permitted level of 100 mg/L.

4.2 Methodology and approach for dietary exposure assessment

The general FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009). A summary of the general FSANZ approach to conducting the dietary exposure assessment for this application is at Appendix 1.

Dietary exposure assessments require data on the concentration of the chemical of interest in the food requested and consumption data for the foods that have been collected through a national nutrition survey. The dietary exposure assessment was undertaken using FSANZ's dietary modelling computer program Harvest³. The Harvest model used to assess dietary exposure was a food additive model as it groups consumption of foods, and allows concentrations to be assigned, according to the food categories in Schedule 15 of the Code. As food additive permissions in the Code apply to both Australia and New Zealand, dietary exposure assessments were undertaken for both countries.

4.3 Food consumption data used

The food consumption data used for the dietary exposure assessments were:

- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS): a 24-hour recall survey of 3,275 New Zealand children aged 5-14 years, with a second 24-hour

³ Harvest is FSANZ's custom-built platform to calculate dietary exposures.

recall undertaken for 15% of respondents. The assessment only used data from Day 1 of the survey.

- 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS): a 24-hour recall survey of 4,721 New Zealanders aged 15 years and above, with a second 24-hour recall undertaken for 25% of respondents. The assessment only used data from Day 1 of the survey.
- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), a component of the 2011-13 Australian Health Survey (2011-13 AHS): a 24-hour recall survey of 12,153 Australians aged 2 years and above, with a second 24-hour recall undertaken for 64% of respondents. Only those respondents who had two days of food consumption data (n=7,735) were used in the assessment of dietary exposure (ABS, 2015). Data from the two days are averaged in order to better estimate longer term or chronic estimates of food consumption or dietary exposure.

Dietary exposure assessments based on food consumption data from national nutrition surveys provide the best estimation of actual consumption of a food and the resulting estimated dietary exposure assessment for the Australian and New Zealand populations. However, national nutrition survey data have some limitations. The design of these nutrition surveys vary and the key attributes of each are set out in Appendix 1.

The hazard assessment did not identify any target or at-risk groups for which there were specific safety considerations in relation to exposure to potassium polyaspartate. In addition, the food category requested in the application for addition of potassium polyaspartate is consumed by all age groups of the Australian and New Zealand populations (for example, in cooked dishes such as casseroles). Therefore the dietary exposure assessments were conducted for the general Australian and New Zealand populations based on the dietary survey data available. For Australia, the population group used for the dietary exposure assessment was the population aged 2 years and above. For New Zealand the population groups were children (aged 5-14 years) and adults (aged 15 years and above).

4.4 Assumptions and limitations of the dietary exposure assessment

The aim of the dietary exposure assessment was to make the best estimate of dietary potassium polyaspartate exposure. Where significant uncertainties in the data exist, FSANZ uses conservative assumptions to ensure that the estimated dietary exposure is not an underestimate.

Assumptions made in the dietary exposure assessment included:

- All wine included in category 14.2.2 Wine, sparkling wine and fortified wine contained potassium polyaspartate at the proposed maximum permitted level of 100 mg/L.
- 1 mL of wine, sparkling wine and fortified wine = 1 g.
- As wine is used in cooking (e.g. casseroles) as well as consumed as a beverage, the dietary exposure assessment includes wine, sparkling wine and fortified wine used in recipes.

In addition to the specific assumptions made in relation to this dietary exposure assessment, there are a number of limitations associated with the nutrition surveys per se. A discussion of these limitations is included in Section 6 of the *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ, 2009).

4.5 Dietary exposure assessment results

The estimated mean and 90th percentile consumption of wine, sparkling wine and fortified wine for consumers range from 13 g/day to 265 g/day and from 28 g/day to 594 g/day respectively across the population groups assessed (refer to Figure 2. Table 4 for detailed results).

