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Research report

Research on the safety evaluation of existing additives

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Research report

Research on the safety evaluation of existing additives

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A. Summary

When the Food Sanitation Act (Act No. 233 of 1947) was revised in 1995, approvement was granted to continue the use, etc. of natural additives listed in the list of existing additives (Notification No. 120 of the Ministry of Health and Welfare in 1996), and it was also stated to review their safety. Regarding these existing additives, the report of the FY 1996 Health and Welfare Science Grant Research, "Research on the safety evaluation of existing natural additives" (Senior Researcher: Hayashi Yuzo; hereinafter, "Hayashi Group Report"), includes the results of investigation regarding the basic safety of existing additives conducted based on international evaluation results, approval status in Europe and the United States, safety study results, etc. As a result, the additives were classified into: (i) "additives that require future investigation of their safety, including the implementation of new toxicity studies;" and (ii) "additives that require no immediate investigation of safety based on their origin, method of preparation, and definition." This research was aimed to evaluate the safety of 38 additives for which overseas evaluation results have been obtained as of 2017 among the 109 additives classified into the latter.

Among 38 existing additives evaluated as part of this project, 29 additives have been evaluated in overseas assessment reports as those without an allocated acceptable daily intake (ADI), Generally Recognized As Safe (GRAS), or intake not limited, and they were evaluated as having no safety concerns. Four additives had an allocated ADI and it was considered that there are no safety concerns with the current use. Concerning the other 5 additives, it was considered that there are no safety concerns with their use as food additives based on the results of acute toxicity studies, repeated-dose toxicity studies and mutagenicity studies, as well as the actual state of use.

Although some of the existing additives evaluated in this project and distributed in Japan are different in origin and method of preparation from those evaluated in overseas assessment reports, it was considered that there are no safety concerns as long as they are used as food additives.

B. Objective

When the Food Sanitation Act (Act No. 233 of 1947) was revised in 1995, approvement was granted to continue the use, etc. of natural additives listed in the list of existing additives (Notification No. 120 of the Ministry of Health and Welfare in 1996), and it was also stated to review their safety. Regarding these existing additives, the FY 1996 Health and Welfare Science Grant Research, "Research on the safety evaluation of existing natural additives" (Senior Researcher: Hayashi Yuzo; hereinafter, "Hayashi Group Report"), includes the results of investigation regarding the basic safety of existing additives conducted based on international evaluation results, approval status in Europe and the United States, safety study results, etc. As a result, the additives were classified into: (i) "additives that require future investigation of their safety, including the implementation of new toxicity studies;" and (ii) "additives that require no immediate investigation of safety based on their origin, method of preparation, and definition." This research is aimed to evaluate (discuss) the safety of 38 additives for which overseas evaluation results have been obtained as of 2017 among the 109 additives classified into the latter.

C. Methods

Concerning 38 additives for which overseas assessment reports were available among the 109 existing additives that were classified as (ii) "additives that require no immediate investigation of safety based on their origin, method of preparation, and definition," acute toxicity studies, repeated-dose toxicity studies, mutagenicity studies and other toxicity studies were summarized, and their position in overseas assessment reports was evaluated. The additives that are subject to evaluation are listed below.

α-Acetolactate decarboxylase
Isoamylase
Invertase
Exomaltotetraohydrolase
Esterase
Cassia gum
Carboxypeptidase
Xylanase
Chitosan

Cristobalite
Glucosamine
α-Glucosidase
α-Glucosyltransferase
Glutaminase
Diatomaceous earth
Yeast cell wall
Vegetable sterol
Hydrogen
Powdered stevia
Crude potassium chloride (sea water)
Crude magnesium chloride (sea water)
Taurine (extract)
Theobromine
Copper
d-γ-Tocopherol
d-δ-Tocopherol
Transglucosidase
Trehalose
Peroxidase
Phytase
Phosphodiesterase
Phospholipase
Polyphenol oxidase
Muramidase
Charcoal
Lactoperoxidase
Lactoferrin concentrates
D-Ribose

D. Results

Additives of which the principal components are monosaccharides or polysaccharides are evaluated as ADI not specified (Cassia gum and Trehalose) or GRAS (Chitosan, Yeast cell wall and D-Ribose). Glucosamine, which is used as a thickening stabilizer, is considered to have no safety concerns, because it showed LD50

> 15,000 mg/kg body weight in acute toxicity studies, NOAEL of 2,130 mg/kg body weight/day in a 52-week repeated-dose toxicity study, and was negative in mutagenicity studies. The EFSA evaluates glucosamine hydrochloride from *Aspergillus oryzae* as safe.

Concerning Vegetable sterol, which is similar to animal sterol, the JECFA had established the group ADI as 40 mg/kg body weight/day, and there are no safety concerns with the current use.

Concerning *d*-γ-Tocopherol and *d*-δ-Tocopherol, which are classified as vitamin E, the JECFA had established a group ADI of 0.15-2 mg/kg body weight/day based on the data of *dl*- and *d*-α-tocopherol. The EFSA cannot establish the ADI because available toxicity data is limited. However, vitamin E is a necessary nutrient that is commonly taken as food, and it is considered that there are no safety concerns with its current use as a food additive and the concentration at which tocopherols is used.

Theobromine, which is a xanthine-derived alkaloid, is an additive similar to caffeine, and the EFSA evaluated that there are no safety concerns with the use of Theobromine as a flavoring agent. There are no safety concerns about theobromine considering the current situation of distribution and intake, because there are very limited use results of theobromine in Japan.

The principal component of Cristobalite and Diatomaceous earth, which are classified as minerals, is Silicon Dioxide. The JECFA evaluates cristobalite as a kind of silicate containing silicon dioxide and calcium silicate, and states that the ADI is not specified. The FDA evaluates diatomaceous earth as GRAS.

The FDA considers Hydrogen, which is an element, as GRAS. Charcoal is similar to activated carbon and is used similar description, and the JECFA states that the ADI is not limited.

The scope of this project includes 17 enzymes that are mainly used as food manufacturing agents. Among these enzymes, 7 additives (α-Acetolactate decarboxylase, Exomaltotetraohydrolase, Xylanase, α-Glucosyltransferase, Glutaminase, Phospholipase and Lactoperoxidase) are evaluated as without an allocated ADI, and 8 additives (Isoamylase, Esterase, Carboxypeptidase, α-Glucosidase, Transglucosidase, Peroxidase, Phosphodiesterase and Polyphenol oxidase) are evaluated as GRAS or without an allocated ADI. For Phytase, the use of Phytase derived from *Aspergillus niger* as a food grade enzyme is allowed in the Australian Food Standards Code. The JECFA considered that allergic reactions to Muramidase (lysozyme), which is formed from egg white, are weaker than those to other proteins such as egg white albumin and albumin in animals and humans, and

concludes based on the available data that there are no concerns that the additional small amount of intake from cheese is harmful to consumer health. Lactoferrin concentrate, which is a component of formula for special use, is evaluated as GRAS, and the FDA responds that there are no concerns.

It states that there are no safety concerns for taurine at the current levels of intake because the estimated intake of Taurine (extract) is below the acceptable intake of structure class I.

Concerning Powdered stevia, which is used as a sweetener, the JECFA evaluated steviol glycoside (stevioside), and judged that the ADI of stevioside is 0-4 mg/kg body weight/day.

Crude potassium chloride (sea water) and Crude magnesium chloride (sea water) manufactured from sea water are evaluated by the JECFA as “not limited” because it shows no toxic effect when used as a food additive.

Although Copper has been evaluated as metal, there is little information on the safety of copper as a simple substance, and it is evaluated as a compound. The JECFA does not establish the ADI for Copper, while the NOEL was evaluated to be about 5 mg/kg body weight/day in a 1-year repeated-dose study in dogs, and the temporary maximum tolerable daily intake (MTDI) is evaluated to be 0.05-0.5 mg/kg based on this. Copper gluconate is used as a nutrient, and the FDA evaluates it as GRAS. The acceptable upper limits (UL) for copper gluconate is established as 9 mg/person/day in Japan. Although the upper limits (UL) for copper gluconate have been established at 9 mg/person/day in Japan, special consideration should be given to patients with inborn errors of copper metabolism (Wilson's disease) and infants to children, and appropriate precautions should be taken to prevent overdose.

E. Discussion

Among the 38 existing additives evaluated in this project, 29 additives have been evaluated in overseas assessment reports as without an allocated ADI, as GRAS or as intake not limited, and the ADI was established for 4 additives, and it is considered that there are no safety concerns for all of these with the current use. For 5 additives for which no safety assessment conclusions have been made in terms of intake, the following considerations were made based on information on toxicity studies, quality, distribution amounts, and estimated intakes.

For Glucosamine, it was considered that there are no safety concerns with its use as a food additive based on the results of acute toxicity studies, repeated-dose toxicity

studies and mutagenicity studies.

Phytase and Muramidase are enzymes and are not considered to affect human health in general as long as they are properly manufactured at food grade.

As for Theobromine, there is no evidence of high intake in Japan, and there is no concern about its safety as a food additive in terms of exposure.

Taurine (extract) belongs to “amino acids and related substances,” and it is considered that there are no safety concerns for Taurine (extract) at the current levels of intake based on the estimated intake.

Some additives that are distributed in Japan are different in origin and method of preparation from those evaluated in overseas assessment reports, and it was considered that there are no safety concerns when they are used as food additives.

F. Conclusion

The safety of 38 additives for which overseas assessment reports were available among 109 existing additives that are classified as (ii) “additives that require no immediate investigation of safety based on their origin, method of preparation, and definition,” in the FY 1996 Health and Welfare Science Grant Research Report, the “Hayashi Group Report,” was evaluated. The additives evaluated in this project include enzymes, monosaccharides, polysaccharides, minerals, elements, salts, etc. Although some of those additives evaluated in the overseas assessment reports are different from those distributed in Japan in terms of their origin and methods of preparation, there is no concern about their safety as long as they are used as food additives by judging comprehensively from the information in the overseas assessment report and the information on toxicity tests.

Annex

α-Acetolactate decarboxylase

English name: α-Acetolactate decarboxylase
CAS No. 9025-02-9
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

α-Acetolactate decarboxylase is an enzyme that is obtained from the culture of bacteria (only *Bacillus licheniformis, Bacillus subtilis*, and genus *Serratia*), and it removes the carboxy group from α-acetolactate. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

It is used as a processing aid in the fermentation and alcohol industry to avoid the formation of α-diacetyl, which has an unpleasant taste, from α-acetolactate during fermentation.

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

(i) A 13-week repeated-dose test was performed in CD rats (20 males and females each per group) by dietary administration with non-stable ALDC (92.9% TOS) or glutaraldehyde-stable d-ALDC (92.8% TOS) of α-Acetolactate decarboxylase respectively. Toxic effects attributable to the test substance were not observed, but a slight increase in platelet count was observed in males in the 500 mg/kg group.[1)]

(ii) Sprague-Dawley rats (10 males and females each per group) received 90-day

dietary administration with α-acetolactate decarboxylase, and the NOAEL was considered to be 1,018 mg TOS/kg BW/day, which was the highest dose.[2)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in mammalian cells, a micronucleus test, and a gene mutation test were performed, and all the results were reported to be negative.

Ames test: Negative; TA100, TA1535, TA98, TA1537 30-10,000 μg/plate (with and without metabolic activation)[3)]
Ames test: Negative; TA100, TA1535, WP2*uvr*A (pKM101), TA98, TA1537 156-5,000 μg/plate (with and without metabolic activation)[2)]
Chromosomal aberration test in mammalian cells: Negative; Human lymphocytes, 44-5,000 μg/mL (with and without metabolic activation) (20-hour and 44-hour treatment)[3)]
Micronucleus test in mammalian cells: Negative; Human lymphocytes, 3,000-5,000 μg/mL (with and without metabolic activation) (3-hour treatment), 100-3,000 μg/mL (without metabolic activation) (24-hour treatment)[2)]
Gene mutation test in mammalian cells (HGPRT gene): Negative; L5178Y cells, 1.58-5,000 μg/mL (with and without metabolic activation) (2-hour treatment)[3)]

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA performed toxicity studies with the two kinds of α-acetolactate decarboxylase shown below:

(i) α-Acetolactate decarboxylase is obtained by submerged fermentation of a bacterium (*Bacillus subtilis*) carrying the gene cording for α-acetolactate decarboxylase from *Bacillus brevis.*[4)]
(ii) α-Acetolactate decarboxylase is produced with a genetically modified *Bacillus licheniformis* strain NZYM-JB strain.[2)]

The results showed that α-Acetolactate decarboxylase is an enzyme with little toxicity and that there is no need to perform additional toxicological studies, and the provisional ADI is not specified).[3), 5)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Broadmeadow, A. (1990) ALDC: Toxicity study by dietary administration to CD rats for 13-weeks. Unpublished report No.90/0691 from Life Science Research Ltd (Submitted to WHO by Novo NordiskA/S, Denmark).
(Reference 1 is cited in Reference 3)

2) EFSA (European Food Safety Authority), 2018.Safety evaluation of the food enzyme acetolactate decarboxylase from a genetically modified Bacillus licheniformis (strain NZYM-JB), EFSA Journal · November 2018

3) JECFA: Safety evaluation of certain food additives (1998), WHO FOOD ADDITIVES SERIES 40

4) JECFA: Safety evaluation of certain food additives and contaminants (1999), WHO Technical Report Series 884

5) JECFA: Evaluation of certain food additives (2000), WHO Technical Report Series 891

Isoamylase

English name: Isoamylase
CAS No. 9067-73-6
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Isoamylase is an enzyme that is obtained from the culture of bacteria (only genus *Bacillus*, *Flavobacterium odoratum, Naxibacter* sp., and *Pseudomonas amyloderamosa*), and it hydrolyzes α-1,6-glucoside bonds in starch and related polysaccharides. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

It is used in the preparation of food derived from starch (glucose syrup, maltose/maltitol, trehalose, cyclodextrin, and resistant starch).

3. Summary of safety studies

1) Acute toxicity study

Mouse (ddy-N) oral LD_{50} > 17,000 mg/kg BW[1)]

2) Repeated-dose toxicity study

Wistar rats (20 males and females each per group) received 13-week treatment with isoamylase by gavage. Toxic effects attributable to the test substance were not observed, and the NOEL was considered to be 370 mg TOS/kg BW/day, which was the highest dose.[2)]

3) Mutagenicity study

An Ames test and a chromosomal aberration test were performed, and all the results were reported to be negative.

<Overseas reports>[3)]
Ames test: Negative; TA100, TA1535, WP2*uvr*A, TA98, TA1537, 62-5,000 μg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; Human lymphocytes, 1,250-5,000 μg/mL (with and without metabolic activation) (with and without metabolic activation: 3-hour treatment) (without metabolic activation: 20- and 44-hour treatment)

<Domestic reports>[4)]
Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: 2,000 mg/kg BW

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA evaluated isoamylase as Isoamylase from *Pseudomonas amylodermosa* and considers that the ADI is not specified.[3)]
This isoamylase is produced by the MU 1174 strain with high isoamylase productivity selected from the chemically mutated wild SB-15 strain of *Pseudomonas amyloderamosa*.[5)] Isoamylase was collected from the secretions in the liquid fermentation medium during the pure culture process of the MU 1174 strain and concentrated, and then stabilized, standardized, and normalized with maltose, glucose, water, acylglycerol, and benzoic acid.[6)]
The US FDA states that it is safe to use Isoamylase for human consumption and is generally recognized as safe (GRAS).[7)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Morimoto, H., Noro, H. & Ohtaki, H.(1979) Acute toxicity test with isoamylase (of Pseudomonas amyloderamosa origin). Unpublished report No.12110175-3

from Japan Food Research Laboratories, Tokyo, Japan. Submitted to WHO by Bioresco Ltd, Basel, Switzerland.

