Ver. 1.0.0

Research report

Research on the safety evaluation of existing additives

FY 1996

Version history:

Ver. 1.0.0 February 9, 2022

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**Supervised by Food Chemistry Division, Environmental Health Bureau,
Ministry of Health and Welfare**

Research on the safety evaluation of existing additives
- FY 1996 health and welfare science grant research report -

Hayashi Yuzo
Senior Researcher
Visiting Professor of Kitasato University School of Pharmacy

April 1998
Japan Food Additives Association

Editor's Preface

For the regulation of food additives, all additives are, in principle subject to the designation system of the Ministry of Health and Welfare, due to the amendment of the Food Sanitation Act in May 1995. However, the scope of natural additives that have already been distributed is defined as a list of existing additives, the distribution of which has been approved to continue, as a transitional measure.

Unlike the additives designated pursuant to Article 6 of the Food Sanitation Act, those listed as such existing additives are not designated as "those not likely to harm human health" by the Ministry of Health and Welfare; however, their distribution was approved as a transitional measure. Therefore, confirmation of their basic safety is strongly required.

Accordingly, the Ministry of Health and Welfare requested that a research group, including Visiting Professor Hayashi Yuzo of the Kitasato University School of Pharmacy as Senior Researcher (former head of Biological Safety Research Center, National Institute of Health Sciences), investigate the status of evaluation of these additives by international organizations and in Europe and the United States, and to collect and evaluate the safety study results of individual additives using a health and welfare science research grant awarded for the period from FY 1995 to FY 1996.

Now, this study has been reported in detail. Needless to say, a continuous review of the safety assurance of additives is required, along with continuing technological development. I believe that the achievements of this study will form the basis for ensuring the safety of existing natural additives, in the future.

While it is clear that the responsibility for ensuring the safety of individual food additives should primarily rest with the manufacturers and importers of those additives, I anticipate that this report will be widely used by the relevant parties, including the associated manufacturers and importers, to help to ensure the safety of food additives.

Kurokawa Tatsuo
Director, Food Chemistry Division, Environmental Health Bureau,
Ministry of Health and Welfare

Research on the safety evaluation of existing additives

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Visiting Professor of Kitasato University School of Pharmacy

Summary

In this study, the basic safety of existing natural additives was researched by investigating existing international evaluation results, as well as approval status in Europe and the United States, and by collecting safety study results and evaluating the study results for 489 existing natural additives publicly notified on April 16, 1996.

Study collaborators:

Kanai Mieko	Lecturer, Department of Home Economics, Sagami Women's Junior College
Sofuni Toshio	Director, Division of Genetics and Mutagenesis, Biological Safety Research Center, National Institute of Health Sciences
Tanaka Satoru	Visiting Researcher, National Institute of Health Sciences
Hirose Akihiko	Senior Researcher, Division of Risk Evaluation, Biological Safety Research Center, National Institute of Health Sciences
Yamada Takashi	Director, Division of Food Additives, National Institute of Health Sciences

A. Objective

Due to the amendment of the Food Sanitation Act in May 1995, the designated system of food additives, previously targeted only chemically synthesized additives, expanded into all additives except for natural flavors, etc. Upon this amendment, the additives except those that are chemically synthesized (excluding natural flavors, etc.; hereinafter, referred to as “natural additives”) which already have been distributed, manufactured, used, were defined by the list of existing additives, and were then permitted the continuation of sales, manufacture, import, etc., as a transitional measure.

However, unlike additives that have been previously designated, the natural additives included on the list of existing additives have not been checked individually for safety, therefore, confirmation of their safety is requested in the Diet, etc.

This study aimed to clarify the basic safeties of existing natural additives by investigating related international evaluation results and approval status in Europe and the United States, and also collecting domestic and overseas study results and performing an evaluation analysis of these results for 489 natural additives listed as existing additives.

B. Methods

Among 489 existing additives, 466 additives listed in the provisional list, dated August 1995 were investigated in the FY 1995 health and welfare science grant research study regarding their evaluation status by international organizations and in Europe and the United States and the implementation status of domestic and overseas safety studies. In this fiscal year, 23 additives that were additionally entered into the list of existing additives were then investigated regarding (1) safety evaluation status in the Joint FAO/WHO Expert Committee on Food Additives (hereinafter referred to as "JECFA"), (2) authorization status in the United States, and (3) authorization status in the EU, along with the collection of (4) safety study results, and the results were combined with the research results obtained in FY 1995.

The safety evaluation status by JECFA was determined according to the minutes of JECFA meetings which are held regularly once per year in principle, and the minutes were disclosed by the World Health Organization (WHO) by February 1996. In addition, the authorization status for the distribution of the additives was investigated based on the authorization status in the United States and Europe, according to the Code of Federal Regulations (hereinafter referred to as "CFR"), 1997 edition, for the United States, and the 1994 Directives on food additives (94/36/EC and 94/34/EC for colors and sweeteners, respectively), 1995 Directive on food additives (95/2/EC for food additives other than colors and sweeteners), and 1988 Directive on extraction solvents (88/344/EEC) for the EU. In addition, additives for which distribution is authorized in the United States are officially listed only in the CFR, in accordance with which this research study was performed. However, it should be noted that the distribution of those additives not listed in the CFR is not necessarily prohibited; for example, it is also specified in the CFR that "substances generally recognized as safe" (hereinafter referred to as "GRAS substances") are not limited to those listed in the GRAS list.

In any case, unlike chemically synthesized additives, it is difficult to identify natural additives based on structural formula, etc., and such additives are distributed under different names in different countries and regions. Therefore, in this study, we endeavored to specify the situations regarding additives that were considered to be scientifically and substantially equivalent.

Moreover, among additives without international evaluations and have no approval neither in Europe nor the United States, 41 additives for which the materials of both the 28-day or longer repeated-dose toxicity study results and the mutagenicity study results were available were individually subjected to the evaluation of safety study results.