The estimated dietary exposures to potassium polyaspartate were calculated for ‘consumers’ of potassium polyaspartate and are reported for mean and 90th percentile exposures in two ways:

- in milligrams of potassium polyaspartate per day, derived from each individual’s ranked daily exposures
- in milligrams of potassium polyaspartate derived on a per kilogram body weight basis using each individual’s body weight.

The estimated mean and 90th percentile exposure to potassium polyaspartate for consumers range from 1 mg/day to 26 mg/day and from 3 mg/day to 59 mg/day respectively across the population groups assessed. When expressed on a kilogram body weight basis, the estimated mean and 90th percentile exposures range from 0.031 mg/kg body weight/day to 0.35 mg/kg body weight/day and from 0.072 mg/kg body weight/day to 0.79 mg/kg body weight/day respectively (refer to Figure 2.

Table 4 for detailed results). The estimated mean and 90th percentile daily exposures to potassium polyaspartate on a per kilogram body weight basis are summarised in Figure 2.

Table 4 Estimated mean and P90 wine, sparkling wine and fortified wine consumption and potassium polyaspartate dietary exposures for Australia and New Zealand consumers only

Country	Age group	No. of resp.	Proportion consumers to respondents	Estimated consumption of wine, sparkling wine and fortified wine (g/consumer/day)		Estimated dietary exposure to potassium polyaspartate (mg/day)		Estimated dietary exposure to potassium polyaspartate (mg/kg body weight/day)	
				Mean	P90	Mean	P90	Mean	P90
Australia*	2 years and above	7,735	20	265	594	26	59	0.35	0.79
New Zealand[∇]	5-14 years	3,275	3	13	28	1	3	0.031	0.072
	15 years and above	4,721	18	249	537	25	54	0.34	0.76

* 2011-12 Australian National Nutrition and Physical Activity Survey. Based on consumption data from respondents with two days of data only.

[∇] 2002 New Zealand National Children’s Nutrition Survey and the 2008–09 New Zealand Adult Nutrition Survey. Based on day 1 consumption data only from all respondents.