2) Lina, B.A.R. (2000) Sub-chronic (13-wk) oral toxicity study with isoamylase in rats. Unpublished report No.V99.646 from TNO Nutrition and Food Research Institute, Zeist, Netherlands. Submitted to WHO by Bioresco Ltd, Basel, Switzerland.
(References 1 and 2 are cited in Reference 6)

3) JECFA: Safety evaluation of certain food additives and contaminants (2008), WHO Food Additive Series 59

4) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

5) JECFA: Evaluation of certain food additives and contaminants (2007), WHO Technical Report Series 947

6) JECFA: Olempska-Beer, Z. (2007) Isoamylase from pseudomonas amyloderamosa, Chemical and Technical Assessment.
http://www.fao.org/fileadmin/templates/agns/pdf/jecfa/cta/68/Isoamylase.pdf

7) FDA: GRAS Notice GRN 85

Invertase

English name: Invertase
CAS No. 9001-57-4
JECFA No. Not available
Other names: Saccharase
Sucrase
Structural formula:-

1. Origin and method of preparation

Invertase is an enzyme that is obtained from the culture of filamentous fungi (only *Aspergillus aculeatus, Aspergillus awamori, Aspergillus niger*, and *Aspergillus japonicus*), yeasts (only *Kluyveromyces lactis* and *Saccharomyces sereviciae*), or bacteria (only genera *Arthrobacter* and *Bacillus*), and it hydrolyzes a residue on the non-reducing end of β-D-fructofuranoside. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Manufacture of chocolates and confectionery

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated-dose toxicity

3) Mutagenicity study

No information available on mutagenicity

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA evaluated Invertase as invertase from *Saccharomyces cerevisiae* and considers that there are no safety concerns (acceptable) in the current situation of use.[1)]

4. Conclusion

Based on the opinion of JECFA, it is considered that there are no safety concerns about this existing additive that is distributed in Japan because it is derived from yeasts that are used in food manufacturing.

5. References

1) JECFA: Evaluation of certain food additives and contaminants (2001), WHO Technical Report Series 909

Exomaltotetraohydrolase

English name: Exomaltotetraohydrolase
CAS No. 37288-44-1
JECFA No. Not available
Other names: Glucan 1,4-α-maltotetraohydrolase
4-α-D-glucan maltotetraohydrolase
Structural formula:-

1. Origin and method of preparation

Exomaltotetraohydrolase is an enzyme that is obtained from the culture of actinomycetes (only *Streptomyces thermoviolaceus* and *Streptomyces violaceoruber*) or bacteria (only *Pseudomonas stutzeri*), and it acts on starch by hydrolyzing maltotetraose units from the non-reducing end. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Delaying deterioration and maintaining quality of consumption quality of bread

3. Summary of safety studies

1) Acute toxicity study

Rat oral LD_{50} > 2,000 mg/kg BW[1)]

2) Repeated-dose toxicity study

Wistar Han™:HsdRccHan™:WIST rats (10 males and females each per group) received 90-day treatment with maltotetraohydrolase by gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 79 mg total protein/kg BW/day (90.9 mg TOS/kg BW/day), which was the highest dose.[2)]

3) Mutagenicity study

An Ames test and a chromosomal aberration test were performed, and all the results were reported to be negative.[3)]

Ames test: Negative; TA100, TA1535, WP2*uvr*A, TA98, TA1537 50-5,000 μg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; Human lymphocytes, 19.5-625 μg/mL (with and without metabolic activation) 4-hour treatment, 19.5-312.5 μg/mL (without metabolic activation) 24-hour treatment

4) Others

The Food Safety Commission of Japan checked the safety of the inserted gene, the toxicity and allergenicity of the protein produced from the inserted gene, base sequencing after gene transfer, etc., concerning "Exomaltotetraohydrolase produced using the MDT06-228 strain prepared by introducing the modified Exomaltotetraohydrolase gene (*sas3* gene) derived from the *Pseudomonas stutzeri* IAM 1504 strain to the *Bacillus licheniformis* BRA7 strain as the host" based on "Standards for Safety Assessment of Food Additives produced Using Genetically Modified Microorganisms" (Food Safety Commission of Japan Decision of March 25, 2004) and found no factors that newly affect the safety compared to existing additives, and thus judged that it has no risks for human health.[4)]

5) Position in overseas assessment reports

JECFA states that the ADI of maltotetraohydrolase produced by modified *Bacillus licheniformis* is not specified[3)].
FSANZ similarly states that the ADI is not specified[5), 6)].

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Pooles, A. (2008). SAS 3 amylase (Bacillus licheniformis) (GICC 03279):Acute oral toxicity in the rat – fixed dose method.(SPL Project No.2420/0003, SafePharm Laboratories, United Kingdom).
(Cited in Reference 5)

2) Dhinsa, N.K. & Brooks, P. (2008).SAS 3 amylase (Bacillus licheniformis)(GICC 03279):Ninety day repeated dose oral (gavage) toxicity study in the rat (SPL

Project No.2420/0008, SafePharm Laboratories, United Kingdom).
(Cited in References 3 and 5)

3) JECFA: Safety evaluation of certain food additives and contaminants (2015), WHO Food Additives Series 71

4) Food Safety Commission of Japan: Genetically modified food assessment report "Exomaltotetraohydrolase produced using the MDT06-228 strain" (2017)

5) Food Standard Australia New Zealand (FSANZ): Risk Assessment Report, Application A1033 maltotetraohydrolase as a processing aid (enzyme) (2009).

6) Food Standard Australia New Zealand (FSANZ): Approval Report, Application A1033 maltotetraohydrolase as a processing aid (enzyme) (2010).

Esterase

English name: Esterase
CAS No. 9013-79-0
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Esterase is an enzyme obtained from the liver of animals, fish, and the culture of filamentous fungi (only genus *Aspergillus*), yeasts (only genera *Candida* and *Torulopsis*), or bacteria (only genus *Pseudomonas*), and it hydrolyzes esters. Food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment) may be contained.

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

Wistar rats (10 males and females each per group) received 91-day treatment with pectinesterase derived from *T.reesei* RF6201 by gavage according to OECD TG 408. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 1,000 mg TOS/kg BW/day, which was the highest dose.[1)]

3) Mutagenicity study

An Ames test and a chromosomal aberration test were performed, and all the results were reported to be negative.[1)]

Ames test: Negative; TA100, TA1535, TA98, TA1537, TA102, 33-5,000 μg/plate

(with and without metabolic activation)
Chromosomal aberration test: Negative; V79 cells, maximum of 5,310 μg/mL (with and without metabolic activation)

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The FDA evaluates that pectinesterase of *Trichoderma Reesei* derived from modified *Aspergillus tubingensis* is "generally recognized as safe (GRAS)".[1)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) AB ENZYMES GmbH, GRAS NOTICE FOR PECTIN ESTERASE FROM A GENETICALLY MODIFIED STRAIN OF TRICHODERMA REESEI (2014) (FDA: GRAS Notice GRN 558)

Cassia gum

English name: Cassia gum
CAS No. 11078-30-1
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Cassia gum is obtained from the crushed endosperm of the seed of *Cassia tora* LINNE of the Fabaceae family. It consists mainly of polysaccharides.

2. Major use

Thickener, emulsifier, foam stabilizer, humectant, texturizing agent for processed cheese, frozen dessert, meat and poultry product

3. Summary of safety studies

1) Acute toxicity study

Rat (Wistar-Han-Schering) oral LD_{50} > 5,000 mg/kg BW[1)]
Mouse (KM) oral LD_{50} > 10,000 mg/kg BW[2)]

2) Repeated-dose toxicity study

Crl:CD(SD)BR Sprague-Dawley rats (5 males and females each per group) received 28-day dietary administration with crudely purified cassia gum. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 50,000 mg/kg food (4,590 mg/kg BW/day), which was the highest dose[3)].

Beagle dogs (4 males and females each per group) received 90-day dietary administration with crudely purified cassia gum, and the NOAEL was considered to be 25,000 mg/kg food (3,290 mg/kg BW/day), which was the highest dose.[4)]

Cats (5 males and females each per group) received 13-week dietary administration with crudely purified cassia gum. The test substance did not show any toxicological effects, and the NOEL was considered to be 25,000 mg/kg food (2,410 mg/kg body weight/day), which was the highest dose.[5)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, a mouse lymphoma test, a bone-marrow micronucleus test, and a morphological abnormality test with sperm were performed, and all the results were reported to be negative.[6)]

Ames test: Negative; TA100, TA1535, *E.coli* WP2*uvr*A, TA98, TA1537, TA97, TA102, 5,000 μg/plate (with and without metabolic activation)[6)]
Chromosomal aberration test: Negative; Human lymphocytes, 10.0 μg/mL [6)]
Mouse lymphoma test: Negative; Mouse lymphoma L5178Y $TK^{+/-}$ 10.0 μg/mL [6)]
Bone-marrow micronucleus test: Male and female KM mice: Negative; 2,500 mg/kg BW, oral (administered twice within 30 hours, sampled at 6 hours after the final administration)[6)]
Morphological abnormality test with sperm: Negative; KM mouse, 2,500 mg/kg BW/day, 5-day oral administration (sampling at 30 days after the final administration)[6)]

4) Others

In a 2-generation reproductive toxicity test of OECD TG416, in which Ico:OFA.SD Sprague-Dawley rats (25 males and females each per group) received dietary administration with crudely purified cassia gum, the NOEL was considered to be 50,000 mg/kg food (5,280 mg/kg BW/day), which was the highest dose.[7)]
In a prenatal developmental toxicity test of OECD TG414, in which pregnant SD rats (28 animals) received dietary administration with crudely purified cassia gum, the NOAEL was considered to be 1,000 mg/kg BW/day, which was the highest dose.[8)]

5) Position in overseas assessment reports

JECFA evaluates it as a crudely purified product, and the provisional ADI is not specified.[9, 10)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Schöbel, C. (1986) Acute toxicity of Mucigel X-18-H in male rats after a single i.g. application with approximative LD50 determination. Unpublished report No. FC 4/86 from Schering. Submitted to WHO by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium.

2) Weidu, H. (2006) Shanghai Institute for Preventive Medicine testing report: Summary of one and two stage toxicological tests on RheoRanger SR. Unpublished report No.0021 from Shanghai Institute for Preventive Medicine, Shanghai, China. Submitted to WHO by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium.

3) Zühlke, U. (1990) Diagum CS-Twenty-eight day oral (dietary administration and gavage) range-finding subchronic toxicity study in the rat. Unpublished report No.711-14/48 from Hazleton Laboratories Deutschland GmbH, Münster, Germany.

4) Schuh, W. (1990) Diagum-CS-Systemic tolerance study in Beagle-dogs after daily oral (dietary) administration over a period of 90 days. Unpublished report No. IC 4/90 from Schering. Submitted to WHO by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium.

5) Virat, M. (1984) 13 week toxicity study in the cat by the oral route. Unpublished report No.411233 from Institut Français de Toxicologie, Saint Germain sur l'Arbresle, France. Submitted to WHO by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium.

6) JECFA, Safety evaluation of certain food additives, WHO Food additives series 62(2010)
(References 1-5, 7 and 8 are cited in Reference 9)

7) McIntyre, M.D. (1990) Diagum CS-Two generation oral (dietary administration) reproduction toxicity study in the rat. Unpublished report No.710791 from Hazleton – Institut Français de Toxicologie, Saint Germain sur l'Arbresle, France. Submitted to WHO by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium

8) Müller, W. (1989) Diagum CS-Oral (gavage) teratogenicity study in the rat. Unpublished report No.725-14/50 from Hazleton Laboratories Deutschland GmbH, Münster, Germany. Submitted to WHO by Lubrizol Advanced Materials Europe BVBA, Brussels, Belgium.

9) JECFA: Safety evaluation of certain food additives and contaminants (2009), WHO Technical Report Series 956

10) JECFA: Evaluation of certain food additives and contaminants (2016), WHO Technical Report Series 1000

Carboxypeptidase

English name: Carboxypeptidase
CAS No. 9046-67-7(EC 3.4.16.1 derived from *Aspergillus*)
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Carboxypeptidase is an enzyme that is derived from testa and pericarp (bran) of wheat (*Triticum aestivum* L.) or the culture of filamentous fungi (only genus *Aspergillus*), yeasts (only *Pseudozyma hubeinsis* and *Saccharomyces cerevisiae*), and actinomycetes (only Streptomyces *avermitilis*, *Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces themoviolaceus*, and *Streptomyces violaceoruber*), and it degrades proteins and peptides from the carboxy end. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

Wistar rats (10 males and females each per group) received 90-day treatment with carboxypeptidase by gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 11,000 mg/kg BW/day (1,056 mg TOS/kg BW/day, 48,851 CPGU/kg BW/day), which was the highest dose.[1)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, and a bone-marrow micronucleus test were performed, and all the results were reported to be negative.[1)]

Ames test: Negative; TA100, TA1535, *E.coli* WP2*uvr*A, TA98, TA1537 5,000 μg/plate (with and without metabolic activation)[1)]
Chromosomal aberration test: Negative; Human lymphocytes, 5,000 μg/mL (with and without metabolic activation)[1)]
Bone-marrow micronucleus test: Negative; NMRI BR mice 2,000 mg/kg BW, oral[1)]

4) Others

The Expert Committee on Genetically Modified Foods of the Food Safety Commission of Japan investigated carboxypeptidase produced using the genetically modified *Aspergillus niger* PEG strain, which has high carboxypeptidase productivity, and judged that safety assessment as an additive manufactured using genetically modified microorganisms is not necessary, because it is manufactured using microorganisms that fall under "cases where the DNA ultimately introduced to the host through recombinant DNA technology is only DNA from a microorganism belonging to the same taxonomic species as the concerned microorganism".[2)]

5) Position in overseas assessment reports

FDA evaluates this additive as generally recognized as safe (GRAS).[1)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) FDA: GRAS Notice GRN 345, Carboxypeptidase enzyme preparation from modified *Aspergillus niger* (2010).

2) Food Safety Commission of Japan: Genetically modified food assessment report "Carboxypeptidase produced using PEG strain" (2016)

Xylanase

English name: Xylanase
CAS No. 9025-57-4
JECFA No. Not available
Other names: Not available
Structural formula: -

1. Origin and method of preparation

Xylanase is an enzyme that is obtained from the culture of filamentous fungi (only *Aspergillus aculeatus*, *Aspergillus awamori*, *Aspergillus niger*, *Disporptrichum dimorphosporum*, *Humicola insolens*, *Rasamsonia emersonii*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, and *Trichoderma viride*) or actinomycetes (only *Streptomyces avermitilis*, *Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), and it degrades xylan. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Breadmaking (improving dough, bulking, etc.)

3. Summary of safety studies

1) Acute toxicity study

The xylanase enzyme was obtained by collecting the product of pure culture of strains prepared by gene transfer of *Bacillus subtilis* natural xylanase-derived BS1 and BS2 and flour xylanase inhibition-resistant mutant BS3 and concentrating it.[1)] The results of the acute toxicity study are as shown below.

Rat oral
Xylanase BS1: LD_{50} > 2,000 mg/kg BW [200,000 TXU/kg BW][2), 3)]
Xylanase BS2: LD_{50} > 2,000 mg/kg BW [212,000 TXU/kg BW][4)]
Xylanase BS3: LD_{50} > 2,000 mg/kg BW [220,000 TXU/kg BW][5)]

2) Repeated-dose toxicity study

Wistar rats (10 males and females each per group) received 13-week treatment with xylanase BS1 by gavage. No treatment-related changes were observed, and the NOEL was 80,000 TXU/kg BW/day (equivalent to 63 mg/kg BW/day as TOS), which was the highest dose.[3), 6)]

Wistar rats (5 males and females each per group) received 4-week treatment with xylanase BS3 by gavage. No treatment-related changes were observed, and the NOEL was 200,000 TXU/kg BW/day (equivalent to 304 mg/kg BW/day as TOS), which was the highest dose.[7)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, and a bone-marrow micronucleus test were performed, and all the results were reported to be negative.[8), 9)]

<Domestic reports>

Ames test: False positive; 5,000 μg/plate. An increase in the revertant colony count by approximately 2-fold was observed in the TA1535 group without metabolic activation, which was considered to be false positive, because the test substance contained histidine.[8)]

Chromosomal aberration test: Negative; 5,000 μg/mL [8)]

Micronucleus test: Negative; 2,000 mg/kg BW[8)]

<Overseas reports>

Ames test: TA100, TA1535, TA98, TA1537, TA102, 5,000 μg/plate (with and without metabolic activation)[9)]

Chromosomal aberration test: Human lymphocytes, 5,000 μg /mL [9)]

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA evaluated Xylanase as *Bacillus subtilis*-derived xylanase and concludes that ADI is not specified in the current situation of use.[1), 9)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) JECFA: Evaluation of certain food additives and contaminants (2005), WHO Technical Report Series 928

2) Kaaber, K. (1999) Bacillus xylanase — acute oral toxicity study in the rat. Unpublished report No.34762 from Scantox, Lille Skensved, Denmark. Submitted to WHO by Danisco USA Inc., Ardsley, NY, USA.