C. Results

(1) Safety evaluation status by JECFA

To summarize the evaluation results by JECFA for 489 additives, including 23 natural additives that were additionally listed on the list of existing additives, 74 additives have been evaluated, including: 58 additives for which the acceptable daily intake (hereinafter referred to as "ADI") is set or for which the establishment of the ADI was toxicologically considered unnecessary (ADI not specified or ADI not limited) (13 additives for which the ADI was established, and 45 additives for which the establishment of the ADI was toxicologically considered unnecessary); 4 additives for which the ADI is provisionally established; and, 12 additives for which the ADI is not established, but the current use of which is judged to have no toxicological problem (GMP (no problem, as long as properly used in food production), acceptable (acceptable under the current conditions for use)). These additives are shown in Table 1.

(2) Authorization status in Europe and the United States

Among the 23 additives that were added to existing natural additives, there was none that was authorized for use as food additives in the United States or the EU, except for 2 additives that were designated as GRAS substances in the United States. Including these additives, the authorization status of the 489 existing natural additives is summarized as follows: 136 additives were authorized as additives for distribution or designated as GRAS substances in the United States; and 52 additives were authorized as additives for distribution in the EU. These additives are shown in Table 2 and Table 3.

(3) Collection and evaluation of safety study results

Among the 23 additives that were added to existing natural additives, there were 6 additives for which safety study results were available, among which both 28-day or longer repeated-dose toxicity study results and mutagenicity study results were available for 2 additives.

In (1) and (2) above, there were 112 additives, including 6 additives (Table 4) for which safety studies commissioned by the Ministry of Health and Welfare were performed at the National Institute of Health Sciences or safety study results were available from additive-related companies with the cooperation of the Japan Food Additives Association, among those other than additives for which safety had been evaluated by JECFA or additives that were confirmed to have authorization for distribution as additives in Europe and the United States. Among these additives, there were 41 additives that both 28-day or longer repeated-dose toxicity study results and mutagenicity study results were available, and the safety of those additives was investigated by evaluating the study results. A summary of the results is shown in Annex 1. In addition, because 2 additives of curdlan and enzymatically decomposed lecithin were newly listed in CFR 1997 edition, while their safeties were investigated at the same time, these additives are attached in Annex 2, for reference.

D. Discussion

For the 489 natural additives listed in the list of existing additives, the (1) safety evaluation status in JECFA and (2) authorization status in Europe and the United States were reviewed, and (3) safety study results were collected and evaluated.

As a result, 159 out of these 489 additives were confirmed to have been evaluated for safety by JECFA or to have been authorized for distribution as additives in Europe and the United States. Accordingly, the basic safety of these additives is considered to be ensured, as they have been evaluated by JECFA, Europe, and the United States.

For the 41 additives which both 28-day or longer repeated-dose toxicity study results and mutagenicity study results were available among additives which safety study results were available, basic safety could be evaluated based on the study results. Furthermore, as there is no study result that suggests any immediate effect on human health at present, it was considered that there is no immediate need to perform a new safety study.

In addition, from among the 289 additives that have not been evaluated by JECFA, Europe or the United States and for which safety study results are not available, the safety of 150 additives was discussed based on origin, method of preparation, and definition. The details are described in the remarks column of Table 5, which has been prepared for all additives included the list of

existing additives, together with the evaluation results by JECFA, etc. Among these 150 additives, insoluble minerals and enzymes are generally not considered to be hazardous to the maintenance of human health, provided that they are manufactured in a scientifically appropriate manner. In particular, for the former, their criteria for use have been established previously, and the possible concern, from a safety perspective is the dissolution of inorganic salts, which are very limited in quantity, while the latter are mainly used as catalysts in the process of food production or processing, and consist of protein. Moreover, as shown in the remarks column in Table 5, it is considered, for additives that are regarded to be related, based on origin, method of preparation, and definition to those evaluated by JECFA, or those for which safety study results were available, that safety can be evaluated with reference to the evaluation results of the related additives. For these additives for which discussion is described in the remarks column in Table 5, it is considered that there is no need to perform a further investigation of safety, such as the immediate implementation of safety studies, at the present stage.

For the remaining 139 additives among the 289 additives that have not been evaluated by JECFA, Europe or the United States and for which safety study results were not available (*i.e.,* excepting the above 150 additives for which a discussion was performed on safety), the materials required to confirm their basic safety have not yet been collected, and accordingly, an investigation of safety, including the implementation of safety studies, is required. As it is considered that these additives can be classified as shown in Table 6 based on origin, method of preparation, and definition (9 major classifications; 25 classifications, including minor classifications), it is necessary to give priority to the maintenance of the health of the national population, such as by testing the representative additives within each classification, so that safety can be confirmed rapidly and effectively.

E. Conclusion

Among 489 existing natural additives, 159 additives have already been internationally evaluated, and their basic safety has been confirmed. Moreover, it is considered that there is no present need to investigate safety immediately for 41 additives based on the evaluation of study results available, and for 150 additives based on their origin, method of preparation, and definition.

For the 139 additives other than those described above, it is necessary to confirm their safety rapidly and effectively, by classifying them by origin, method of preparation, and definition.

[Tables 1~6 are omitted]

Reference: Research on the safety evaluation of existing additives (FY 1999)

159 existing additives for which have been internationally evaluated (JECFA, FDA, etc.): Existing Additives (Internationally Evaluated) FY 1996.pdf

Annex 1. Summary of safety studies

Aspergillus terreus glycoprotein

1. Food additive name:

Aspergillus terreus glycoprotein

2. Origin, method of preparation, and definition:

Aspergillus terreus glycoprotein is obtained from the fermentation culture solution of glucose, starch, and soybean meal by a filamentous fungus (*Aspergillus terreus*) by bacterial elimination, followed by fractionation with ammonium sulfate and desalting. It consists mainly of glycoprotein.