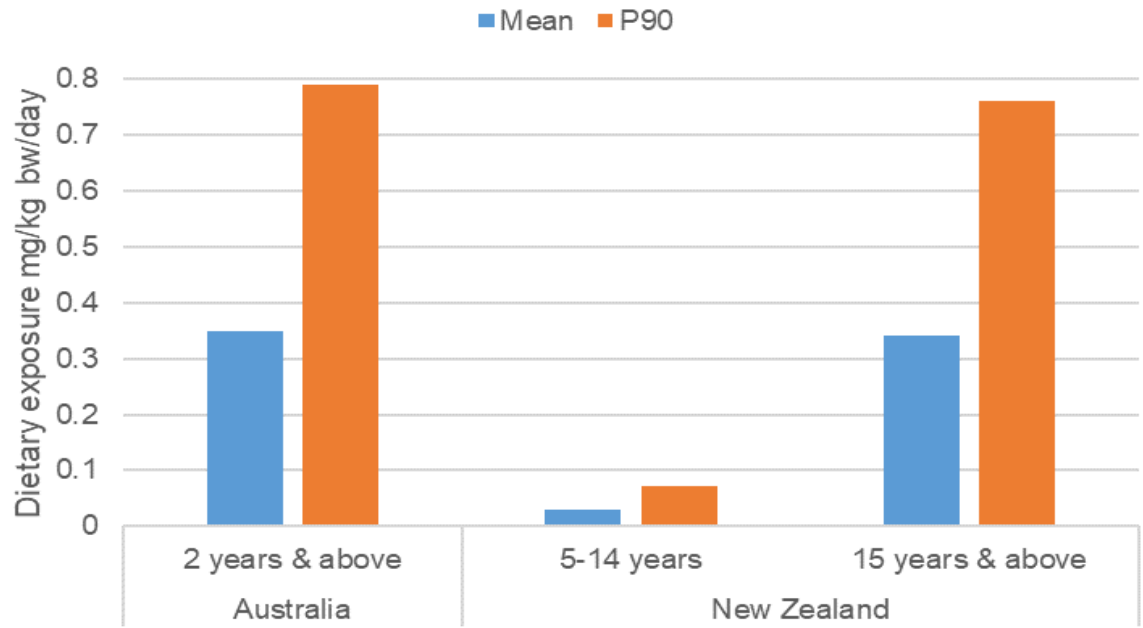


Figure 2 Estimated mean and 90th percentile (P90) daily dietary exposures (mg/kg body weight/day) to potassium polyaspartate for Australia and New Zealand consumers only

5 Hazard and dietary exposure assessment discussion

The submitted toxicological data were considered adequate to determine the hazard of potassium polyaspartate. Potassium polyaspartate is the potassium salt of polyaspartic acid, produced from L-aspartic acid and potassium hydroxide. The applicant's proposed specifications are for a minimum purity of 98.0% on a dry matter basis.

Minimal proteolytic digestion of potassium polyaspartate was observed *in vitro*, and an *in vitro* study found no evidence of absorption across a human intestinal cell monolayer.

The amount of free aspartic acid released in the *in vitro* intestinal digestion study was less than 6%. Aspartic acid is a macronutrient and normal component of protein such that dietary intakes from the use of polyaspartate (0.10 mg/kg bw/day⁴) will be negligible compared to the intake in the normal diet (highest median and 99th percentile estimates of usual daily intake in the USA are 9.2 g/day and 15.4 g/day, respectively, equivalent to 131 and 220 mg/kg bw/day, respectively for a 70 kg adult [Institute of Medicine 2005]).

Potassium polyaspartate was not mutagenic, clastogenic or aneugenic *in vitro*. No adverse effects were observed in 14-day and 90-day repeated dose oral toxicity studies in rats at doses up to 1000 mg/kg bw/day, the highest dose tested.

No chronic toxicity/carcinogenicity studies of potassium polyaspartate were submitted. Such studies are not considered necessary based on *in vitro* evidence indicating gastrointestinal digestion and absorption is likely to be minimal, the absence of genotoxicity and no evidence from subchronic studies of lesions that could lead to neoplasia through non-genotoxic mechanisms.

No reproductive or developmental toxicity studies of potassium polyaspartate were identified. Based on the likely negligible digestion and absorption of potassium polyaspartate, the lack of genotoxicity and no evidence of adverse effects on reproductive tissues or the oestrus cycle in the 90-day toxicity study, such studies are not considered necessary.

The NOAEL in the 90-day repeated dose oral toxicity study in rats (1000 mg/kg bw/day) is more than 1200-fold higher than the highest 90th percentile exposure to potassium polyaspartate in the dietary exposure assessment (0.79 mg/kg bw/day).

Based on the reviewed data, it is concluded that in the absence of any identifiable hazard, an Acceptable Daily Intake (ADI) 'not specified' is appropriate for potassium polyaspartate.

5.1 Hazard and dietary assessment conclusions

Based on the available toxicological evidence and the dietary exposure assessment there are no public health and safety concerns from the use of potassium polyaspartate as a food additive in wine at the proposed levels.

⁴ Based on dietary exposures to potassium polyaspartate estimated by FSANZ from additive uses in wine only, and assuming 100% breakdown, and converting potassium polyaspartate to aspartic acid based on the ratio of molecular weights (1100 g/mol for potassium polyaspartate [number average molecular weight used as the most conservative value], 133.1 g/mol for aspartic acid).

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Appendix 1 Dietary exposure assessments at FSANZ

A dietary exposure assessment is the process of estimating how much of a food chemical a population, or population sub group, consumes. Dietary exposure to food chemicals is estimated by combining food consumption data with food chemical concentration data. The process of doing this is called 'dietary modelling'.