3) Harbak, L. & Thygesen, H.V. (2002) Safety evaluation of a xylanase expressed in Bacillus subtilis. Food Chem. Toxicol., 40,1-8.

4) Bollen, L.S. (2003a) Xylanase BS2 — acute oral toxicity study in the rat. Unpublished report No.51932 from Scantox, Lille Skensved, Denmark. Submitted to WHO by Danisco USA Inc., Ardsley, NY, USA.

5) Bollen, L.S. (2003b) Xylanase BS3 — acute oral toxicity study in the rat. Unpublished report No.51228 from Scantox, Lille Skensved, Denmark. Submitted to WHO by Danisco USA Inc., Ardsley, NY, USA.

6) Glerup, P., (1999) Bacillus xylanase — a 13-week oral (gavage) toxicity study in rats. Unpublished report No.34387 from Scantox, Lille Skensved, Denmark. Submitted to WHO by Danisco USA Inc., Ardsley, NY, USA.1999

7) Kaaber, K. (2003) Xylanase BS3 — a 4-week oral (gavage) toxicity study in rats. Unpublished report No.51173 from Scantox, Lille Skensved, Denmark. Submitted to WHO by Danisco USA Inc., Ardsley, NY, USA.

8) Hayashi, et al.: Food hygiene and safety science 46, 177-184 (2005)

9) JECFA: Safety evaluation of certain food additives (2006), WHO Food additives series 54
(References 2-6 are cited)

Chitosan

English name: Chitosan
CAS No. 9012-76-4
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Chitosan is “chitin” that has been deacetylated with warm to hot sodium hydroxide solution and consists of the polymer of D-glucosamine.

2. Major use

Processing aid for the manufacture of wines, beers, ciders, spirits, and edible ethanol

3. Summary of safety studies

1) Acute toxicity study

Rat (SD) oral LD_{50} > 2,000 mg/kg BW[1)]
Mouse (Kunming) oral LD_{50} > 1,000 mg/kg BW[2)]

2) Repeated-dose toxicity study

Sprague-Dawley rats (9 males and females each per group) received 28-day treatment with oligomer chitosan by gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 2,000 mg/kg BW/day, which was the highest dose.[3)]

In a 90-day dietary administration test in F344 rats (10 males and females each per group) with oligosaccharide chitosan, malnutrition was occasionally observed in addition to weight gain suppression associated with decreased food intake in the 1% group, which was the highest dose, and the NOAEL was considered to be 0.2% (124.0 mg/kg BW/day for males and 142.0 mg/kg BW/day for females).[4)]

Sprague-Dawley [Crl:CD(SD)] rats (10 males and females each per group) received 26-week treatment with chitosan by gavage. No deaths were observed. Decreased weight gain by approximately 10% was observed in males and females in the 4,500 mg/kg group. No effects were observed in groups of 1,500 mg/kg and less.[5)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, and a rec assay were performed, and all the results were reported to be negative.[6), 7)] An Ames test and a bone-marrow micronucleus test were also performed with chitosan oligomer, and all the results were reported to be negative.[7)]

<Domestic reports>

No detailed descriptions available [7)]

<Overseas reports>

Ames test: Negative; TA100, TA1535, *E.coli* WP2*uvr*A, TA98, TA1537, 1,000 μg/plate (with and without metabolic activation), 5,000 μg/plate (oligomer, with and without metabolic activation)[7)]

Bone-marrow micronucleus test: Negative; ICR and Kunming mice, oligomer at 1%w/v administered in drinking water for 180 days, and oligomer at 5,000 mg/kg BW, oral[7)]

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

In the FSANZ evaluation, there are no public health or safety problems with the use of fungal chitosan as a processing aid, because no toxicity was observed in 13 cases of 6-month oral intake in humans (mean daily concentration of 3.5 g).[11)] The FDA evaluated it as “generally recognized as safe (GRAS)”.[7), 8), 9), 10)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Kitozyme (2008) Final Report Acute Oral toxicity in the rat. Study no TAO423-PH-08/0064: CONFIDENTIAL. Prepared by S. Seguier – Phycher Bio development (FR)

2) Qin C, Gao J, Wang L, Zeng L, Liu Y (2006) Safety evaluation of short-term exposure to chitooligomers from enzymic preparation. Food Chem Toxicol.44(6):855 – 861.

3) Kim SK, Park PJ, Yang HP, Han SS (2001) Subacute toxicity of chitosan oligosaccharide in Sprague-Dawley rats. Arzneimittelforschung 51(9):769-774.

4) Naito Y, Tago K, Nagata T, Furuya M, Seki T, Kato H, Morimura T, Ohara N (2007) A 90-day ad libitum administration toxicity study of oligoglucosamine in F344 rats. Food Chem Toxicol.45(9):1575-1587.

(References 1-4 are cited in Reference 14)

5) NTP (2009) National Toxicology Program. Testing Status of Agents at NTP. Chitosan. Study no.C20226.Available at:

https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox093_508.pdf

6) Hayashi et al.: Environ. Mutagen Res., 22, 27-44 (2000)

7) FDA: GRAS Notice GRN 397, Chitosan from *Aspergillus niger*

8) FDA: GRAS Notice GRN 73

9) FDA: GRAS Notice GRN 170

10) FDA: GRAS Notice GRN 443

11) Jull AB, Ni Mhurchu C, Bennett DA, Dunshea-Mooij CA, Rodgers A (2008) Chitosan for overweight or obesity. Cochrane Database Syst Rev. Jul 16;(3):CD003892.

12) EFSA: TECHNICAL REPORT (2013), Outcome of the consultation with Member States and EFSA on the basic substance application for chitosan hydrochloride and the conclusions drawn by EFSA on the specific points raised

13) Food Standard Australia New Zealand (FSANZ): Winemakers Federation of Australia (2012): Application for a NEW PROCESSING AID. Fungal chitosan from *Aspergillus niger*

14) Food Standard Australia New Zealand (FSANZ): Risk and Technical

Assessment Report, Application A1077-Fungal Chitosan as a Processing Aid (2013).

Cristobalite

English name: Cristobalite
CAS No. 14464-46-1
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Cristobalite is cristobalite that has been mined from a deposit, milled, dried, and burned at 800-1200°C, or treated with hydrochloric acid and burned.

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

Rat oral LD_{50} = 3.16 mg/kg BW[1)]
Mouse oral LD_5 > 5,000 mg/kg BW[2)]

2) Repeated-dose toxicity study

Wistar rats (20 males and females each per group) received oral administration with pellets containing amorphous silica (> 98.3% SiO_2) at a silica content of 100 mg/kg BW/day daily for 2 years. Toxic effects attributable to the test substance were not observed.[3)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, and a micronucleus test were performed as silica, and all the results were reported to be negative. There is also a report that quartz has genotoxicity. However, it is uncertain.[4)]

Ames test: No detailed description available [4)]
Chromosomal aberration test: Negative; 1,600 μg/mL [4)]
Micronucleus test: Negative; 500 mg/kg BW, intraperitoneal[4)]

4) Others

The IARC states that crystalline silica inhaled in the form of quartz or cristobalite as occupational exposure is carcinogenic in humans (Group 1) and that amorphous silica cannot be classified as carcinogenic in humans (Group 3).[5)]

5) Position in overseas assessment reports

JECFA evaluates cristobalite as a kind of silicate containing amorphous silicon dioxide and calcium silicate and states that the ADI is not specified (JEFCA 1985 29th meeting, WHO Food Additives Series 20).[6)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Elsea, J.R. (1958a) Unpublished report, January 8, from Hazleton Laboratories, Inc.

2) Kimmerle (1968) Unpublished report submitted by Bayer
(References 1 and 2 are cited in Reference 3)

3) JECFA: Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents. WHO Food Additives Series No.5 (1974)

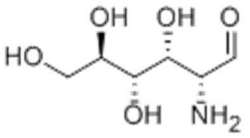
4) JECFA, Concise International Chemical Assessment Document 24(2000). CRYSTALLINE SILICA, QUARTZ.

5) JECFA: International Agency for Research on Cancer (IARC)-Summaries & Evaluations. SILICA Crystalline silica-inhaled in the form of quartz or cristobalite from occupational sources (Group 1) Amorphous silica (Group 3) (1997)

6) JECFA: Toxicological evaluation of certain food additives and contaminants. WHO Food Additives Series 20(1985)

Glucosamine

English name: Glucosamine
CAS No. 3416-24-8(66-84-2 for hydrochloride)
JECFA No. Not available
Other names: Not available

Structural formula: 

1. Origin and method of preparation

Glucosamine is obtained from “chitin” by hydrolyzing with hydrochloric acid and separating. Its component is glucosamine.

2. Major use

Thickening stabilizer, food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

Mouse oral LD_{50} > 15,000 mg/kg BW[1)]

Male and female rats (strain unknown) received glucosamine hydrochloride at 5,000 mg/kg BW. It did not show any toxic effects and was not considered to have high acute oral toxicity.[2)]

2) Repeated-dose toxicity study

In a 52-week repeated-dose toxicity test in rats (strain unknown), the NOAEL was considered to be 2,130 mg/kg BW/day (not disclosed).[3)]

F344 rats (40 males and females each per group) received 90-day dietary administration with glucosamine at 0.5%, 1.67%, and 5%. In the 5% administered group, females showed an increase in liver weight, while males showed an increase in urine volume and a decrease in urine specific gravity associated with an increase in drinking water intake, an increase in kidney weight, and an increase in the degree

of eosinophilic bodies histopathologically. The experimenter considered that the NOAEL was 1.67% (1,075 mg/kg BW/day for males and 1,158 mg/kg BW/day for females).[4)]

3) Mutagenicity study

An Ames test and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[3), 5)] (There are also positive results of an *in vivo* chromosomal aberration test. However, the test was not performed by a standard test method and was judged as less reliable by the EFSA[3)])

<Domestic reports>[5)]
Ames test: 5,000 μg/plate

<Overseas reports>[3)]
Ames test: Negative; TA100, TA1535, WP2*uvr*A, TA98, TA1537, 100-5,000 μg/plate (with and without metabolic activation)
Micronucleus test: Negative; Mouse, 50-2,000 mg/kg BW, oral

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The EFSA evaluates glucosamine hydrochloride from *Aspergillus oryzae* as safe.[3)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Kohzaburo Seki: On the General Pharmacological Actions of Glucosamine Hydrochloride. 10th Hokubukai (Iwate) Article 56, 64S (1960)

2) Japan Confectionery Research Center: N-Acetylglucosamine/Glucosamine,

Series on Technology Utilization of Innovative Food Ingredients (2000)

3) EFSA, SCIENTIFIC OPINION, Opinion of the safety of glucosamine hydrochloride from Aspergillus niger as food ingredient, Scientific Opinion of the Panel on Dietetic Products, Nutrition and Allergies, (Question No EFSA-Q-2008-306), The EFSA Journal 1099,1-19(2009)

4) Mayumi Kawabe, et al.: 90-day Repeated Dose Toxicity Study of Glucosamine in F344/DuCrj Rats. Japanese Journal of Food Chemistry 12(1): 15-22 (2005)

5) Asanoma and Tamura: Annual Report of Nagoya City Public Health Research Institute 52, 39-44 (2006)

α-Glucosidase

English name: α-Glucosidase
CAS No. 9001-42-7
JECFA No. Not available
Other names: Maltase
Structural formula:-

1. Origin and method of preparation

α-Glucosidase is an enzyme that is derived from the culture of filamentous fungi (only genera Absidia, *Acremonium*, and *Aspergillus*), yeasts (only genus *Saccharomyces*), actinomycetes (only *Streptomyces avermitilis*, *Streptomyces griseus*, and *Streptomyces violaceoruber*), or bacteria (only genus *Bacillus*, *Burkholderia ginsengisoli*, *Halomonas aquamarina*, and genus *Pseudomonas*), and it hydrolyses the α-D-glucoside bond present at the non-reducing end of maltose and oligosaccharides. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

Rat oral LD_{50} > 2,000 mg/kg BW (2,350 mg TOS/kg BW)[1)]

2) Repeated-dose toxicity study

SPF-bred Wistar rats (10 males and females each per group) received 13-week treatment with α-glucosidase by gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 63.64 mg/kg BW/day (74.8 mg TOS/kg BW/day), which was the highest dose.[2)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[3)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The FDA states that the use of the α-glucosidase enzyme produced by filamentous fungi having the α-glucosidase gene derived from *Aspergillus niger* as a processing aid is generally recognized as safe (GRAS).[4)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Harlan Labs-Study No.C57481, Acute Oral Toxicity Study in Rats. December 15(2009), FDA: GRAS Notice GRN 703

2) Harlan Labs-Study No: C57558, An 18-week Oral (Gavage) Toxicity Study in Wistar Rats. April 15(2010), FDA: GRAS Notice GRN 703

3) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

4) FDA: GRAS Notice GRN 703, Alpha-glucosidase Enzyme Preparation Derived from *Trichoderma reesei* Expressing the Alpha-glucosidase Gene from *Aspergillus niger* Is Generally Recognized As Safe For Use in Food Processing, Notification Submitted by Danisco US Inc.(operating as DuPont Industrial Biosciences) April 24(2017)

α-Glucosyltransferase

English name: α-Glucosyltransferase
CAS No. 9032-09-1
JECFA No. Not available
Other names: 4-α-Glucanotransferase
6-α-Glucanotransferase
Structural formula:-

1. Origin and method of preparation

α-Glucosyltransferase is an enzyme that is obtained from the tuber of potatoes (*Solanum tuberosum* L.), actinomycetes (only *Streptomyces avermitilis*, *Streptomyces cinnamoneus*, *Streptomyces griseus*, *Streptomyces thermoviolaceus*, and Streptomyces *violaceoruber*), or bacteria (only *Agrobacterium radiobacter,* genus *Arthrobacter*, genus *Bacillus*, genus *Erwinia*, *Geobacillus pallidus*, *Geobacillus stearothermophilus*, *Gluconobacter oxydans*, *Leuconostoc mesenteroides*, *Paenibacillus alginolyticus*, genus *Pimelobacter*, genus *Protaminobacter*, genus *Pseudomonas*, genus *Serratia*, *Sporosarcina globispora*, and genus *Thermus*), and it transfers the glucosyl group or glucan chains. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

Rat (Wistar) oral LD_{50} > 2,000 mg/kg BW[1)]

2) Repeated-dose toxicity study

Sprague-Dawley [Crl:CD(SD)] rats (10 males and females each per group) received 13-week treatment with the 1,4-α-D-glycan branching enzyme produced by *Bacillus subtilis* that expressed the gene of the 1,4-α-D-glycan branching enzyme derived from *B. stearothermophilus* by gavage. The NOAEL was considered to be

870 mg/kg BW/day, which was the highest dose.[2)]
Sprague-Dawley [Crl:CD(SD)] rats (10 males and females each per group) received 13-week treatment with the 1,4-α-D-glycan branching enzyme produced by *Bacillus subtilis* that expressed the gene of the 1,4-α-D-glycan branching enzyme derived from *Aquifex aeolicus* by gavage. The NOAEL was considered to be 900 mg/kg BW/day, which was the highest dose.[2)]
Rats (strain unknown, 10 males and females each per group) received 13-week treatment with branching glycosyltransferase by gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 769 mg TOS/kg BW/day, which was the highest dose.[3)]

3) Mutagenicity study

No information available on mutagenicity.