3. Major use:

Food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study

The acute oral LD_{50} is over 6,000 mg/kg in rats, and over 7,500 mg/kg in mice. [1), 2)]

(2) Repeated-dose study

In a 3-month repeated-dose test in SD rats by gavage (300, 600, 1,200, and 2,400 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2,400 mg/kg/day. [3)]

(3) Mutagenicity study

All the results from a reverse mutation test in bacteria and a chromosomal aberration test in mammalian cells were judged as negative. [4), 5)]

(4) Ocular mucosal irritation test

Primary irritation in the cornea, conjunctiva, and iris in the eye was investigated in Japanese White rabbits with 3.0% solution in water and the results were judged as negative. [6)]

(References)

1. Imai Kiyoshi, et al.: Acute toxicity study in rats with MST (mutastein) by oral treatment, The Clinical Report, 20(12), 131, Sep. 1986

2. Imai Kiyoshi, et al.: Acute toxicity study in mice with MST (mutastein) by oral treatment, The Clinical Report, 20(12), 133, Sep. 1986

3. Yamaguchi Kazuki, et al.: Oral 3-month toxicity study in rats with MST (mutastein), The Clinical Report, 20(12), 135, Sep. 1986

4. Tanaka Noriho, et al.: Chromosomal test of MST (mutastein) in Chinese hamster cell cultures, The Clinical Report, 20(12), 127, Sep. 1986

5. Iwahara Shigeo, et al.: Mutagenicity study report of MST (mutastein) in bacteria, The Clinical Report, 20(12), 123, Sep. 1986

6. Hara Yasuo, et al.: Mucastin's ocular mucosal irritation test, The Clinical Report, 23(2), 71,

Jan. 1989

Madder colour

1. Food additive name:
   Madder colour

2. Origin, method of preparation, and definition:
   Madder colour is obtained from the roots of *Rubia tinctorum* LINNE of the Rubiaceae family. It is produced by extraction with water at either room or warm temperature or with hydrous ethanol. It consists mainly of alizarin and rubberythric acid as principal coloring components. It is yellow to red-purple.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be 3,505-3,509 mg/kg in mice. [1)]

(2) Repeated-dose study
In a 90-day repeated-dose test in $B6C3F_1$ mice by dietary administration (0.3, 0.6, 1.25, 2.5, and 5.0%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 7.5 g/kg/day. [2)]

(3) Mutagenicity study
Positive results were obtained in reverse mutation tests in bacteria. [3), 4), 6)] While TA100-S9 showed positive results from 0.1 mg/plate, +S9 showed positive results from 1.0 mg/plate, indicating that the activity was decreased by the addition of S9. [4)] A DNA repair test in bacteria showed a weakly positive result at a high dose of 100 μL/mL. [3)] The results from mouse micronucleus tests were judged as negative. [5), 7)]

(4) Medium-term multiorgan carcinogenicity study
In a medium-term multiorgan carcinogenicity test performed in F344 rats by dietary administration (0, 2.5, and 5.0%) with N-nitrosodiethylamine (DEN), N-methyl-N-nitrosourea (MNU), and N-bis (2-hydroxypropyl) nitrosoamine (DHPN), no tumor promoter effect was observed at any of the target sites. [8)]

(References)

1. Acute toxicity study in mice, 1975, internal data (unpublished)

2. Tanaka Takuji, et al.: Study on the acute and subacute toxicity of a novel natural color MADDER ROOT from Rubia tinctorum, Japanese Journal of Food Chemistry, 1(1) 17, 1994

3. Hachiya Noriyuki, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives (1981-1983), Toxicology Forum, 8(1), 91-105, 1985

4. Mutagenicity study, 1980, internal data (unpublished)

5. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 7) - FY 1985

Ministry of Health and Welfare Research Grant -, Toxicology Forum, 9(6), 628-633, 1986

6. Asanoma Masaharu, et al.: Mutagenicity of natural additives in Salmonella (2nd report), Annual Report of Nagoya City Public Health Research Institute, 30, 1984

7. Madder Red Color: Mouse Micronucleus Test, 1996, internal data (unpublished)

8. A. Hagiwara, et al., Two Different Constituents of Madder Colors Lack Tumor Promoting or Carcinogenic Potential in a Medium-term Multi-organ Carcinogenesis Bioassay in Rats, Japanese Journal of Food Chemistry, Vol. 4(2), 99-106, 1997

Acylase

1. Food additive name:
   Acylase

2. Origin, method of preparation, and definition:
   Acylase is derived from the culture solution of filamentous fungi *Aspergillus ochraceus* or *Aspergillus melleus* by extraction with water, or by elimination of fungi at cool or room temperature, with or without subsequent ethanol treatment at a cool temperature.

3. Major use:
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 15,000 mg/kg in mice and rats. [1)]

(2) Repeated-dose study
In a 5-week repeated-dose test in Wister rats by gavage (200, 800, and 3,200 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 3,200 mg/kg/day. [2)] In a 26-week repeated-dose test in Wister rats by gavage (200, 800, and 3,200 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 3,200 mg/kg/day. [3)]

(3) Mutagenicity study
The results from reverse mutation tests in bacteria were judged as positive at higher doses (not less than 1.25 mg/plate). [4), 5)] All the results from a DNA repair test in bacteria, [5)] a chromosomal aberration test in cultured cells [6)] and a mouse micronucleus test [7)] were judged as negative.

(References)

1. Acute toxicity study of acylase, 1975. 8, internal data (unpublished)

2. Safety study (II) of acylase produced by *Aspergillus sp*. - 5-week subacute toxicity study in rats by oral gavage-, 1975. 10, internal data (unpublished)

3. Safety study (III) of acylase produced by *Aspergillus sp*. - 26-week chronic toxicity study in rats by oral gavage-, 1975. 10, internal data (unpublished)

4. Kuroda Koichi, et al.: Mutagenicity of natural additives, Annual report of Osaka City Institute of Public Health and Environmental Sciences, 47, 24-30, 1985

5. N. Hachiya, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives (1981-1983), Toxicology Forum, 8(1), 91-105, 1985

6. M. Ishidate, et al.: Mutagenicity Study Results of Food Additives (Part 6) - FY 1984 Ministry of Health and Welfare Research Grant -, Toxicology Forum, 8(6), 705-708, 1985