Dietary exposure = food chemical concentration x food consumption

FSANZ's approach to dietary modelling is based on internationally accepted procedures for estimating dietary exposure to food chemicals. Different dietary modelling approaches may be used depending on the assessment, the type of food chemical, the data available and the risk assessment questions to be answered. In the majority of assessments, FSANZ uses the food consumption data from each person in the national nutrition surveys to estimate their individual dietary exposure. Population summary statistics such as the mean exposure or a high percentile exposure are derived from the ranked individual person's exposures from the nutrition survey.

An overview of how dietary exposure assessments are conducted and their place in the FSANZ Risk Analysis Process is provided on the FSANZ website at:

<http://www.foodstandards.gov.au/science/riskanalysis/Pages/default.aspx>

FSANZ has developed a custom-built computer program 'Harvest' to calculate dietary exposures. Harvest replaces the program 'DIAMOND' that had been used by FSANZ for many years. Harvest has been designed to replicate the calculations that occurred within DIAMOND using a different software package.

Further detailed information on conducting dietary exposure assessments at FSANZ is provided in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009), available at:

[http://www.foodstandards.gov.au/science/exposure/documents/Principles%20 %20practices %20exposure%20assessment%202009.pdf](http://www.foodstandards.gov.au/science/exposure/documents/Principles%20%20practices%20exposure%20assessment%202009.pdf)

1.1 Food consumption data used

The most recent food consumption data available were used to estimate exposures to potassium polyaspartate for the Australian and New Zealand populations. The national nutrition survey (NNS) data used for these assessments were:

- The 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)
- The 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS)
- The 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS).

The design of each of these surveys varies somewhat and key attributes of each are set out below. Further information on the National Nutrition Surveys used to conduct dietary exposure assessments is available on the FSANZ website at:

<http://www.foodstandards.gov.au/science/exposure/Pages/dietaryexposureandin4438.aspx>

1.1.1 2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)

The 2011–12 Australian National Nutrition and Physical Activity Survey (NNPAS) undertaken by the Australian Bureau of Statistics is the most recent food consumption data for Australia. This survey includes dietary patterns of a sample of 12,153 Australians aged 2 years and above. The survey used a 24-hour recall method for all respondents, with 64% of respondents also completing a second 24-hour recall on a second, non-consecutive day. The collection dates of the data were May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census). Only those respondents who had two days of food consumption data were used to estimate potassium polyaspartate exposures. Consumption and respondent data from the *Confidentialised Unit Record Files* (CURF) data set (ABS, 2015) form part of the Harvest core data set. These data were used weighted in Harvest.

1.1.2 2002 New Zealand National Children’s Nutrition Survey (2002 NZ CNS)

The 2002 NZ CNS was a cross-sectional and nationally representative survey of 3,275 New Zealand children aged 5-14 years. The collection period for the data was during the school year from February to December 2002. The survey used a 24-hour food recall and provided information on food and nutrient intakes, eating patterns, frequently eaten foods, physical activity patterns, dental health, anthropometric measures and nutrition-related clinical measures. It was also the first children’s nutrition survey in New Zealand to include a second day diet recall data for about 15% of the respondents, and dietary intake from both foods (including beverages) and dietary supplements. Only the Day 1 24-hour recall data for all respondents (excluding supplements) were used for this assessment. These data are used weighted in Harvest.

1.1.3 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS)

The 2008 NZ ANS provides comprehensive information on the dietary patterns of a sample of 4,721 respondents aged 15 years and above. Collection of data for the survey occurred on a stratified sample over a 12-month period between October 2008-October 2009. The survey used a 24-hour recall methodology with 25% of respondents also completing a second 24-hour recall. The information collected in the 2008 NZ ANS included food and nutrient intakes, dietary supplement use, socio-demographics, nutrition related health, and anthropometric measures. Only the Day 1 24-hour recall data for all respondents (excluding supplements) were used for this assessment. These data are used weighted in Harvest.