4) Others

JECFA states that ADI is not specified for 1,4-α-D-glycan branching enzyme produced by *Bacillus subtilis*.[4), 5)]
The Food Safety Commission of Japan judges that "6-α-glucanotransferase produced using the *Bacillus subtilis* BR151(pUAQ2) strain" has no risks for human health.[6)]

5) Position in overseas assessment reports

Branching glucosyltransferase produced by *Bacillus subtilis* as well as 1,4-α-D-glycan branching enzyme for the purpose of processing aid are applied to the FDA as GRAS.[3), 7)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Choi SS, Danielewska-NikielB, Ohdan K, Kojima I, Takata H, Kuriki T (2009a). Safety evaluation of highly-branched cyclic dextrin and a 1,4-α-glucan branching enzyme from *Bacillus stearothermophilus*. Regul Toxicol Pharmacol 55(3):281-290

2) Choi SS, Danielewska-Nikiel B, Kojima I, Takata H (2009b). Safety evaluation of 1, 4-α-glucan branching enzymes from *Bacillus stearothermophilus* and *Aquifex aeolicus* expressed in *Bacillus subtilis*. Food Chem Toxicol 47(8):2044-2051
(References 1 and 2 are cited in FDA: GRAS Notice GRN 361)

3) FDA: GRAS Notice GRN 274, A branching glycosyltransferase produced by *Bacillus subtilis* expressing the *Rhodothermus obamensis* branching glycosyltransferase gene. December (2008)

4) JECFA: Evaluation of certain food additives and contaminants (2009), WHO Technical Report Series 956

5) JECFA: Safety evaluation of certain food additives and contaminants (2010), WHO Food Additives Series 62

6) The Expert Committee on Genetically Modified Foods, Food Safety Commission of Japan: Final report, A 13-week repeated-dose oral toxicity study of BE-02 in rats (internal report), (Draft) Genetically modified food assessment report, 6-α-glucanotranferase produced using the BR151(pUAQ2) strain, November 2011

7) FDA: GRAS Notice GRN 406, Generally Recognized as Safe (GRAS) Exemption Claim for 1,4-α-D-Glucan Branching Enzyme for Use as a Processing Aid in Food Production, 2011

Glutaminase

English name: Glutaminase
CAS No. 9001-47-2(EC 3.5.1.2)
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Glutaminase is an enzyme that is obtained from the culture of filamentous fungi (only genus *Aspergillus*), yeasts (only genus *Candida*), or bacteria (only *Bacillus amyloliquefaciens*, *Bacillus circulans*, and *Bacillus subtilis*), and it hydrolyses L-glutamine to form L-glutamic acid and ammonia. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

Wistar rats (20 males and females each per group) received 13-week dietary administration with glutaminase derived from *Bacillus amyloliquefaciens*. Although decreases were observed in weight gain and food intake in the 2% (w/v) group, it was considered that the addition of high-dose salt to food affected preference. The NOAEL was considered to be 1,239 mg/kg BW/day for males and 1,432 mg/kg BW/day for females at 2%.[1)]

Sprague-Dawley SPF rats [Crj:CD(SD)IGS] (12 males and females each per group) received 90-day treatment with glutaminase protein (protein glutaminase, CAS No. 62213-11-0) produced by *Chryseobacterium* proteolyticum by gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was

considered to be 2,538 mg/kg BW/day (93 mg TOS/kg BW/day).[2), 3)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative except for the Ames test in Japan.[4), 5)] The positive results of the domestic Ames test are considered to be caused by histidine contained as a contaminant.[4)]

<Domestic reports>[4)]
Ames test: False positive; 5,000 µg/plate
Chromosomal aberration test: Negative; 5,000 µg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

<Overseas reports>[5)]
Ames test: Negative; TA100, TA1535, TA98, TA1537, 62-5,000 µg/plate (with and without metabolic activation), WP2*uvr*A, 62-5,000 g/mL (with and without metabolic activation)
Chromosomal aberration test: Negative; CHO K-1 cells, 0.05-200 µg/mL (with and without metabolic activation)

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The FSANZ evaluates glutaminase enzyme from *B.amyloliquefaciens* for the purpose of processing aid and states that there are no health concerns and that the ADI is not specified.[5)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Appel, M.J. (1999). Sub-chronic (13-week) oral toxicity study with Glutaminase

in rats. TNO report V99.442.Lab:TNO Nutrition and Food Research Institute, Zeist, The Netherlands (unpublished).
(Cited in Reference 5)

2) FDA: GRAS Notice GRN 267, GRAS Notification for Protein-Glutaminase derived from *Chryseobacterium proteolyticum* (2008)

3) The Expert Committee on Food Additives, Food Safety Commission of Japan: Food additive assessment report, Protein glutaminase produced using the Chryseobacterium proteolyticum 9670 strain (2011)

4) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

5) Food Standard Australia New Zealand (FSANZ): Supporting document 1, Risk and technical assessment report (at Approval) – Application A1109, Glutaminase from Bacillus amyloliquefaciens as a Processing Aid (Enzyme), (2016)

Diatomaceous earth

English name: Diatomaceous earth
CAS No. 61790-53-2
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Diatomaceous earth is amorphous silicon dioxide derived from diatom. There are dried, calcined, and flux-calcined products that are referred to as diatomaceous earth (dried), diatomaceous earth (calcined), and diatomaceous earth (flux-calcined), respectively. Calcined diatomaceous earth is calcined at 800-1200°C, while flux-calcined diatomaceous earth is calcined at 800-1200°C with the addition of a small amount of alkaline carbonate salt. Specifications (excluding the description) for calcined diatomaceous earth apply correspondingly to acid-pickled diatomaceous earth among flux-calcined diatomaceous earth.

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

Wistar rats (15 males and females each per group) received 90-day dietary administration with diatomaceous earth at 1%, 3%, and 5%. An increase in weight gain was observed in the 5% group, while toxic effects attributable to the test substance were not observed in the 1% and 3% groups.[1)]

3) Mutagenicity study

An Ames test was performed, and the results were reported to be negative.[2)]

Ames test: Negative; 5,000 μg/plate [2)]

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The FDA evaluated it as a mixture with Perlite that is “generally recognized as safe (GRAS)”.[3)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Bertke E.M., The Effect Of Ingestion Of Diatomaceous Earth In White Rats: A Subacute Toxicity Test. Toxicol Appl Pharmacol.6:284-91(1964)

2) Hayashi et al.: Environ. Mutagen Res., 22, 27-44 (2000)

3) FDA: GRAS Notice GRN 087(2002)

Yeast cell wall

English name: Yeast cell wall
CAS No. 8013-01-2(Yeast extract)
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Yeast cell wall is obtained from the cell wall of yeasts of genus *Saccharomyces* (only *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, or Saccharomyces *pastorianus*), and it mainly consists of polysaccharides.

2. Major use

Thickening stabilizer, food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

Mouse oral LD_{50} > 2,000 mg/kg BW[1)]
Rat oral LD_{50} > 8,000 mg/kg BW[1)]

2) Repeated-dose toxicity study

Wistar rats (5 males and females each per group) received 10-day treatment with yeast extract (generally containing 8%-12% of yeast cell walls) at 2,000 mg/kg BW/day by gavage. Toxic effects attributable to the test substance were not observed.[1)]
Wistar rats (5 males and females each per group) received 27-day treatment with yeast extract (generally containing 8%-12% of yeast cell walls) at 50, 175, and 650 mg/kg BW/day by gavage. Toxic effects attributable to the test substance were not observed.[1)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[2)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The FSANZ states that there are no safety concerns and that the use of yeast cell walls up to the maximum allowable concentration of 400 mg/L is acceptable as a food additive for stability treatment of wine.[3)]
The FDA states that the use of *Saccharomyces cerevisiae* cell walls for stability of wine is generally recognized as safe (GRAS).[1)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) FDA: GRAS Notice GRN 284, GRAS Notification for Mannoprotein derived from an extract of bakers yeast (*Saccharomyces cerevisiae)*, March 6(2009)

2) Hayashi, et al.: Food hygiene and safety science 46, 177-184 (2005)

3) Food Standard Australia New Zealand (FSANZ): FINAL ASSESSMENT REPORT, APPLICATION A605, YEAST MANNOPROTEINS AS A FOOD ADDITIVE FOR WINE, March 19(2008)

Vegetable sterol

English name: Vegetable sterol
CAS No. Not available because the substance is a mixture
JECFA No. Not available
Other names: Phytosterols
Structural formula:-

1. Origin and method of preparation

Vegetable sterol is obtained from oilseeds and contain phytosterols as main components. There are two types of this additives: one with high free radical sterols and the other with low free sterols.

2. Major use

Emulsifier

3. Summary of safety studies

1) Acute toxicity study

Rat oral LD_{50} > 2,000 mg/kg BW[1), 2)]

2) Repeated-dose toxicity study

Sprague-Dawley rats received 90-day dietary administration with soybean phytosterols esterified with free fatty acids of olive oil. At 9 g/kg BW/day, which was the highest dose, a decrease in weight gain was observed in males and females, and an increase in the frequency of cardiomyopathy was observed in males. The NOAEL was therefore considered to be 3 g/kg BW/day.[3), 4)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed with free sterol-poor and free sterol-rich phytosterols, and all the results were reported to be negative.[5)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL

Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA established the group ADI as 40 mg/kg BW/day (as a total of free sterol) based on the NOAEL of 4,200 mg/kg BW/day obtained from the results of a 90-day repeated-dose administration test in rats with vegetable sterols, vegetable stanols, and a mixture of their esterified forms.[3), 4)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Appel, M.J. (1998a) Acute oral toxicity study (limit study) with WD-041T97 in rats. Zeist, Netherlands, TNO Nutrition and Food Research Institute (TNO Report V98.212)

2) Appel, M.J. (1998e) Acute oral toxicity study (limit study) with PU-029P97 in rats. Zeist, Netherlands, TNO Nutrition and Food Research Institute (TNO Report V98.217).

   (References 1 and 2 are cited in Reference 3)

3) JECFA: Safety evaluation of certain food additives. WHO Food Additives Series 60(2009)

4) JECFA: Evaluation of certain food additives. WHO Technical Report Series 952(2009)

5) Hayashi, et al.: Food hygiene and safety science 46, 177-184 (2005)

Hydrogen

English name: Hydrogen
CAS No. 1333-74-0
JECFA No. Not available
Other names: Not available
Structural formula: H-H

1. Origin and method of preparation

H_2

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated-dose toxicity

3) Mutagenicity study

No information available on mutagenicity.

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

The EFSA states that there are no problems with safety if it is used for deoxidation in food packages and beverages at room temperature or less.[1)]
The FDA states that the use of hydrogen for deoxidation in beverages is safe and evaluated it as “generally recognized as safe (GRAS)”.[2)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) EFSA: SCIENTIFIC OPINION, Scientific Opinion on the safety assessment of the active substances, palladium metal and hydrogen gas, for use in active food contact materials, EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 12(2):3558(2014)

2) FDA: GRAS Notice GRN 520(2014)

Powdered stevia

English name: Powdered stevia
CAS No. 91722-21-3(Stevia extract)
57817-89-7(Stevioside)
JECFA No. Not available
Other names: Steviol Glycosides (Steviol glycoside which is the main component)
Structural formula:-

1. Origin and method of preparation

Powdered stevia is the powdered leaf of *Stevia rebaudiana* BERTONI of the family Asteraceae. The major sweetening components are steviol glycosides (stevioside and rebaudioside).

2. Major use

Sweetener

3. Summary of safety studies

1) Acute toxicity study

Mouse (male) oral LD_{50} > 500 mg/kg BW[1)]
Rat (male) oral LD_{50} > 500 mg/kg BW[1)]
Dog (male and female) oral LD_{50} > 500 mg/kg BW[1)]

2) Repeated-dose toxicity study

Rats (strain unknown) received 2-year dietary treatment with stevioside. In the 5% group, a decrease in the final survival rate was observed in males, as well as decreases in weight and in renal parenchyma weight in males and females. The NOAEL was considered to be 970 mg/kg BW/day (383 mg/kg BW/day as steviol) in the 2.5% group.[2)]

3) Mutagenicity study

<Domestic reports>[3)]

While there is a report of a positive result in a reverse mutation test in bacteria with stevioside of 50% purity, negative results have been obtained with stevioside of

50% and 85% purity. In addition, all results of the following tests were reported to be negative: a DNA repair test in bacteria, a forward mutation test in bacteria, Umu test, and a chromosomal aberration test in mammalian cells.
Steviol had a positive result in a forward mutation test in bacteria, a DNA repair test in bacteria, and a chromosomal aberration test in mammalian cells under metabolic activation conditions. On the other hand, all results of the following tests were reported to be negative: a reverse mutation test in bacteria, a DNA repair test in bacteria, and a mouse micronucleus test.

<Overseas reports>[4)]

While there is a report of positive results with stevioside of unknown purity in an *in vitro* micronucleus test in human lymphocytes and oral mucosal cells, negative results were reported with stevioside of at least 95% purity in a reverse mutation test in bacteria and a rec assay. A sister chromatid exchange (SCE) test and a chromosomal aberration test were also performed with stevioside of unknown purity, and the results of both were reported to be negative. Positive results were reported for an *in vivo* comet assay with stevioside of 88.62% purity. However, other researchers questioned the validity of the results, and the evidence for genotoxicity was reported to be unconvincing.
Positive results were reported for a forward mutation test in bacteria (*S.typhimurium* TM677) with steviol of unknown purity; the authors reported that the strain showed unique sensitivity to steviol under metabolic activation conditions (rat S9 that was induced only with polychlorinated biphenyl was used) and that there was no relationship with the *in vivo* environment in the body. A bone-marrow micronucleus test was performed in rats, hamsters, and mice with steviol of 90% purity, and all the results were reported to be negative.

- Stevioside
  Ames test: Negative; TA100, TA1535, TA98, TA1537, TA1538, GA46, WP2hcr$^-$, 10-10,000 g/plate (with and without metabolic activation)
  Chromosomal aberration test: Negative; Human lymphocytes, 0.01-10 μmol/L
  SCE test: Negative; Human lymphocytes, 0.01-10 μmol/L
  Rec-assay: Negative; B-subtilis H17(rec$^+$), M45(rec$^-$), 500-2,000 μg/plate (with and without metabolic activation)
  In vitro micronucleus test: Positive; Human lymphocytes, human oral mucosa, 0.01-10 μmol/L
  In vivo comet assay: False positive; Wistar rat liver, brain, peripheral blood, spleen, 4 mg/mL oral

- Steviol
  Forward mutation test: False positive; S-typhimurium TM677 (with and without metabolic activation)
  Bone-marrow micronucleus test: Negative; Rat, hamster, mouse, 4-8 mg/kg BW

Based on the above, it was considered that powdered stevia containing stevioside and steviol has no mutagenicity that is problematic in the body.

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA evaluated steviol glycoside and judged that the ADI of steviol is 0-4 mg/kg BW/day.[2), 5)]

4. Conclusion

It was concluded that there are no safety concerns with this existing additive concerning its impact on human health in the current situation of use.

5. References

1) Bazotte, R.B., Lonardoni, M.T.C., Alvarez, M., Gaeti, W.P. & Amado, C.A.B. (1986) [Determination of the lethal dose LD_{50} of isosteviol in laboratory animals.] Arq. Biol. Tecnol., 29,711 – 722 (in Portuguese).

2) JECFA: Evaluation of certain food additives, WHO Technical Report Series 952(2009)

3) FY 1996 Health Sciences Research Report: Research on the safety evaluation of existing additives
https://www.ffcr.or.jp/houdou/1998/04/9C1A85A276A3290749256A4600080A21.html?OpenDocument

4) JECFA: Safety evaluation of certain food additives. WHO Food Additives Series 60(2009)

5) JECFA: Evaluation of certain food additives, WHO Technical Report Series

1000(2016)

Crude potassium chloride (sea water)

English name: Crude potassium chloride (sea water)
CAS No. 7447-40-7(Potassium chloride)
JECFA No. Not available
Other names: Not available
Structural formula: K-Cl

1. Origin and method of preparation

Crude potassium chloride (sea water) is obtained by concentrating sea water to precipitate and separate sodium chloride and then cooling the filtrate to room temperature to precipitate and separate. It consists mainly of potassium chloride.

2. Major use

Flavoring agent

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated-dose toxicity

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed as potassium chloride, and all the results were reported to be negative.

Ames test: Negative; 5,000 μg/plate [1)]
Chromosomal aberration test: Negative; 5,000 μg /mL [1)]
Micronucleus test: Negative; 2,000 mg/kg BW [1)]

4) Others

There have been no reports that toxicity is a concern.

5) Position in overseas assessment reports

JECFA states that the ADI of salts that freely ionize is to be established based on the related acid or base. Potassium chloride, which is the main component of crude potassium chloride (sea water) is included in the group ADI of hydrochloric acid and its salts together with ammonium salt and magnesium salt.[2)] Hydrochloric acid is evaluated as "Not limited" because it shows no toxic effects when used as a food additive.[3)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Hayashi, et al.: Food hygiene and safety science 46, 177-184 (2005)

2) JECFA: Evaluation of certain food additives, Twenty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. (1980) WHO Food Additives Series No.14, TRS 648

3) JECFA: Evaluation of certain food additives, Nine Report of the Joint FAO/WHO Expert Committee on Food Additives. (1965) WHO Food Additives Series 67.29, TRS 339

Crude magnesium chloride (sea water)

English name: Crude magnesium chloride (sea water)
CAS No. 7786-30-3
JECFA No. Not available
Other names: Not available
Structural formula: Cl-Mg-Cl

1. Origin and method of preparation

Crude magnesium chloride (sea water) is obtained from sea water by precipitating and separating potassium chloride and sodium chloride, and it consists mainly of magnesium chloride.