7. T. Sofuni, et al.: Mutagenicity Study Results of Food Additives (Part 12) - FY 1990 Ministry of Health and Welfare Research Grant -, Japanese Journal of Mutagenicity Tests on Chemicals, 3(4), 206-215, 1994

Krill colour

1. Food additive name:
   Krill colour

2. Origin, method of preparation, and definition:
   Krill colour is obtained from the shell or eye of *Euphausia similis* G. O. SARS or *Euphausia superba* DANA of the Euphausiidae family and can be extracted by compression and separation, by extraction with acetone at room temperature, by extraction with carbon dioxide under pressure, or by extraction with hexane. It consists mainly of astaxanthin as a principal coloring component. It is orange to red.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 2 g/kg in mice. [1)]

(2) Repeated-dose study
In a 4-week repeated-dose test in male SD rats by gavage (0.3 g/kg, 1.0 g/kg, and 3.0 g/kg; astaxanthin concentration of approximately 1.7%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 3 g/kg/day. [2)]

(3) Mutagenicity study
The results from a reverse mutation test in bacteria were judged as negative. [3)]

(References)

1. Acute oral toxicity study on ASTAX1700, 1994, internal data (unpublished)

2. Four-week repeated-dose toxicity study with ASTAX1700 by oral treatment, 1994, internal data (unpublished)

3. Mutagenicity study on ASTAX1700, 1994, internal data (unpublished)

γ -Oryzanol

1. Food additive name:
   γ-Oryzanol

2. Origin, method of preparation, and definition:
   γ- Oryzanol is obtained from rice bran and rice oil which are derived from the seeds of *Oryza sativa* LINNE of the Poaceae family. It is produced from hydrous ethanol fraction after fractioning them by hydrous ethanol at room temperature with either n-hexane or acetone. It consists mainly of sterol, ferulic acid, triterpene alcohol and ferulic acid ester.

3. Major use:
   Antioxidant

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 5.0 g/kg in mice. [1)]

(2) Repeated-dose/carcinogenicity study
In a 181-day repeated-dose test in Wistar rats by gavage (30, 100, 300, and 1,000 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 1,000 mg/kg/day. [2)]

In a 2-year carcinogenicity test in F344 rats by dietary administration (200, 600, and 2,000 mg/kg), neither a toxicological effect caused by administration with the substance nor carcinogenicity were observed. The no observed adverse effect level is considered to be 2,000 mg/kg/day. [6)]

In a 78-week carcinogenicity test in $B6C3F_1$ mice by dietary administration (200, 600, and 2,000 mg/kg), neither a toxicological effect caused by administration with the substance nor carcinogenicity were observed. The no observed adverse effect level is considered to be 2,000 mg/kg/day. [7)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria, [3)] a chromosomal aberration test in mammalian cells, [4)] and a DNA repair test in bacteria [5)] were judged as negative.

(References)

1. Ministry of Health and Welfare FY 1991 Study on reevaluation, etc. of safety of food additives, acute toxicity study report

2. Hazato Hikozaemon, et al.: Chronic toxicity study of γ-oryzanol (Oliver Tablets), The Clinical Report, 8(11), 91-109, 1974

3. Ministry of Health and Welfare FY 1991 Evaluation study of safety of food additives, Mutagenicity study (Ames test) report

4. Ministry of Health and Welfare FY 1991 Study on reevaluation, etc. of safety of food

additives, Mutagenicity study (chromosomal aberration test) report

5. Ministry of Health and Welfare FY 1991 Study on reevaluation, etc. of safety of food additives, Mutagenicity study (Rec-assay)

6. M.TAMAGAWA. et. al.: Carcinogenicity study of γ-oryzanol in F344 rats, Fd. Chem. Toxicol., 30, 41-48, 1992

7. M.TAMAGAWA. et. al.: Carcinogenicity study of γ-oryzanol in B6C3F_1 mice, Fd. Chem. Toxicol., 30, 49-56, 1992

Cacao colour

1. Food additive name:
   Cacao colour

2. Origin, method of preparation, and definition:
   Cacao colour is obtained from the seeds (cacao bean) of *Theobroma cacao* LINNE of the Sterculiaceae family after fermentation, torrefaction, and extraction with a warm weakly alkaline solution, followed by neutralization. It consists mainly of anthocyanin that polymerized by heat. It is brown.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 10,000 mg/kg in mice, and over 5,000 mg/kg in rats. [1), 2)]

(2) Repeated-dose/carcinogenicity study
In a 5-week repeated-dose test in SD rats by gavage (500, 1,000, 2,000, and 4,000 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 4 g/kg/day. [2)]

In a 104-week repeated-dose test and carcinogenicity study in SD rats by dietary administration (0.05% and 5%), neither a toxicological effect caused by administration with the substance nor carcinogenicity was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [2)]

(3) Mutagenicity study
There have been reports of reverse mutation tests in bacteria in which the results were judged as positive [3)] and weakly positive, [4)] at high doses not less than 5 mg/plate. In addition, there was a report in which different results were obtained between lots. [5)] Results that differed by lot were also obtained in a DNA repair test in bacteria, with a positive result at a high dose of 15 mg/disk. [5)] Different lots were also tested in chromosomal aberration tests in cultured cells, and positive results were obtained at higher doses (D_{20} at 0.7-1.83 mg/mL). [3), 5), 6)] While a statistically significant difference was obtained in one mouse micronucleus test, the frequency was very low (0.28%), [7)] and a negative result was obtained for the other lot. [8)]

(References)

1. Shimizu Mitsuru, et al.: Acute Oral Toxicity of Food Additives Other Than Synthesized Chemicals in Mice and Rats, Seikatsu Eisei (Journal of Urban Living and Health Association), 37(5), 215-220, 1993

2. FY 1986 Ministry of Health and Welfare Contract Research

3. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 2) - 1st Screening

Data by FY 1980 Ministry of Health and Welfare Research Grant -, Mutagens & Toxicology, 4(6), 80-89, 1981