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated-dose toxicity

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.

Ames test: Negative; 5,000 μg/plate [1)]
Chromosomal aberration test: Negative; 5,000 μg/mL [1)]
Micronucleus test: 2,000 mg/kg BW[1)]

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA includes magnesium chloride, which is the main component of crude magnesium chloride (sea water) in the group ADI of hydrochloric acid and its salts.[2)] Hydrochloric acid is evaluated as “Not limited” because it shows no toxic effects when used as a food additive.[3)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

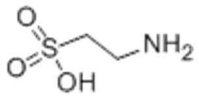
1) Food and Drug Safety Center: FY2002 Survey on genotoxicity concerning existing food additives (2003)

2) JECFA: Evaluation of certain food additives, Twenty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. (1980) WHO Food Additives Series No.14, TRS 648

3) JECFA: Evaluation of certain food additives, Nine Report of the Joint FAO/WHO Expert Committee on Food Additives. (1965) WHO Food Additives Series 67.29, TRS 339

Taurine (extract)

English name: Taurine (extract)
CAS No. 107-35-7
JECFA No. Not available
Other names: 2-Aminoethanesulfonic Acid

Structural formula: 

1. Origin and method of preparation

Taurine (extract) is obtained from fish and shellfish or the organs or meat of mammalians, and it consists mainly of taurine.

2. Major use

Flavoring agent

3. Summary of safety studies

1) Acute toxicity study

Mouse oral LD_{50} > 1,000 mg/kg BW[1), 4)]

2) Repeated-dose toxicity study

Wistar rats aged 7 weeks (9 males and 7 females per group) received 18-month dietary administration with taurine at 0, 0.5, and 5% (equivalent to 0, 500, and 5,000 mg/kg BW/day, respectively). The results showed slight growth inhibition that was not statistically significant in the 5% group. In addition, a moderate increase in the bile duct was observed in the 5% group. The NOEL was considered to be 500 mg/kg BW/day.[2), 4)]

Wistar rats immediately after weaning (6 males per group) received 8-week dietary administration with taurine at 0 or 5% (equivalent to 5,000 mg/kg BW/day). The results showed no significant differences in serum AST, ALT, or ALP between administered groups, or in the relative liver or kidney weight.[3), 4)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, a sister chromatid exchange (SCE) test, and a rec assay were performed, and all the results were reported to be negative.

<Domestic reports>
Ames test: Negative; TA100, TA1535, TA98, TA1537, TA1538, 6,200 μg/plate (with and without metabolic activation)[6)]
Chromosomal aberration test: Negative; CHL cells, 2,000 μg/mL[5), 6)]
Rec-Assay: Negative; B-subtilis M45(Rec^-), wild-type H17(Rec^+) 2,500 μg/disc (with and without metabolic activation)[6)]

<Overseas reports>
Ames test: Negative; TA100, TA98, TA102, 100-50,000 μg/plate (without metabolic activation)[8), 9)]
SCE: Negative; 125 μg/mL[4), 8), 9)]
Chromosomal aberration test: Negative; 125 μg/mL[4), 8), 9)]

4) Others

There have been no reports that toxicity is a concern.

5) Position in overseas assessment reports

JECFA evaluated taurine as "amino acids and related substances" as a group according to the flavor evaluation process. It states that there are no safety concerns with taurine at the current levels of intake because the estimated intake of taurine is below the acceptable level of the structural class I.[4)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Doull, J., Plzak, V. & Brois, S.J. (1962) A Survey of Compounds for Radiation Protection, Arlington, Virginia: United States Air Force, Armed Services Technical Information Agency.pp.1 – 124.

2) Takahashi, H., Mori, T., Fujihira, E. & Nakazawa, M. (1972) Long-term feeding of taurine in rats. Pharmacometrics, 6,529-534.

3) Hwang, D.F., Hour, J.L. & Cheng, H.M. (2000) Effect of taurine on toxicity of oxidized fish oil in rats. Food Chem. Toxicol., 38,585 – 591.

4) JECFA: Safety evaluation of certain food additives, WHO FOOD ADDITIVES SERIES: 54
(References 1-3 are cited in Reference 4)

5) Ishidate, et al.: Toxicology Forum 9, 628-633 (1986)

6) Food Safety Commission of Japan: Feed additive assessment report, June 2008

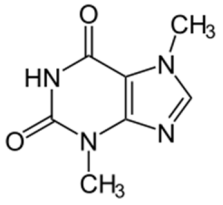
7) Laidlaw SA et al., Antimutagenic effects of taurine in a bacterial assay system. Cancer Res. 49, 660-6604(1989).

8) FDA: GRAS Notice 586, Taurine

9) Cozzi R et al., Taurine and ellagic acid: two differently acting natural antioxidants. Environ. Mol. Mutag., 26,248-254(1995).

Theobromine

English name: Theobromine
CAS No. 83-67-0
JECFA No. Not available
Other names: Not available

Structural formula: 

1. Origin and method of preparation

Theobromine is obtained from the seed of *Theobroma cacao* LINNE of the family Sterculiaceae, the seed of *Cola acuminata* SCHOTT et ENDL. of the family Sterculiaceae, or the leaf of *Camellia sinensis* O.KZE. of the family Theaceae by extraction and separation with water or ethanol. Its component is theobromine.

2. Major use

Bittering agent, etc.

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

Rats, mice, and hamsters (strains unknown) received 28-day dietary administration with theobromine.[1)] Body weight, organ weight, and histopathological examination of some organs were examined. In rats, a decrease in thymus weight was observed at the lowest dose of 0.2% (male: 94 mg/kg BW/day, female: 110 mg/kg BW/day) and higher administered groups, associated with histopathological changes in the 0.6% group. In mice, an increase in the death rate and testicular toxicity associated with histopathological changes were observed in the 1.2% (1,800 mg/kg) group. In hamsters, no toxic effects were observed, even at the highest dose of 1.0% (1,027

mg/kg) group.
Rats (strain unknown) received 49-day dietary administration with theobromine. The results showed a no observed adverse effect level (NOAEL) for testicular toxicity of 88 mg/kg BW/day.[1)] One testis was removed on Day 49. After another 49 days recovery period, no damage was observed in the remaining testis in the 88 mg/kg group, while irreversible toxicity was observed in the 244 and 334 mg/kg groups.
In rats and rabbits, delayed skeletal formation was observed in offspring, and the no observed adverse effect level (NOAEL) was 48 and 21 mg/kg BW/day, respectively.[1)] As a result of reviewing the reproductive toxicity of theobromine, it was concluded that the no observed adverse effect level (NOAEL) in animals was 50 mg/kg BW/day.[1)]

3) Mutagenicity study

No information available. Theobromine is a metabolite of caffeine, and it is considered that the genotoxicity evaluation of caffeine applies.[1)] Caffeine, when used as a flavoring substance, is evaluated to have no concern about genotoxicity.[1)]

4) Others

In rats and rabbits, the no observed adverse effect level (NOAEL) for delay in skeletal development of the offspring was 48 and 21 mg/kg BW/day, respectively.[1)] After reviewing the reproduction and developmental toxicity of theobromine, it was concluded that the no observed adverse effect level (NOAEL) was 50 mg/kg BW/day in animals.[1)]
A randomized, double-blind, placebo-controlled human intervention test was performed in 84 healthy volunteers with single oral doses of theobromine at 250, 500, and 1,000 mg.[1)] Headache and nausea were observed as adverse effects, particularly often in the 1,000 mg administered group. Decreased heart rate was observed in the administered groups with 500 mg and higher. The level of 250 mg/person could be considered as a no observed adverse effect level (NOAEL) in this study.
A placebo-controlled, double-blind cross-over intervention test was performed in 42 healthy volunteers who received 106 and 979 mg/day (1.5 and 14.0 mg/kg BW/day, respectively) of theobromine in beverages for 3 weeks, followed by a 2-week washout period.[1)] Significant changes in blood pressure, etc., were observed in the 14.0 mg/kg BW/day administered group. In addition, a placebo-controlled, double-blind randomized test was performed in 38 volunteers who received 150,

850, and 1,000 mg/day (2.1, 12.1, and 14.2 mg/kg BW/day, respectively) of theobromine in beverages for 4 weeks.[1)] The most commonly reported adverse effects were nausea, emesis, headache, and diarrhea, and these were observed in the 12.1 mg/kg BW/day and higher administered groups.

5) Position in overseas assessment reports

There are no reports in EFSA (2017) that evaluated toxic effects of theobromine by long-term exposure in humans, and therefore the safety evaluation of theobromine was performed based on the data of caffeine.[1)] The reference dose of theobromine as a flavoring agent was set as 0.6 mg/kg BW/day, and 0.3 mg/kg BW/day for children, adolescents, and pregnant or nursing women, based on the reference dose of caffeine (5.7 mg/kg BW/day, and 3 mg/kg BW/day for children, adolescents, and pregnant or nursing women) and the fact that approximately 11% of the oral intake of caffeine is converted to theobromine.[1)] It is stated that the values are based on the assumption that adverse effects of caffeine intake are mostly the effects of theobromine after metabolism, while in fact the pharmacological activity of theobromine is lower than that of caffeine and the values assume maximal adverse effects of theobromine.[1)] While the estimated intake of theobromine from meals (including from caffeine) was below 0.3 mg/kg BW/day in almost all age groups, the 95th percentile in children aged 3-10 reached 0.5 mg/kg BW/day. However, the reference dose is conservative as described above, and intake as a flavoring agent was at almost negligible levels, and therefore it is evaluated that there are no safety concerns with the use of theobromine as a flavoring agent.[1)]

A dose of 1.5-2.1 mg/kg BW/day theobromine has been reported as the dose at which no adverse effects are observed in more than one clinical test in humans; it is used only as reference information because the exposure periods were short and the studies were not for safety evaluation.

IARC (1991) classifies the carcinogenicity of theobromine as Group 3 (Not classifiable for carcinogenicity in humans) due to insufficient data.[2)]

4. Conclusion

There has been a very limited history of use of theobromine in Japan. Therefore, there are no safety concerns with theobromine considering the current situation of distribution and intake.

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Copper

English name: Copper
CAS No. 7440-50-8
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

^{63}Cu, ^{65}Cu

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

Copper chloride:	Rat oral	LD_{50} = 140 mg/kg BW[1)]
Copper sulfide (anhydrous):	Rat oral	LD_{50} = 300 mg/kg BW[1)]
	Rabbit oral	LD_{100} = 50 mg/kg BW[2)]
	Dog oral	LD_{100} = 165 mg/kg BW[3)]
	Sheep oral	LD_{100} = 9-20 mg/kg BW[4)]
	Horse oral	LD_{100} = 125 mg/kg BW[5)]
Copper sulfide (hydrate):	Rat oral	LD_{50} = 960 mg/kg BW[6)]
Copper nitrate:	Rat oral	LD_{50} = 940 mg/kg BW[1)]
Copper acetate:	Rat oral	LD_{50} = 710 mg/kg BW[6)]
Copper carbonate:	Rat oral	LD_{50} = 159 mg/kg BW[1)]
Copper oxide:	Rat oral	LD_{50} = 470 mg/kg BW[6)]

2) Repeated-dose toxicity study

Mice (strain unknown) received 14-day treatment with copper sulfate in drinking water at a concentration from 0.006% (1.52 mg/kg BW/day) to 1.6% (407 mg/kg BW/day). The results showed no toxic effects in groups at 0.2% or less, while delayed growth compared to the control group was observed in groups at 0.4% and higher and significant weight loss was observed in the 0.8 and 1.6% groups. Moreover, death was observed in 80% of animals in the 1.6% group.[7)]

Rats (strain unknown) aged 21 days received 4-week dietary administration with copper sulfate at a concentration of 0, 500, 1,000, 2,000, and 4,000 ppm. The results showed that food intake decreased in the administered groups, and the mean daily intake of copper was 5, 8, 11, and 8 mg/rat in the respective groups. All animals died within 1 week in the 4,000 ppm group, and 1 of 8 animals in the 2,000 ppm group died in the 4th week. The cause of death in the 4,000 ppm group was considered to be decreased food intake. In the 500 ppm group, the growth rate decreased mildly and the copper concentration in the liver increased mildly while no other abnormalities were found.[8)]

Male rats (strain unknown, 90-110 g) aged 90 days received 20-day repeated oral administration with copper sulfate at a dose of 100 mg/kg BW/day and were euthanized after fasting for 24 hours after the final day of treatment. The results showed significant body weight loss in the administered group, and hematological examination showed significant decreases in HGB, HCT, and RBC. Histopathological examination showed hepatocyte necrosis in the centrilobular zone and fibrosis in the peripheral zone of the liver as well as necrosis and hyperplasia of renal tubules in the medulla region of the kidney. Copper deposition was observed in the liver and was severe in hepatocytes in the centrilobular zone and mild in the peripheral zone. Copper deposition in the kidney was observed in the epithelial cells of the distal tubules, interstitium, and medulla.[9)]

Male and female rats (strain unknown) received 44-week dietary administration with copper sulfide at 0.135 and 0.406% (0.053 and 0.16% as copper, respectively) or with copper gluconate at a concentration of 1.14% (0.16% as copper) (25 animals per group). The results showed weight gain suppression from the 26th week in the 0.406% copper sulfide group and the 1.14% gluconate group. The death rate increased in the 0.406% copper sulfide group, and 90% died in the copper gluconate group. Hematological examination and urine analysis showed an increase in the blood nonprotein nitrogen concentration in the 0.406% copper sulfide group and the copper gluconate group and no change in the ascorbic acid value. Hypertrophy of the uterus, the ovary, and the seminal vesicle was observed in the copper gluconate group. Enlargement, dilation, hypertrophy, and ulcer of the stomach, discoloration (bloody coloring) of the intestinal mucosa, and discoloration (copper coloring) of the kidney and the liver were observed in the copper gluconate group and the 0.406% copper sulfide group. Histopathological examination showed abnormalities in the liver, kidney, and testis in the copper gluconate group and the

0.406% copper sulfide group. An increase in the copper concentration in tissues was observed in the liver, kidney, and spleen in all administered groups, and it was the most significant in the liver. The iron concentration in tissues decreased in the copper gluconate group and the 0.406% copper sulfide group.[10)]

Weaned male rats (strain unknown) received dietary administration with copper sulfide at a concentration of 0.2% as copper and were euthanized after 1, 2, 3, 6, 9, and 15 weeks to investigate the effects on the liver, kidney, and the blood enzyme activities. Changes in the liver and kidney were observed in 3 stages. At first the copper concentration in tissues increased gradually, showing cell damage; the copper concentration in tissues then reached the maximum (liver: 0.3360%, kidney: 0.1447%), showing severe cytolysis; and finally, the copper concentration in tissues decreased (liver: 0.2144%, kidney: 0.114%), leading to tissue regeneration and restoration. It was therefore considered that there are some adaptive metabolic responses in rats to administration with copper at a high concentration. The copper concentration in the liver and kidney of the control group was 0.0018% and 0.0034%, respectively.[11)]

Rabbits received 105-day dietary administration with copper acetate at a concentration of 0.2%. The results showed induration and necrosis of the liver at various levels. The copper concentration in the liver was from 0.097 to 0.237%. The frequency of hepatic cirrhosis increased with extension of the administered period.[12)]

Pigs (2,000 animals) received 10.5-month dietary administration with copper sulfide at a copper concentration of 0.07%. As a result, 400 pigs died, and symptoms immediately before death were anorexia, body weight loss or gain suppression, debility, and pallor. Visual examination and histopathological examination showed pigmentation (yellowish brown to orange) and centrilobular necrosis in the liver, ulcers of the cardiac region of the stomach, watery blood, reddening of the bone marrow, and myeloid metaplasia in the spleen. Hematological examination showed microcytic hypochromic anemia, an increase in the red blood cell glutathione concentration, an increase in serum total iron binding capacity, and a decrease in the serum iron concentration. The copper concentration in the liver of the animals that showed the changes was from 0.01 to 0.017%, whereas it was from 0.00008 to 0.00063% in the control group.[13)]

Sheep aged 6 to 12 weeks received dietary administration with copper at a concentration of 0.008%. The results showed spongy degeneration of the central nervous system, especially the midbrain, pons, and cerebellar white matter.[14)]

Male and female dogs received 1-year dietary administration with copper gluconate at a concentration of 0.012, 0.06, and 0.24% (the respective doses are 3, 15, and 60 mg/kg BW/day). The results showed mild changes in liver function in 1 of 12 animals in the 0.24% group, which disappeared after a washout period of 12 weeks. In the 0.24% group, copper accumulation was observed in the liver, kidney, and spleen. No deaths, visual changes, or histological changes related to administration were observed in any of the treatment groups.[15)]

3) Mutagenicity study

No information on mutagenicity is available.
The genotoxicity of copper gluconate [16), 17)], copper sulfate [17)], and copper iodide [18)] are reported as negative based on the Ames test and a test on yeasts.