4. Fujita Hiroshi, et al.: Mutagenicity of natural food additives in the Ames test, Annual Report of the Tokyo Metropolitan Research Laboratory of Public Health, 47, 309-313, 1996

5. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 7) - 1st Screening Data by FY 1985 Ministry of Health and Welfare Research Grant -, Toxicology Forum, 9(6), 628-633, 1986

6. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives- 1st Screening Data (1st) by FY 1979 Ministry of Health and Welfare Research Grant -, Mutagens & Toxicology, Vol. 12, 82-90, 1980

7. Hachiya Noriyuki, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives (1981-1983), Toxicology Forum, 8(1), 91-105, 1985

8. Hachiya Noriyuki, et al.: Micronucleus test with natural additives, Japanese Journal of Mutagenicity Tests on Chemicals, 1(1), 13-17, 1992

Enzymatically hydrolyzed guar gum

1. Food additive name:
   Enzymatically hydrolyzed guar gum

2. Origin, method of preparation, and definition:
   Enzymatically hydrolyzed guar gum is obtained from "guar gum" by hydrolyzation with enzymes (α-galactosidase and hemicellulase). It consists mainly of polysaccharides.

3. Major use:
   Thickening stabilizer

4. Summary of safety study results:

(1) Single-dose study
   The acute oral LD_{50} is considered to be over 6,000 mg/kg in mice and rats. [1)]

(2) Repeated-dose study
   In a 13-week repeated-dose test in SD rats by dietary administration (0.2, 1.0, and 5.0%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 3.1 g/kg/day. [2)]

(3) Mutagenicity study
   Results from a reverse mutation test in bacteria were judged as negative. [3)]

(References)

1. Acute oral toxicity study of K-13 (enzymatically hydrolyzed guar gum) in mice and rats, 1988, internal data (unpublished)

2. Thirteen-week toxicity study of K-13 (enzymatically hydrolyzed guar gum) in male and female rats, 1989, internal data (unpublished)

3. Mutagenicity study of K-13 (enzymatically hydrolyzed guar gum), 1990, internal data (unpublished)

Quercetin

1. Food additive name:
   Quercetin

2. Origin, method of preparation, and definition:
   Quercetin is obtained by hydrolyzing “rutin (extract)” with an enzyme or an acidic aqueous solution. It consists mainly of quercetin.

3. Major use:
   Antioxidant

4. Summary of safety study results:

(1) Repeated-dose/carcinogenicity study
In a 64-week repeated-dose test in F344 rats by dietary administration (0.1 and 0.2%), no toxicological effect caused by administration with the substance was observed. [3)]

In a 410-day repeated-dose test in rats by dietary administration (0.25, 0.5, and 1%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 500 mg/kg/day. [7)]

In a carcinogenicity test in ddY mice by dietary administration (2%), no carcinogenicity was observed. [1)]

In a 23-week carcinogenicity test in A/JJms mice by dietary administration (5%) using lung tumor as an indicator, no carcinogenicity was observed. [2)]

In a 540-day carcinogenicity test in F344 rats by dietary administration (0.1%), no carcinogenicity was observed. [4)]

In a 540-day carcinogenicity test in ACI rats by dietary administration (1 and 5%), a significant suppression of body weight gain was observed in only the 5% administered group, and no carcinogenicity was observed. [5)]

In an 850-day carcinogenicity test in ACI rats by dietary administration (10%), no carcinogenicity was observed. [5)]

In a 735-day carcinogenicity test in golden hamsters by dietary administration (10%), no carcinogenicity was observed. [6)]

(2) Reproduction study
Effects on reproductive capacity (such as delivery, rate of viable offspring, and nursing rate) were investigated in a 64-day repeated-dose test in F344 rats by dietary administration (0.1 and 0.2%), and no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 100 mg/kg/day. [3)]

(3) Teratogenicity study
In a teratogenicity test in SD rats by single oral administration (2, 20, 200, and 2,000 mg/kg) on day 9 of gestation, and by repeated oral administration (2, 20, 200, and 2,000

mg/kg) from day 6 to 5 of gestation, the fetal weight was significantly lower in the 200 and 2,000 mg/kg single oral administered groups and the 2 and 2,000 mg/kg repeated oral administered groups than in the control group; however, there was no difference in the number of viable fetuses, and no teratogenicity was observed. [10)]

(4) Mutagenicity study
Positive results have been reported for reverse mutation tests in Salmonella strain TA100 or TA98. [3), 8)] In a mouse micronucleus test, both the investigation results of red blood cells in the bone marrow and the peripheral blood with quercetin at 100-1000 mg/kg by gavage or intraperitoneal administration, and quercetin at 5% and 10% as a dietary administration were judged as negative. [9)] Results from a sister chromatid exchange test using peripheral blood lymphocytes in rabbits receiving quercetin at 250 mg/kg by intraperitoneal administration were judged as negative. [9)]

(References)

1. Saito, D. et al.: Test of carcinogenicity of quercetin, a widely distributed mutagen in food, Teratogenesis, Carcinogenesis, and Mutagenesis, 1, 213-221, 1980

2. Hosaka, S. et al.: Carcinogenicity test of quercetin by pulmonary adenoma bioassay in strain A mice, Gann, 72 (2), 327-328, 1981

3. Stoewsand, G. S. et al.: Quercetin: a mutagen, not a carcinogen, in Fischer rats, J. Toxicol. Environ. Health, 14, 105-114, 1984

4. Takanashi, H. et al.: Carcinogenicity test of quercetin and kaempferol in rats by oral administration, J. Food Safety, 5, 55-60, 1983

5. Hirono, I. et al.: Carcinogenicity examination of quercetin and rutin in ACI rats, Cancer Letters, 13, 15-21, 1981

6. Morino, K. et al.: Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration, Carcinogenesis, 3 (1), 93-97, 1982

7. Ambrose, A. M. et al.: Comparative toxicities of quercetin and quercitrin, Amer. Pharm. Assoc. XLI (3), 119-122, 1952

8. Nagao, M. et al.: Mutagenicities of 61 flavonoids and 11 related compounds, Environ. Mutagen., 3, 401-419, 1981

9. MacGregor, J. T. et al.: In vivo exposure to plant flavonols: influence on frequencies of micronuclei in mouse erythrocytes and sister-chromatid exchange in rabbit lymphocytes, Mutat. Res., 124, 255-270, 1983