Copper gluconate
Ames test: Negative; TA97, TA102, 10-1,000 μg/plate (with and without metabolic activation)[17)]
Copper sulfate
Ames test: Negative; TA97, TA102, 10-1,000 μg/plate (with and without metabolic activation)[17)]

4) Others

Green hair and green urine have been reported in patients with copper intoxication due to occupational exposure, and copper fume fever or brass chills due to inhalation of dust or fumes have also been reported (exposure level unknown).[18)] Jaundice and severe hemolytic anemia associated with increases in serum copper, ceruloplasmin, and aspartate transaminase have been reported in children who received repeated application of copper sulfate to severe and extended skin burns (exposure level unknown).[19)] Headache, chills, nausea, perspiration, and malaise during or after dialysis have been reported as copper poisoning in dialysis patients (exposure level unknown).[19-22)] It has been reported that such parts as tubes containing copper were included in the dialysis devices and that these symptoms disappeared by replacing them with other parts.[23)]

The case study of acute copper poisoning by Chuttani et al. includes 48 patients who had emergency hospitalization and 5 cases of death (exposure route and exposure level unknown).[24] Although the accurate amount of copper ingestion for each case of hospitalization is not clear, it was considered to be 1-112 g based on self-reporting by patients. Clinical symptoms observed were: metallic taste, burning sensation in the upper abdomen, nausea, and green vomit in all cases; diarrhea, hemoglobinuria, and hematuria in 30% of the cases; and jaundice, oliguria, and anuria in 8% of the cases. Seven of 48 cases of the hospitalized patients died within 24 hours after copper ingestion because of shock or complications in the liver or kidney. The copper concentration in whole blood correlated with the severity of poisoning (mild symptoms: copper concentration 287 ± 126.8 ug/dL, severe symptoms: copper concentration 798 ± 396 ug/dL). Histopathological examination showed ulcers in the stomach and the intestinal mucosa and dilation of the central vein in the liver, and cell death at various levels and bile thrombus were observed in the liver. Congestion in glomeruli, swelling and necrosis of the epithelium of renal tubules, and hemoglobin casts were observed in the kidney.

In the report by Singh, et al., continuous increase in the copper concentration in whole blood was observed in 40 cases of copper sulfide poisoning; hemolysis related to the high blood copper concentration was observed in 18 cases (40%) among them; and severe intravascular hemolysis was observed in 3 of 4 cases of death (exposure route and exposure level unknown).[25]

Stain, et al. have reported the case of a 44-year-old female with acute copper sulfide poisoning.[26] In this case, 10% copper sulfide at 2 and 10 cc (2 g in total) (exposure route unknown) as an emetic for the treatment of alcohol-diazepam poisoning was administered. Autopsy showed acute hemorrhagic necrosis in the small intestine and indistinct fused yellow spots in the liver, and the copper concentration in the liver was reported to be 0.075% (the normal copper concentration in the liver is 0.0008%). Acute tubular necrosis and casts were observed in the kidney.

Chugh, et al. have reported that acute renal failure was observed in 11 of 29 cases of acute copper sulfide poisoning (ingested amount: 1-50 g) (exposure route unknown).[27] Serious intravascular hemolysis was observed in all 11 cases with acute renal failure, and this was considered to be the most relevant factor. Histopathological examination showed the disappearance and regeneration of renal

tubules, edema of the interstitium, and sporadic inflammatory cell infiltration. Dilated renal tubules lined with squamous epithelium were observed in cases which recovered from symptoms.

The World Health Organization (WHO) concluded that the lethal dose of copper salts including copper sulfide, copper chloride, copper carbonate, copper hydroxide, and basic copper chloride in humans was approximately 200 mg/kg BW.[28)] It is clear that there are marked individual differences in copper sensitivity.

Copper poisoning due to exposure via food or beverages has also been reported. In the report by Nicholas, 20 workers were exposed to copper via tea, and nausea, emesis, and diarrhea were observed as the symptoms (exposure level unknown).[29)] The source of pollution was a gas water heater, and the copper concentration in the tea was 0.003%.

McMullen reported 10 cases that were exposed to copper via soft drinks (orange squash and lime juice cordial) (exposure level unknown).[30)] Copper tubes plated with chromium were attached to the spouts of these soft drinks, and the tubes were discolored to green. The copper concentration was 0.019% in the orange squash and 0.0222% in the lime juice cordial. It was considered that copper had dissolved in the tube because these soft drinks were acidic. Witherell, et al. have also reported gastroenteritis caused by exposure to copper via an acidic beverage (exposure level unknown).[31)]

Reports on chronic copper poisoning are limited. A case suspected of chronic copper poisoning has been reported by Salmon, et al., in which behavior changes, diarrhea, and progressive marasmus were observed in a 15-month-old boy for 5 weeks before hospital admission (exposure route and exposure level unknown).[32)]

In the report by Pratt, et al., 7 persons received copper gluconate as copper at a dose of 10 mg/person/day for 12 weeks. The results showed no effect on copper concentrations in the serum, urine, and hair, or the serum zinc, Mg, HCT, triglyceride, aspartate transaminase, alanine transaminase, lactate dehydrogenase, CHO, and ALP levels. Symptoms such as nausea, diarrhea, and heartburn were of a similar degree as in the control group.[33)]

5) Position in overseas assessment reports

JECFA evaluates copper as a food pollutant, and the ADI (acceptable daily intake) is not established, while the PMTDI (provisional maximum tolerable daily intake) is 0.05-0.5 mg/kg BW.[34] It is stated that the copper concentration in food meets the nutritional requirement (adults: 2-3 mg/day, children: 0.5-0.7 mg/day), that copper is not carcinogenic in humans and animals, and that copper chloride does not show toxicity in rodent fetuses. It is also stated that copper is unlikely to show adverse effects in areas of high exposure to copper except for patients with Wilson's disease and that copper accumulation is unlikely to show adverse effects.

The EU established the tolerable upper intake level (UL) as 5 mg/person/day with an uncertainty factor of 2 considering potential individual differences based on the results of a report by Pratt, et al., in which no toxic effects were observed after 12-week treatment with copper at 10 mg/person/day.[33] In addition, lower ULs are separately established by age group for humans aged 17 years and younger.[35]

4. Conclusion

The Food Safety Commission of Japan has performed food safety assessment for copper gluconate, and the results are described as shown below.[37]

In Japan, glucono-delta-lactone, gluconic acid, zinc gluconate, potassium gluconate, calcium gluconate, ferrous gluconate, and sodium gluconate, in addition to copper gluconate, are designated as gluconate food additives. JECFA evaluated gluconates (glucono-delta-lactone, calcium gluconate, magnesium gluconate, potassium gluconate, and sodium gluconate) in 1998 as ADI “not specified”.

The UL for copper was evaluated in consideration thereof that it is appropriate to evaluate copper gluconate as copper intake and that copper is an essential element for humans. The LOAEL is not established for copper because no reports were found concerning side effects when adults receive copper.

When humans receive 10 mg copper gluconate daily for 12 weeks, no effects were observed. The Institute of Medicine (IOM) of the United States and the EU evaluate this value to be the NOAEL.

JECFA does not establish the ADI for copper, while the NOEL was evaluated to be approximately 5 mg/kg BW/day in a dog 1-year repeated-dose test in 1982, and the provisional MTDI was evaluated to be 0.05-0.5 mg/kg based on this.

The UL in Japan has been established to be 9 mg/person/day so far, which is smaller than the NOAEL of 10 mg/person/day in the 12-week administration test in humans, and changing this is considered unnecessary based on the information obtained this time. The UL for copper gluconate was therefore evaluated to be 9

mg/person/day as copper.
Since the UL evaluated in this study is for adults, appropriate precautions should be taken to prevent excessive copper intake in infants to children.
It is also stated that care must be taken to prevent excessive copper intake in adults in the future, and that the actual intake must be ascertained, and measures should be taken based on the results.

5. References

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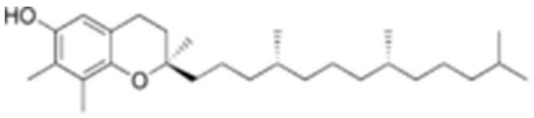
d-γ-Tocopherol

English name: *d*-γ-Tocopherol
CAS No. 54-28-4
JECFA No. Not available
Other names: γ-Vitamin E

Structural formula: 

1. Origin and method of preparation

d-γ-Tocopherol is separated from vegetable oil obtained from oilseeds or mixed tocopherol concentrate (which consists mainly of *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol obtained from vegetable oil) and consists mainly of *d*-γ-tocopherol. It may include edible fat and oil.

2. Major use

Antioxidant, nutrition enhancer

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

There is no information on studies with *d*-γ-tocopherol, which is the natural form, and only information on studies using *dl*-γ-tocopherol, which is the synthetic form, is available.

Wistar rats (6 males and females each per group) received 13-week treatment with *dl*-γ-tocopherol at 0, 800, and 1,600 (females only) mg/kg BW/day using soybean oil as the solvent by gavage in addition to an untreated group. Significant decreases in platelet count, total lipids, and T-CHO and an increase in CK were observed in both administered groups of females. Increases in BIL, ALT, and the absolute and relative weight of the liver and spleen were observed in females in the 1,600 mg/kg group. In the male 800 mg/kg group (the only administered group), increases in CK and ALT were observed. Aggregation of macrophages in the mesenteric lymph

nodes was observed in male and female administered groups. The authors judged that aggregation of macrophages and increased liver weight were physiological and adaptive reactions, respectively, to the test substance. The NOAEL could not be obtained.[1)]

Syrian golden hamsters (10 males and females each per group) received *dl*-γ-tocopherol at 0 and 2,000 mg/kg BW/day using soybean oil as the solvent by oral gavage for at least 28 days in addition to an untreated group. Significant extension of APTT and PT was observed in male and female administered groups. Significant decreases in total lipids, T-CHO, and PL were observed in the female administered group. The authors describe these as well-known effects of tocopherol. An increase in Cre was observed in the male treatment group.[2)]

Syrian golden hamsters (10 females per group) received 13-week treatment with *dl*-γ-tocopherol at 0 and 800 mg/kg BW/day using soybean oil as the solvent by oral gavage in addition to the untreated group. Significant extension of APTT and PT as well as significant increases in BIL, ALP, and γ-glutamyltransferase were observed. Aggregation of macrophages was also observed in the mesenteric lymph nodes. Although the number of animals and administered groups were limited,[1)] the EFSA panel considers that the NOAEL in the study is not more than 800 mg/kg BW/day.[3)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[4)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

There are no reports of toxicity concerns about this existing additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA established the group ADI of 0.15-2 mg/kg BW/day based on the data of *dl*- and *d*-α-tocopherol. It is stated that it contains *d*-alpha-, *d*-beta-, *d*-gamma-, and *d*-delta-tocopherols as mixed tocopherol concentrate.[5)]

The European Food Safety Authority (EFSA) states that the ADI cannot be

established because of limited availability of toxicity data. However, it states that vitamin E is a required nutrient that is widely taken as food and there are no safety concerns with tocopherols (E 306-E 309, tocopherol-rich extract of natural origin (E 306), synthetic α-tocopherol (*all-rac-α-tocopherol; dl-α-tocopherol; E 307), synthetic γ-tocopherol (dl-γ-tocopherol; E 308) and synthetic δ-tocopherol*) at the current usage and concentration of use as food additives.[3)]

4. Conclusion

It is considered that there are no safety concerns about the existing additive distributed in Japan.

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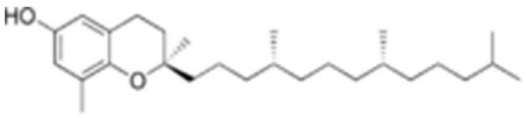
3) EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2015.Scientific Opinion on the re-evaluation of tocopherol-rich extract (E 306), α-tocopherol (E 307), γ-tocopherol (E 308) and δ-tocopherol (E 309) as food additives. EFSA Journal 2015;13(9):4247,118 pp.doi:10.2903/j.efsa.2015.4247 Available online: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4247 (References 1 and 2 are cited in Reference 3)

4) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

5) JECFA: 30th meeting (1986) WHO Food Additives Series 21, TRS 751 http://www.inchem.org/documents/jecfa/jecmono/v21je05.htm http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/additive-469-m1.pdf

d-δ-Tocopherol

English name: *d*-δ-Tocopherol
CAS No. 119-13-1
JECFA No. Not available
Other names: δ-Vitamin E

Structural formula: 

1. Origin and method of preparation

d-δ-Tocopherol is separated from vegetable oil obtained from oilseeds or mixed tocopherol concentrate (which consists mainly of *d*-α-tocopherol, *d*-β-tocopherol, *d*-γ-tocopherol, and *d*-δ-tocopherol obtained from vegetable oil) and consists mainly of *d*-δ-tocopherol. It may include edible fat and oil.

2. Major use

Antioxidant, nutrition enhancer

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated-dose toxicity

3) Mutagenicity study

An Ames test, a chromosomal aberration test, an *in vivo* micronucleus test, and an SCE test were performed, and all the results were reported to be negative.[1), 2)]

<Domestic reports>[1)]
Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

<Overseas reports>[2)]
Ames test: Negative; TA100, TA1535, TA98, TA97, TA102, 5-5,000 μg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; Human lymphocytes, 75-1,800 μg/mL (with and without metabolic activation)
Bone-marrow micronucleus test: Negative; Mouse, 30 or 1,000 mg/kg BW/day, 50-week oral
SCE test: Negative; Mouse, 30 or 1,000 mg/kg BW/day, 50-week oral

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA established the group ADI of 0.15-2 mg/kg BW/day based on the data of *dl*- and *d*-α-tocopherol. It is stated that it contains *d*-alpha-, *d*-beta-, *d*-gamma-, and *d*-delta-tocopherols as mixed tocopherol concentrate.[3)]
Although the European Food Safety Authority (EFSA) cannot establish an ADI due to the limited toxicity data available, it states that vitamin E is a necessary nutrient that is widely consumed as foods and that there is no safety concern for tocopherols (E 306-E 309), tocopherol-rich extract of natural origin (E 306), synthetic α-tocopherol (*all-rac-α-tocopherol; dl-α-tocopherol; E 307*), *synthetic γ-tocopherol* (*dl-γ-tocopherol; E 308*) *and synthetic δ-tocopherol*) in their current use as food additives and at the concentrations used.[2)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

2) EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), 2015.Scientific Opinion on the re-evaluation of tocopherol-rich extract (E 306), α-tocopherol (E 307), γ-tocopherol (E 308) and δ-tocopherol (E 309) as

food additives. EFSA Journal 2015;13(9):4247,118 pp.doi:10.2903/j.efsa.2015.4247 Available online: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4247

3) JECFA: 30th meeting (1986) WHO Food Additives Series 21, TRS 751, http://www.inchem.org/documents/jecfa/jecmono/v21je05.htm http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/Monograph1/additive-469-m1.pdf

Transglucosidase

English name: Transglucosidase
CAS No. 9030-12-0
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Transglucosidase is obtained from the culture of filamentous fungi (only *Aspergillus niger* and *Aspergillus usamii*) or bacteria (only *Sulfolobus solfataricus*) and is an enzyme that hydrolyzes the glucoside bond of maltose and oligosaccharide and simultaneously transfers the glucosyl bond. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated-dose toxicity

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[1)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

5) Position in overseas assessment reports

The US FDA states that transglucosidase obtained from the culture of filamentous fungi (*Trichoderma reesei*) to which the transglucosidase gene derived from *Aspergillus niger* is introduced is GRAS (GRN 315 and 703).[2, 3)]

Food Standards Australia New Zealand (FSANZ) states that ”From the available information, it is concluded that the use of transglucosidase from this source as a processing aid would pose no significant public health and safety risk” concerning transglucosidase derived from *Aspergillus niger* strains that have not been genetically modified.[4)]

The French Food Safety Agency (AFSSA) judges that there is no health risk for consumers with extension of the scope of approval for α-glucosidase (transglucosidase) derived from non-genetically modified *Aspergillus niger* strains to be used in the manufacturing of cooked food including starch when it is used under the conditions proposed by the applicant.[5)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

2) FDA: GRAS Notice GRN 315.U.S.Food and Drug Administration.2010.

3) FAD: GRAS Notice GRN 703.U.S.Food and Drug Administration.2017.

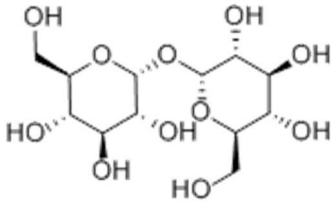
4) Final Assessment Report: Application A466 Food Enzyme, Transglucosidase. Food Standard Australia New Zealand.2003.

5) Agence française de sécurité sanitaire des aliments.2009.

Trehalose

English name: Trehalose
CAS No. 99-20-7 (anhydride)
6138-23-4 (dihydrate)
JECFA No. Not available
Other names: Not available

Structural formula: (anhydride)



1. Origin and method of preparation

Trehalose is obtained from the culture filtrate or the body of basidiomycetes (such as *Agiricus*), bacteria (such as *Arthrobacter*, *Brevibacterium*, *Pimelobacter*, *Pseudomonas, Thermus*), or yeasts (such as *Saccharomyces*) by extraction with water or alcohol, by separating it from enzymatically saccharified starch solution, or by enzymatic treatment with maltose. Its component is trehalose.

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

Acute toxicity studies were performed in mice, rats, and dogs by oral administration. No deaths were observed and no toxic effects caused by the test substance were observed in any of the studies.

Mouse (CD-1) oral	LD_{50} > 5,000 mg/kg BW[1)]
Rat oral	LD_{50} > 16,000 mg/kg BW[2)]
Beagle dog oral	LD_{50} > 5,000 mg/kg BW[3)]

2) Repeated-dose toxicity study

In a 13-week repeated-dose test in HanIbm.NMRL mice (20 males and females each per group) received 13-week by dietary administration with trehalose (99.2% purity) at concentrations of 0, 5,000, 15,000, and 50,000 mg/kg (equivalent to 0, 760, 2,200, and 7,300 mg/kg BW/day, respectively in males and 0, 910, 2,700, and 9,300 mg/kg BW/day, respectively in females). Toxic effects attributable to the test substance were not observed, and the NOEL was considered to be 7,300 mg/kg BW/day, which was the highest dose for males.[4)]

In a 14-day repeated-dose test in Beagle dogs (3 males and females each per group) by oral administration of trehalose (purity not described) capsules at a dose of 5,000 mg/kg BW/day, no toxicological effects caused by administration with the test substances were observed.[5)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[6)]

Ames test: Negative; TA100, TA1535, WP2*uvr*A, TA98, TA15373, 10-5,000 µg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; CHO cells, 1,250-5,000 µg/mL (with and without metabolic activation)
Micronucleus test: Male and female mice: Negative; 1,250-5,000 mg/kg BW

4) Others

A 2-generation reproductive toxicity study was performed in Wistar rats. Toxic effects attributable to the test substance were not observed, and the NOEL was considered to be 5,000 mg/kg BW/day, which was the highest dose.[7)]
Developmental toxicity studies were also performed in Wistar rats and New Zealand White rabbits. Toxic effects attributable to the test substance were not observed in either study, and the NOEL was considered to be 6,900 mg/kg BW/day and 2,800 mg/kg BW/day, respectively, which were the highest doses.[8), 9)]

5) Position in overseas assessment reports

JECFA evaluates it as ADI not specified.[10)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Atkinson, J.E. & Thomas, B.J. (1994a) An acute toxicity study of trehalose in the albino mouse. Unpublished report No.434 from Frederick Research Centre, Frederick, Maryland, USA. Submitted to WHO by Bioresco Ltd, Switzerland.

2) McRae, L.A. (1995) Trehalose crystals. Acute oral toxicity to the rat. Unpublished report from Huntingdon Research Centre Ltd, Huntingdon, United Kingdom. Submitted to WHO by Bioresco Ltd, Switzerland.

3) Atkinson, J.E. & Thomas, B.J. (1994c) An acute toxicity study of trehalose in the beagle dog. Unpublished report No.436 from Frederick Research Centre, Frederick, Maryland, USA. Submitted to WHO by Bioresco Ltd, Switzerland.

4) Schmid, H., Biedermann, K., Luetkemeier, H., Weber, K. & Wilson, J. (1998) Subchronic 13-week oral toxicity (feeding) study with trehalose in mice. Unpublished report (RCC Project 639213) from Research Consulting Company, Ittingen, Switzerland. Submitted to WHO by Bioresco Ltd, Switzerland.

5) Atkinson, J.E. & Thomas, B.J. (1994f) A 14-day toxicity study of trehalose in the beagle dog (Study No.460), Unpublished report No.460 from Frederick Research Centre, Frederick, Maryland, USA. Submitted to WHO by Bioresco Ltd, Switzerland.

6) JECFA: WHO Technical Report Series 46 (2000)
(References 1-5 and 7-10 are cited in Reference 6)

7) Wolterbeek, A.P.M. & Waalkens-Berendsen, D.H. (1999a) Oral two-generation r eproduction study with trehalose in rats. Unpublished report No. V99.280 from TNO Nutrition and Food Research Institute, Zeist, Netherlands. Submitted to WHO by Bioresco Ltd, Switzerland.

8) Waalkens-Berendsen, D.H. (1998) Oral embryotoxicity/teratogenicity study with trehalose in rats. Unpublished report No. V98.551 from TNO Nutrition and Food Research Institute, Zeist, Netherlands. Submitted to WHO by Bioresco Ltd, Switzerland.

9) Wolterbeek, A.P.M. & Waalkens-Berendsen, D.H. (1999b) Oral embryotoxicity/teratogenicity study with trehalose in New Zealand white rabbits. Unpublished report No.V98.797 from TNO Nutrition and Food Research Institute, Zeist, Netherlands. Submitted to WHO by Bioresco Ltd, Switzerland.

10) JECFA: WHO Technical Report Series 901 (2001)

Peroxidase

English name: Peroxidase
CAS No. 9003-99-0
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Peroxidase is an enzyme obtained from cucumber (*Cucumis sativus* L.), horseradish (*Armoracia rusticana* P. Gaertn, B. Mey, and Scherb), Japanese radish (*Raphanus sativus* L.), or soybean (*Glycine max* (L.) Merr.), or the culture of basidiomycetes (*Coprinus cinereus*), filamentous fungi (only genus *Alternaria*, *Aspergillus oryzae* and, genus *Oidiodendron*), actinomycetes (only *Streptomyces thermoviolaceus* and *Streptomyces violaceoruber*), or bacteria (only genus *Bacillus*), and reductively decomposes hydrogen peroxide. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

Wistar rats (10 males and females each per group) received 90-day dietary treatment with peroxidase from genetically modified *Aspergillus niger* (tox-batch: DBL.GRZ.0914) at a dose of 0, 0.7%, 2%, and 4% (equivalent to 6, 17, 35 mg TOS or 457, 1,306, 2,611 DBLU/g food, respectively). Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 2,300 mg/kg BW/day (approximately 2,000 mg TOS or 150,000 DBLU/kg BW/day).[1)]

3) Mutagenicity study

An Ames test and a chromosomal aberration test were performed, and all the results were reported to be negative.[2)]

Ames test: Negative; TA100, TA1535, WP2*uvr*A, TA98, TA1537, 62-5,000 μg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; Human lymphocytes, 625-5,000 μg/mL (with and without metabolic activation)

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

In 2012, the US FDA, based on the data from DSM, stated that peroxidase from genetically modified *Aspergillus niger* is GRAS for the intended conditions of use.[1),2)]

The European Food Safety Authority (EFSA) published scientific opinion concerning safety evaluation of peroxidase of food enzymes derived from the soybean hull.[3)] The peroxidase is intended for use in the baking process. Exposure to the total organic solids (TOS) of food enzyme from dietary intake based on the maximum recommended use level was estimated based on individual data of the Comprehensive European Food Consumption Database of the EFSA. This estimated exposure is approximately 10 times smaller than exposure to the soybean fraction corresponding to the TOS of the food enzyme based on the results of intake of all foods derived from soybeans. Since the food enzyme is derived from the edible part of the soybean, it meets the requirements for guidelines for the evaluation of food enzymes, and the “Scientific Panel on Food Contact Materials, Enzymes, Flavorings and Processing Aids” (CEF Panel) concluded that it is not necessary to provide toxicological data.
Possible allergenicity was evaluated by searching the Uniprot database (translator's note: database that provides information on protein sequences and functions) for similarity between the amino acid sequence of soybean peroxidase and that of known allergens, and no match was found. Peroxidase derived from the soybean hull is not listed as an allergen in allergen databases. However, several proteins in soybeans and soybean hulls are known as respiratory or food allergens.
The CEF panel concluded that the food enzyme does not cause safety concerns

under the conditions of its intended use based on the origin, manufacturing process, the composition and biochemical data provided, and estimated intake of the food enzyme derived from the edible part of the soybean. However, the CEF panel noted that the food enzyme may contain allergenic soybean protein and therefore adverse reactions cannot be excluded in persons who are highly sensitive to soybean allergies.[4)]

4. Conclusion

It was concluded that there are no safety concerns for its impact on human health in the current situation of use, although there is a possibility that it may be an allergen because its origin includes soybeans.

5. References

1) DSM (2011) Unpublished report submitted to FDA, United States.

2) FDA (2012): Agency response letter GRAS notice No.GRN 000402, https://wayback.archive-it.org/7993/20171031002315/ https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm332201.htm#main

3) EFSA Panel on food contact materials, enzymes, flavourings and processing aids (2017) Safety evaluation of the food enzyme peroxidase obtained from soybean (Glycine max) hulls. EFSA Journal, 15(12), 5519. http://www.efsa.europa.eu/en/efsajournal/pub/5119

4) Food Safety Commission of Japan: Information concerning food safety syu04850090149. (2017) http://www.fsc.go.jp/fsciis/foodSafetyMaterial/show/syu04850090149

Phytase

English name: Phytase
CAS No. 9001-89-2(6-Phytase)
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Phytase is obtained from the culture of filamentous fungi (only *Aspergillus niger*) and is an enzyme that degrades phytic acid. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Phytase is a collective term for enzymes that degrade phytic acid to release inorganic phosphorus and is used as a processing aid for starch.[1)]

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

SD rats (10 males and females each per group) received 13-week treatment with phytase (6-phytase, SP 938, PPQ 5938, produced by *Aspergillus niger* from the phytase gene separated from *Peniophora lycii*) at a dose of 0, 1, 3, or 10 mg/kg BW/day (equivalent to 0, 0.11, 0.32, or 1.07 mg TOS/kg BW, respectively). Toxic effects attributable to the test substance were not observed, and the NOEL was considered to be 10 mg/kg BW/day.[1)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test in cultured cells, and a micronucleus test were performed. Positive results in the Ames test and structural aberration in the chromosomal aberration test were observed. However, the positive Ames test

was considered to be false positive because the test substance contained histidine, and the micronucleus test results were negative. It is therefore considered that the test substance has no clastogenicity in the living body.[3)]

Ames test: False positive; 5,000 μg/plate. The results were positive for TA100 (with and without metabolic activation), TA1535 (with metabolic activation), and TA98 (with and without metabolic activation), which were considered to be false positive because histidine is contained in the test substance.
Chromosomal aberration test: Positive; Structural aberration (without metabolic activation); 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

No information available

5) Position in overseas assessment reports

In the Australian Food Standards Code, phytase derived from *Aspergillus niger* is evaluated and use as a food grade enzyme is allowed.[1)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Australia New Zealand Food Authority, Full Assessment Report, Application A371-Phytase as a processing aid.

2) Hayashi, et al.: Food hygiene and safety science 46, 177-184 (2005)

Phosphodiesterase

English name: Phosphodiesterase
CAS No. 9025-82-5
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Phosphodiesterase is an enzyme obtained from the culture of filamentous fungi (only *Aspergillus niger*, *Leptographium procerum*, and *Penicillium citrinum*) or actinomycetes (only *Streptomyces aureus, Streptomyces avermitilis, Streptomyces cinnamoneus, Streptomyces griseus, Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*), and it hydrolyzes the phosphodiester bond in nucleic acids, etc. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration.

2) Repeated-dose toxicity study

Wistar rats (10 males and females each per group) received 90-day treatment with phosphodiesterase derived from *Leptographium procerum* at doses of 0, 100, 300, and 1,000 mg TOS/kg BW using purified water as solvent by oral gavage.[1)] The results showed that toxic effects attributable to the test substance were not observed in any of the treatment groups, and the NOAEL was considered to be 1,000 mg TOS/kg BW/day (10,428 mg enzyme preparation/kg BW/day), which was the highest dose.

3) Mutagenicity study

An Ames test and a chromosomal aberration test in cultured cells were performed, and all the results were reported to be negative.[1)]

Ames test: Negative; TA100, TA1535, *E.coli* WP2*uvr*A, TA98, TA1537, 5,000 µg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; Human lymphocytes, 5,000 µg/mL (with and without metabolic activation)

4) Others

No information available

5) Position in overseas assessment reports

The US FDA (2014) states that phosphodiesterase I derived from *Leptographium procerum* is generally recognized as safe (GRAS).[1)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) FDA: GRAS Notice GRN 505: GRAS notification for phosphodiesterase I produced with a strain of *Leptographium procerum*. https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=505&sort=GRN_No&order=DESC&startrow=1&type=basic&search=505

Phospholipase

English name: Phospholipase
CAS No. 9043-29-2(Phospholipase A1)
JECFA No. Not available
Other names: Lecithinase
Structural formula:-

1. Origin and method of preparation

Phospholipase is an enzyme obtained from the pancreas of animals, cabbage (*Brassica oleracea L.*) or soybean (*Glycine max (L.)Merr*.) or the culture of basidiomycetes (only genus *Corticium*), filamentous fungi (only *Aspergillus oryzae* and *Aspergillus niger*), actinomycetes (only genus *Actinomadura*, *Kitasatospora sp*., genus *Nocardiopsis*, *Streptomyces avermitilis*, *Streptomyces cinnamoneus*, *Streptomyces griseus, Streptomyces lividans, Streptomyces polychromogenes, Streptomyces thermoviolaceus*, and *Streptomyces violaceoruber*) or bacteria (only genus *Bacillus)*, and it hydrolyzes lecithin. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

SD rats (10 males and females each per group) received 13-week repeated-dose treatment with phospholipase A1 liquid enzyme concentrate expressed by *Aspergillus oryzae* to which a gene from *Fusarium venenatum* was introduced (batch PPW 23436; dry content 6.8% w/w; TOS content 5.6% w/w; specific gravity 1.027 g/mL) at a dose of 0, 57.5, 190, and 575 mg TOS/kg BW by oral gavage. Toxic effects attributable to the test substance were not observed, and the NOEL

was considered to be 575 mg TOS/kg BW/day, which was the highest dose.[1)]

3) Mutagenicity study

An Ames test and a chromosomal aberration test in cultured cells were performed, and all the results were reported to be negative.[2)]

Ames test: Negative; TA100, TA1535, *E.coli* WP2*uvr*ApKM101, TA98, TA1537, 5,000 μg/plate (with and without metabolic activation)
Chromosomal aberration test: Negative; Human lymphocytes, 5,000 μg/mL (with and without metabolic activation)

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA evaluates phospholipase A1 (derived from the *Aspergillus oryzae* strain to which a gene from *Fusarium venenatum* is introduced) as a food additive and as "the ADI (acceptable daily intake) could not be established when used according to GMP specifications".[2)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Salanti, Z.(2004) Phospholipase-A 13-week oral (gavage) toxicity study in rats. Unpublished report No.54663 from Scantox, Ejby, Lille Skensved, Denmark. Submitted to WHO by Novozymes A/S, Bagsværd, Denmark.