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Gardenia blue

1. Food additive name:
   Gardenia blue

2. Origin, method of preparation, and definition:
   Gardenia blue is obtained from the fruits of *Gardenia augusta* MERRILL var. *grandi flora* HORT., *Gardenia jasminoides* ELLIS, of the Rubiaceae family. It is produced by isolation after adding β-glucosidase to a mixture of iridoid glycosides from gardenia fruits by extraction with water at a slightly warm temperature and protein degradation products. It is blue.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5,000 mg/kg in mice, and over 10,000 mg/kg in rats. [1), 2), 3), 4)]

(2) Repeated-dose study
In a 5-month repeated-dose test in ddY mice by dietary administration (1, 2, and 4%), no toxicological effect caused by administration with the substance was observed. [3)]
In a 13-week repeated-dose test in F344 rats by dietary administration (0.6, 1.25, 2.5, and 5%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [5)]

(3) Mutagenicity study
While the results from reverse mutation tests in bacteria were judged as negative, [3), 6), 7), 8)] there was also a report in which a positive result was obtained at high doses of 5-200 mg/plate. [9)] All the results from a DNA repair test in bacteria, [3)] a chromosomal aberration test in cultured cells, [7)] and a mouse micronucleus test [10), 11)] were judged as negative.

(References)

1. Acute oral toxicity study, 1987, internal data (unpublished)

2. Acute oral toxicity study, 1978, internal data (unpublished)

3. Safety of “natural colour gardenia blue 75,” internal data (unpublished)

4. Noguchi Tsutomu, et al.: Acute Oral Toxicities of Natural Food Additives, Seikatsu Eisei (Journal of Urban Living and Health Association), 32, 110-115 (1988)

5. Imazawa Takayoshi, et al.: A 13-week Subchronic Toxicity Study of Gardenia Blue in F344 Rats, Bulletin of National Institute of Health Sciences, 114, 27-32, 1996

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10. Hachiya Noriyuki, et al.: Micronucleus test with natural additives, Japanese Journal of Mutagenicity Tests on Chemicals, 1(1), 13-17, 1992

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Gardenia red

1. Food additive name:
   Gardenia red

2. Origin, method of preparation, and definition:
   Gardenia red is obtained from the fruits of *Gardenia augusta* MERRILL var. *grandi flora* HORT of the Rubiaceae family. It is produced by isolation after adding β-glucosidase to a resulting mixture of the ester hydrolysate of iridoid glycosides from gardenia fruits by extraction with water at a slightly warm temperature and protein degradation products. It is red.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5,000 mg/kg in mice. [1), 2)]

(2) Repeated-dose study
In a 21-week repeated-dose test in $B6C3F_1$ mice by dietary administration (0.5%, 1.5%, and 4.5%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 5.3 g/kg/day. [2)]

(3) Mutagenicity study
Both the results from a reverse mutation test in bacteria and a DNA repair test were judged as negative. [2)]
In a chromosomal aberration test in cultured cells, a positive result was obtained at an extremely high dose (D_{20}: 15.33 mg/mL). [3)] In a mouse micronucleus test, high doses of up to 5 g/kg were tested, and the results were judged as negative. [4)]

(References)

1. Noguchi Tsutomu, et al.: Acute Oral Toxicities of Natural Food Additives, Seikatsu Eisei (Journal of Urban Living and Health Association), 32(3), 110-115, 1988

2. Yoshizumi Satoshi, et al.: Physicochemical properties and safety of enzyme-treated natural gardenia colour, Food Industry, 23(22), 41, 1980

3. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 6) - by FY 1984 Ministry of Health and Welfare Research Grant -, Toxicology Forum, 8(6), 705-708, 1985

4. Hachiya Noriyuki, et al.: Micronucleus test with natural additives, Japanese Journal of Mutagenicity Tests on Chemicals, 1(1), 13-17, 1992

Gardenia yellow

1. Food additive name:
   Gardenia yellow

2. Origin, method of preparation, and definition:
   Gardenia yellow is obtained from the fruits of *Gardenia augusta* MERRILL *var. grandi flor*a HORT., aka *Gardenia jasminodes* ELLIS, of the Rubiaceae family. It is produced by extraction with water at room temperature or hydrous ethanol, or hysrolyzing the resulted extract. It consists mainly of crocin and crocetin as principal coloring components. It is yellow.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5,000 mg/kg in rats. [1)]

(2) Repeated-dose/carcinogenicity study
In a 12-week repeated-dose test in C57BL mice with administration in drinking water (0.05, 0.1, 0.2, 0.4, 0.8, and 1.6%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 1.6 g/kg/day. [2)]

In a 95-week carcinogenicity test in C57BL mice with administration in drinking water (0.2 and 0.8%), neither a toxicological effect caused by administration with the substance nor carcinogenicity was observed. The no observed adverse effect level is considered to be 0.8 g/kg/day. [2)]

(3) Mutagenicity study
Results from a reverse mutation test in bacteria [3)] were judged as negative.

(References)

1. Shimizu, et al.: Acute Oral Toxicity of Food Additives Other Than Synthesized Chemicals in Mice and Rats, Seikatsu Eisei (Journal of Urban Living and Health Association), 37, 215-220, 1993

2. Fujimoto Nariaki et. al: Chronic toxicity study of Gardenia Yellow color in C57BL mice, J. Toxicol. Pathol. 7, 455-460, 1994

3. Safety of a natural yellow colour (gardenia yellow), 1981, internal data (unpublished)

α-Glucosyltransferase treated stevia

1. Food additive name:
   α-Glucosyltransferase treated stevia

2. Origin, method of preparation, and definition:
   α -Glucosyltransferase treated stevia is obtained by glucosylating "Stevia extract" with α -glucosyltransferase etc. It consists mainly of α -glucosylstevioside as a principal sweetening component.