2) JECFA: WHO Technical Report Series 947. WHO Food Additives Series 59(2007)

Polyphenol oxidase

English name: Polyphenol oxidase
CAS No. 9002-10-2
JECFA No. Not available
Other names: Phenolase
Structural formula:-

1. Origin and method of preparation

Polyphenol oxidase is an enzyme obtained from the culture of basidiomycetes (only genus *Cyathus*, *Polyporus cinereus, Pycnoporus coccineus, Polyporus versicolor*, and genus *Trametes*), filamentous fungi (only genus *Alternaria*, *Aspergillus niger,* genus *Coriolus*, and *Myrothecium verrucaria*), or actinomycetes (only *Streptomyces avermitilis*), and it oxidizes the hydroxyl group of polyphenols. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

Laccase expressed in *Aspergillus oryzae* to which a gene from Myceliophthora *Thermophila* is introduced (batch PPX 5720) has been investigated.[1)]
Rat oral $LD(C)_{50}$ > 12 mL/kg BW (equivalent to 39025LAMU or 2.07 g TOS/kg BW)

2) Repeated-dose toxicity study

CD rats (10 males and females each per group, 30-37 days of age) received 13-week treatment with laccase (batch PPX 5720) at a dose of 0, 0.1, 1, or 10 mg/kg BW/day (equivalent to 0, 325, 3,252, or 32,521 LAMU/kg BW/day or 0, 0.017, 0.17, or 1.7 g/kg BW/day TOS, respectively). No treatment-related changes were observed, and the NOEL was considered to be 10 mg/kg BW/day, which was the highest dose.[1, 2, 3)]

3) Mutagenicity study

No information available on mutagenicity.

4) Others

Patch tests were performed 9 times in which 100 volunteers received application with 0.5 mL of 10% (w/v) laccase solution (batch PPX 5720). The patch was 2x2 cm in size and was applied every 24 hours from Monday to Friday for 3 weeks. At 2 weeks after the final application, 10% (w/v) laccase solution (batch PPX 5720) was applied to the arm on which the patches were applied, removed after 24 hours, and the response was scored after 48 and 96 hours. In 3 of 100 subjects, the possibility of skin irritation was shown, although they also reacted to 1-2 kinds of other enzymes investigated simultaneously. None of the 3 subjects showed skin irritation in an examination after approximately 1 month.[1, 3)]

5) Position in overseas assessment reports

JECFA has evaluated it in 2003 as laccase expressed in *Aspergillus oryzae* to which a gene from *Myceliophthora Thermophila* was introduced and judged that ADI is not specified when it is used according to GMP.[1)]

The FDA has evaluated it in 2003 as laccase expressed in *Aspergillus oryzae* to which a gene from *Myceliophthora Thermophila* was introduced and judged that it is generally recognized as safe (GRAS) when it is used at the minimum amount required according to GMP for refreshment products (such as breath mints and chewing gum) and other foods.[4)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) JECFA: WHO Technical Report Series 922, Food Additives Series 52(2003)

2) Bolton N.(1997) Laccase, PPX 5720:Toxicity study by oral (gavage) administration to CD rats for 13 weeks. Unpublished report No. NLE186/9703426 from Huntingdon Life Sciences Ltd., Suffolk, England. Submitted to WHO by Novozymes A/S, Bagsvaerd, Denmark.

3) Brinch, D.S. & Pedersen, P.B. (2002) Toxicological studies on laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae*. *Regul. Toxicol. Pharmacol*., 35,296 – 307.

4) GRAS Notice GRN 122 (2003):GRAS notification for Laccase enzyme preparation produced by Aspergillus oryzae expressing the gene encoding a laccase from *Myceliophthora Thermophila* use in breath freshening products (such as breath mints and chewing gum) as an enzyme.

Muramidase

English name: Muramidase
CAS No. 9001-63-2
JECFA No. Not available
Other names: Lysozyme
Structural formula:-

1. Origin and method of preparation

Muramidase is an enzyme obtained from actinomycetes (only genus *Actinomyces* and genus *Streptomyces*) and bacteria (only genus *Bacillus*), and it hydrolyzes mucopolysaccharides. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

Reported as lysozyme hydrochloride.

Mouse oral LD_{50} > 4,000 mg/kg BW[1)]
Rat oral LD_{50} > 4,000 mg/kg BW[1)]

2) Repeated-dose toxicity study

Reported as lysozyme hydrochloride.
New Zealand rabbits (male, 2 groups, 10 animals per group) received lysozyme hydrochloride (500 mg/kg BW/day) or egg white (200 mg/kg BW/day) for 4 weeks (5 times/week) intravenously, and no toxic effects caused by lysozyme hydrochloride were observed.[1)]

3) Mutagenicity study

No information available on mutagenicity

4) Others

The formation of immunoglobulins was investigated in 15 full-term infants and 18 premature infants who received egg white lysozyme hydrochloride (10 mg/100 mL) mixed into formula to replace breast milk lysozyme hydrochloride (2 mg/mL) from Week 1 to Week 8. The control group was set to include 13 full-term infants and 13 premature infants who received formula, and 20 neonates who received breast milk. Lysozyme hydrochloride intake is equivalent to 60-90 mg/day assuming that formula intake by a neonate is 600-900 mL/day. No abnormalities were observed in the health status of the neonates, and no differences were observed in the production of serum immunoglobulins between the lysozyme hydrochloride group and the control group. Secretory IgA was observed in feces in the lysozyme hydrochloride group and the breast milk control group of full-term infants, while it was present in trace amounts in the other groups (the formula without lysozyme hydrochloride group of full-term infants, the formula without lysozyme hydrochloride group of premature infants, and the formula with lysozyme hydrochloride group of premature infants). Lysozyme hydrochloride treatment partially replaced the passive transfer of secretory IgA from breast milk. Antibodies were not observed in the serum of infants who received lysozyme hydrochloride.

5) Position in overseas assessment reports

JECFA [3)] considered that allergic reactions to lysozyme hydrochloride formed from egg white is weaker than those to other proteins such as egg white albumin and albumin in animals and humans. It was concluded based on available data for investigation that there are no concerns about the risk of additional intake in a small amount from cheese on consumers' health. Lysozyme hydrochloride is obtained from edible animal tissues that are generally used as food, and it can be designated as a class I enzyme and considered as food. Therefore, the use in food processing is considered to be acceptable when used according to the standards for manufacturing and quality control.

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Bianchi, C. (1982) Antigenic properties of hen egg white lysozyme (Fleming's lysozyme) and notes on its acute/sub-acute toxicity. Curr. Therap. Res., 31:494-505.

2) Lodinová, R. & Jouja.V. (1977) Influence of oral lysozyme administration on serum immunoglobulin and intestinal secretory IgA levels in infants. Acta. Pediatr. Scand, 66:709-712.

3) JECFA: 39th report of the Joint FAO/WHO Expert Committee on Food Additive (1992) WHO Food Additives Series 30, WHO Technical Report Series 828 http://www.inchem.org/documents/jecfa/jecmono/v30je04.htm https://apps.who.int/iris/bitstream/handle/10665/40033/WHO_TRS_828.pdf;jsessionid=60E83A381A9E12205DD6B06F56BCFB8A?sequence=1

Charcoal

English name: Charcoal
CAS No. 7440-44-0(Charcoal, activate)
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Charcoal is obtained by carbonizing the stem of *Phyllostachys bambusoides* SIEB.et ZUCC. of the family Poaceae or *Phyllostachys heterocycla* MITF. of the family Poaceae, or the trunk, branches, or seeds of *Betula platyphylla* SUKAT.var.*japonica* HARA of the family Betulaceae, *Pinus koraiensis* SIEB.et ZUCC., or *Quercus phylliraeoides* of the family Fagaceae, etc.

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

No information available on oral administration

2) Repeated-dose toxicity study

No information available on repeated administration

3) Mutagenicity study

No information available on mutagenicity.

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

For Charcoal, activated, the EFSA states that an ADI or a TDI could not be established, but the present use could be accepted.[1)]

For substances with similar origin, the method of preparation, and definition, JECFA evaluates activated carbon as ADI not limited, and vegetable carbon as ADI could not be established.[2)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) The EFSA Journal (2004) Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to a 5th list of substances for food contact materials 109,15-1

2) JECFA: WHO Technical Report Series 759(1987)

Lactoperoxidase

English name: Lactoperoxidase
CAS No. 9003-99-0
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Lactoperoxidase is an enzyme obtained from mammalian milk and reductively decomposes hydrogen peroxide. It may contain food (only for the purpose of filling, powdering, diluting, stabilizing, storage, or potency adjustment) or additives (only for the purpose of filling, powdering, diluting, stabilizing, storage, pH adjustment, or potency adjustment).

2. Major use

Enzyme

3. Summary of safety studies

1) Acute toxicity study

While there are no disclosed data on lactoperoxidase as such, an acute toxicity study in rats has been performed with Milk Basic Protein (MBP®) which contains 54.3% lactoferrin and 40.6% lactoperoxidase.[1, 2)]

Rat (Crj:CD(SD)IGS) oral
MBP® LD_{50} > 2,000 mg/kg BW

2) Repeated-dose toxicity study

Although there is no disclosed data of lactoperoxidase as such, in a 90-day repeated-dose oral toxicity study in Crj:CD(SD)IGS rats (10 males and females each per group) with MBP® (solvent: distilled water) at a dose of 0, 200, and 2,000 mg/kg BW/day, the NOAEL of MBP® in rats was considered to be 2,000 mg/kg BW/day both for males and females.[1, 3)]

3) Mutagenicity study

No information available on mutagenicity.

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

JECFA evaluated the Lactoperoxidase/Thiocyanate/Hydrogen peroxide system for milk preservation as ADI not specified.[4)]

FSANZ designates lactoperoxidase and sodium thiocyanate as processing aids under Specification 1.3.3. It is not a serious risk for most persons at the current level of use, while consumers who are allergic to milk proteins need to recognize its presence in meat products, and the potential risk has to be properly addressed by labeling.[5)]

MBP® is evaluated in the GRAS Notice (GRN) No.196 and was considered to be "No Questions" by the FDA in September 2006.

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Kruger, C.L., K.M. Marano, Y. Morita, Y. Takada, H. Kawakami, T. Kobayashi, M. Sunaga, M. Furukawa, K. and Kawamura. 2005. Safety evaluation of a milk basic protein fraction. Food and Chemical Toxicology.

2) Safety Research Institute for Chemical Compounds Co., Ltd.(2000a.A single dose oral toxicity study of milk basic protein (MBP) in rats. Study Number SR-99 100.Safety Research Institute for Chemical Compounds Co., Ltd.363-24 Shin-ei, Kiyota-ku, Sapporo 004-0839, Japan. Unpublished.

3) Safety Research Institute for Chemical Compounds Co., Ltd.2000c.A 13-week oral repeated dose toxicity study of milk basic protein (MBP) in rats. Study Number SR-9918.Safety Research Institute for Chemical Compounds Co., Ltd.

363-24 Shin-ei, Kiyota-ku, Sapporo 004-0839, Japan. Unpublished.

4) JECFA: WHO Technical Report Series 789 (1989)

5) Food Standard Australia New Zealand (FSANZ): Final Assessment report Application A404; Lactoreroxidase system (2002)

Lactoferrin concentrates

English name: Lactoferrin concentrates
CAS No. 146897-68-9(Lactoferrin)
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

Lactoferrin concentrate is obtained from mammalian milk and contains lactoferrin as the main component.

2. Major use

Food manufacturing agent

3. Summary of safety studies

1) Acute toxicity study

An acute toxicity study was performed in rats as bovine lactoferrin. No deaths were observed and no toxic effects caused by the test substance were observed.[1)]

Rats (strain unknown) oral LD_{50} > 2,000 mg/kg BW

2) Repeated-dose toxicity study

A 13-week repeated-dose toxicity study was performed in Sprague-Dawley rats (12 males and females each per group) with bovine lactoferrin (purity 95.0%) at a dose of 0, 200, 600, and 2,000 mg/kg by oral gavage. Toxic effects attributable to the test substance were not observed, and the NOAEL was considered to be 2,000 mg/kg BW/day, which was the highest dose both for males and females.[2)]
A 60-week (male) or 65-week (female) dietary treatment study was performed in F344/Crj rats (25 males and females for high-dose and control groups, and 10 males and females each per group for other groups) with bovine lactoferrin at a dose of 0, 0.02, 0.2, 2.0, and 5.0%. The authors reported that there were no clear changes but did not provide detailed data, and the FDA thus states that the NOAEL could not be established.

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[3)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

There are no reports of toxicity concerns about this additive other than those mentioned above.

5) Position in overseas assessment reports

In 2012, EFSA declared it Safe under the proposed use and use levels.[4)]
It is evaluated in GRAS Notice (GRN) No.669 and was answered as "No Questions" by the FDA in September 2016.
It has not been evaluated by JECFA.

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) Nishimura N, 1991. Single dose oral toxicity study of monl-01 and monl-02 in rats (Study Number B1969). Gotemba Laboratory, Bozo Research Center Inc., Setagaya-ku, Tokyo, Japan. Unpublished.

2) Yamauchi K, Toida T, Nishimura S, Nagano E, Kusuoka O, Teraguchi S, Hayasawa H, Shimamura S and Tomita M, 2000.13-Week oral repeated administration toxicity study of bovine lactoferrin in rats. Food Chem Toxicol, 38,503-512.

3) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)

4) European Food Safety Authority. Scientific Opinion on bovine lactoferrin. EFSA Journal 2012; 10(7): 2811. [14 pp.]

D-Ribose

English name: D-Ribose
CAS No. 50-69-1
JECFA No. Not available
Other names: Not available
Structural formula:-

1. Origin and method of preparation

D-Ribose is obtained from the liquid fermentation medium of D-glucose by bacteria (only *Bacillus pumilus* or *Bacillus subtilis*) by separation. Its component is D-ribose.

2. Major use

Sweetener

3. Summary of safety studies

1) Acute toxicity study

No information available

2) Repeated-dose toxicity study

Wistar rats (20 males and females each per group) received 13-week dietary treatment with D-ribose provided by the applicant at a dose of 0, 5, 10, or 20% (equivalent to 0, 3.6, 7.6, or 15.0 g/kg BW/day, respectively, in males and 0, 4.4, 8.5, or 15.7 g/kg BW/day, respectively, in females) mixed in gelatinized potato starch instead of barley. Body weight gain suppression was observed in males in the 20% (15.0 g/kg) group and females in the 10 and 20% (8.5 and 15.7 g/kg) groups, and increased cecum weight associated with dose relationship, etc. was observed in males and females. However, all these were considered to be physiologic changes caused by increased carbohydrate intake. Since no clear treatment-related changes were observed, the NOAEL was considered to be 20% of the highest dose (15 g/kg BW/day).[1)]

3) Mutagenicity study

An Ames test, a chromosomal aberration test, and an *in vivo* micronucleus test were performed, and all the results were reported to be negative.[2)]

Ames test: Negative; 5,000 μg/plate
Chromosomal aberration test: Negative; 5,000 μg/mL
Micronucleus test: Negative; 2,000 mg/kg BW

4) Others

Wistar rats aged 12 weeks were mated, and females (28 animals per group) received 13-week dietary treatment from day 0 to 21 of gestation with D-ribose provided by the applicant at a dose of 0, 5, 10, or 20% (equivalent to 0, 4.25, 7.94, or 9.91 g/kg BW/day, respectively) mixed in gelatinized potato starch instead of barley. Increased cecum weight was observed in the treatment groups. However, it was considered to be a physiologic change caused by increased carbohydrate intake. While no clear treatment-related changes were observed in fetuses or the placenta, the frequency of wavy rib was high in the 10 and 20% (7.94 or 9.91 g/kg) treatment groups. Therefore, the NOAEL for teratogenicity was considered to be 20% of the highest dose (9.91 g/kg BW/day), and the NOAEL for developmental toxicity was considered to be 5% (3.64-4.61 g/kg BW/day).[1)]

Twenty-one non-diabetic healthy adults (12 males and 7 females) received 10 g of D-ribose twice daily for 14 days. Daily diet and exercise were not changed during the study period. The blood was collected for blood chemistry at the start, Day 7, and Day 14. While there were some changes, there were no consistent, clearly treatment-related changes.

5) Position in overseas assessment reports

It was evaluated by the FDA in 2007 and is generally recognized as safe (GRAS) when used in beverages, etc. according to the current GMP.[1)]

4. Conclusion

It is considered that there are no safety concerns about this existing additive distributed in Japan.

5. References

1) FDA: GRAS Notice GRN 243(2007): GRAS notification for D-ribose

2) Hayashi and Tanaka: Food hygiene and safety science 46, 5, 177-184 (2005)