3. Major use:
   Sweetener

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 30,000 mg/kg in mice. [1), 2)]

(2) Repeated-dose study
In a 13-week repeated-dose test in SD rats by dietary administration (1.25, 2.5, and 5.0%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [3), 4)]

(3) Mutagenicity study
All the results from reverse mutation tests in bacteria [5), 6)] and a DNA repair test in bacteria [6)] were judged as negative.

(References)

1. Acute Toxicity Study Report, September 1980, internal data (unpublished)

2. Acute Toxicity Study Report, September 1984, internal data (unpublished)

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4. Kikuchi Hiroaki: A Technical Journal on Food Chemistry & Chemicals, June 1988

5. "Mutagenicity Study Report," January 1981, internal data (unpublished)

6. "Mutagenicity Study Report," October 1984, internal data (unpublished)

Kaoliang colour

1. Food additive name:
   Kaoliang colour

2. Origin, method of preparation, and definition:
   Kaoliang colour is obtained from the fruits and husks of *Sorghum nervosum* BESS. of the Poaceae family, by extraction at a warm temperature with water or hydrous ethanol, or at either room or warm temperature with an alkaline aqueous solution followed by neutralization. It consists mainly of apigeninidin and luteolinidin as principal coloring components. It is red-brown.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 61,800 mg/kg in mice, and over 11,200 mg/kg in rats. [1), 2)]

(2) Repeated-dose study
In a 13-week repeated-dose test in SD rats by dietary administration (0.3, 1, 3, and 10%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 5 g/kg/day. [3)]

(3) Mutagenicity study
The all results from reverse mutation tests in bacteria were judged as negative; [4), 5), 6)] however, results from a DNA repair test in bacteria were judged as weakly positive. [4)] In a chromosomal aberration test in cultured cells, the result was judged as positive at an extremely high dose (D_{20}: 13.35 mg/mL). [7)] A mouse micronucleus test was performed at up to 1 g/kg, and the results were judged as negative. [8)]

(References)

1. Acute toxicity study in mice, 1977, internal data (unpublished)

2. Acute toxicity study of Kaoliang colour, 1975, internal data (unpublished)

3. 90-day repeated-dose toxicity study of Kaoliang colour, 1977, internal data (unpublished)

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5. Yasui Yoko, et al.: Mutagenicity of Commercial Natural Food Color, Food Hygiene and Safety Science, 23(1), 1982

6. Asanoma Masaharu, et al.: Mutagenicity of natural additives in Salmonella (2nd report), Annual Report of Nagoya City Public Health Research Institute, 30, 1984

7. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 5) - by FY 1983 Ministry of Health and Welfare Research Grant -, Toxicology Forum, 7(6), 634-643, 1984

8. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 8) - by FY 1986 Ministry of Health and Welfare Research Grant -, Toxicology Forum, 10(6), 649-654, 1987

Cyclodextrin glucanotransferase

1. Food additive name:
   Cyclodextrin glucanotransferase

2. Origin, method of preparation, and definition:
   Cyclodextrin glucanotransferase is derived from the culture solution of bacteria *Bacillus, Brevibacterium* or *Corynebacterium* by extraction with water at cool to room temperature, or by bacterial elimination followed by concentration at cool to room temperature, with or without subsequent treatment with hydrous ethanol.

3. Use
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
   The acute oral LD_{50} of the cyclodextrin glucanotransferase product from *Bacillus sp.* (220 unit/mL, Blue Value method, pH 5.5) is over 20 mL/kg in mice and rats. [1)]

(2) Repeated-dose study
   In a 3-month repeated-dose test of the cyclodextrin glucanotransferase product from *Bacillus macerans* (690 unit/mL, Blue Value method, pH 5.5) in SD rats by gavage (10 mL/kg each of the stock solution and a 5-fold dilution), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 10 mL/kg/day, as a stock solution. [2)]

(3) Mutagenicity study
   The results from a reverse mutation test in bacteria with the cyclodextrin glucanotransferase product from *Bacillus sp.* were judged as negative. [3)]

(References)

1. The safety study of the stock solution of CGTase (K-CGTase) produced by *Bacillus sp.*, acute oral toxicity, August 1986, internal data (unpublished)

2. The subacute toxicity study of CGTase produced by *Bacillus macerans* in rats, May 1986, internal data (unpublished)

3. The safety study of the stock solution of CGTase (K-CGTase) produced by *Bacillus sp.*, mutagenicity in bacteria, August 1986, internal data (unpublished)

Sandalwood red

1. Food additive name:
   Sandalwood red

2. Origin, method of preparation, and definition:
   Sandalwood red is obtained from the trunks and branches of *Pterocarpus santalinus* LINNE of the Fabaceae family by extraction with water, propylene glycol at a high temperature, or with ethanol at a warm temperature. It consists mainly of santalin as a principal coloring compoment. It is red-purple.

3. Major use:
   Color

4. Summary of safety study results:

(1) Repeated-dose study
In a 90-day repeated-dose test in SD rats by dietary administration (1.25, 2.5, and 5%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [1)]

(2) Mutagenicity study
The results from a chromosomal aberration test in mammalian cells were judged as negative. [2)]

(References)

1. Subacute toxicity study of sandalwood red in rats by oral treatment for 3 months, June 1992, internal data (unpublished)

2. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 6) - by FY 1984 Ministry of Health and Welfare Research Grant -, Toxicology Forum, 8(6), 705-708, 1985

Stevia extract

1. Food additive name:
   Stevia extract

2. Origin, method of preparation, and definition:
   Stevia extract is obtained by extraction with water from the leaves of *Stevia rebaudiana* BERTONI of the Asteraceae family at either room or high temperature, followed by purification. It mainly consists of steviol glycosides (stevioside and rebaudioside, etc.) as principal sweetening components.

3. Major use:
   Sweetener

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} of stevioside is over 8,200 mg/kg in rats and over 8,200 mg/kg in mice. [1), 2)]

The acute oral LD_{50} of purified stevioside extract (stevioside content: 41.4%) is over 42,000 mg/kg in mice. [1)]

The acute oral LD_{50} of crude stevioside crystals (stevioside content: 93% to 95%) is over 15,000 mg/kg in mice. [3)]

(2) Repeated-dose/carcinogenicity study:
In a 104-week repeated-dose/carcinogenicity test in F344 rats, with stevioside as a dietary administration (2.5%, 5%), a dose-related suppression of body weight gain, organ weight loss in kidneys and ovaries were observed. These effects are not considered to be toxicological effects, but rather to be the effect caused by calorie restriction. No carcinogenicity has been observed.[4)]

In a 13-week repeated-dose test in F344 rats with stevioside as a dietary administration (0.31, 0.62, 1.25, 2.5, and 5.0%), suppression of body weight gain was observed in females in administered groups at 2.5% or higher, and males in administered groups at 5%. The no observed adverse effect level is considered to be 0.6 g/kg/day. [5)]

In a 1-month repeated-dose test in Wistar rats with stevioside by gavage (100, 500, and 2,500 mg/kg), organ weight loss in the liver, hepatocellular enlargement, and lymphoid follicles hypertrophy in the spleen were observed in the administered group at 2,500 mg/kg. The no observed adverse effect level is considered to be 500 mg/kg/day. [2)]

In a 22-month (male)/24-month (female) repeated-dose toxicity test in F344 rats with Stevia extract (stevioside: 74.54% and rebaudioside A: 16.27%) as a dietary administration (0.1, 0.3, and 1.0%), mild changes were observed in the 6th month in urinalysis, hematological test, serum biochemical test, and organ weight, while toxicological effects were disappeared after 12 months. No carcinogenicity caused by administration with the test substance was observed. [6)] The no observed adverse effect level is considered to be 550 mg/kg/day.

(3) Teratogenicity/reproduction study:
In a teratogenicity test in Wistar rats administered from gestation day 6 to 15 by gavage (250, 500, and 1000 mg/kg), neither a toxicological effect caused by administration with the substance nor teratogenicity was observed. The no observed adverse effect level is considered to be 1,000 mg/kg/day. [12)]

The effect on pregnancy was investigated by the administration of crude stevia extract, purified extract, or stevioside crystals in a 21-day dietary administration (0.69, 0.35, and 0.15%) in SD rats, followed by mating. The result showed no abnormality in pregnancy rate, litter size, or body weights of dams and neonates. [3)]

An administration test in Wistar rats, before pregnancy and during early pregnancy, was performed in dietary administration with stevioside (0.15, 0.75, and 3.0%). There were no abnormalities in the mating ratio, pregnancy rate, or fetus as a result of continuous administration for 60 days in males and 14 days in females before mating, and 7 days after mating. [14)]

Pregnancy inhibition was investigated in Wistar rats by the administration of an extract of dry stevia leaves, extracted with hot water as drinking water at 15 to 20 g/day/animal for 12 days. The result showed that the administration had no effect in birth rate or litter size. [15)]

A three-generation reproductive study was performed in golden hamsters with stevioside by gavage (0.5, 1.0, and 2.5 g/kg), and histological test from all three generations showed no abnormalities in growth, reproductive function or reproductive tissues. [17)]

A study in Wistar rats observed the effects, before pregnancy and during early pregnancy, of the administration of stevioside (100, 500, and 2,000 mg/kg) and stevia dry leaf extract (700 and 2,100 mg/kg) by gavage. The continuous administration was conducted for 60 days in males and 14 days in females before mating, and 7 days after mating. It showed a mild reduction in pregnancy rate in the administered group with stevioside at 2,000 mg/kg. No significant difference in pregnancy rate was observed between the other administered groups with stevioside or administered groups with stevia dry leaf extract and the control groups. No effect of the substance was observed on the estrus cycle, mating ratio, or fetuses. [18)]

It has also been reported that reductions in both pregnancy rate and litter size were observed in female rats from the result of an investigation of the effect after conception. Those female rats received 10 mL of a 5% solution of the extract of stevia leaves and stems for 12 days and were then mated with untreated males. [13)]

(4) Mutagenicity study:
There has been one positive report in a reverse mutation test in bacteria with stevioside of 50% purity,[7)] while negative results have been obtained with stevioside of 50% and 85% purity.[7), 8), 9), 10)] A negative result was also obtained in a DNA repair test in bacteria with stevia crystals (95-98%).[8)] In addition, all the results with stevioside of 85% purity from a forward mutation test in bacteria, a DNA damage test (Umu test), and a chromosomal aberration test in mammalian cells were judged as negative.[9), 10)]

Steviol, the metabolite of stevioside in enterobacteria in rats, was positive in a forward mutation test in bacteria, a DNA damage test in bacteria, and a chromosomal aberration test in mammalian cells in the presence of S9mix.[9), 11)] On the other hand, steviol was judged as negative in a reverse mutation test in bacteria, a DNA repair test in bacteria, and a mouse micronucleus test. [9), 11)]

(References)

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2. Katayama Osamu, et al.: Practical Application of Stevia and Research and Development Data, ISU Co., Ltd., 1976

3. Akashi Haruo, et al.: Safety of Stevia Dry Leaf Extract, - Report on Results from Toxicity Studies - Food Industry, October 1975.

4. K. TOYODA et al: Assessment of the Carcinogenicity of Stevioside in F344 Rats, Food and Chemical Toxicology, 35 (6), 597-603, 1997

5. Aze Yoshiya, et al.: Subchronic Oral Toxicity Study of Stevioside in F344 Rats, Bulletin of National Institute of Hygienic Sciences, 109, p.48, 1991

6. Yamada Akio, et al: Chronic Toxicity Study of Dietary Stevia Extracts in Fisher 344 Rats, Food Hygiene and Safety Science, 26(2), 169-183, 1985

7. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives, - 1st Screening Data (1st) by FY 1979 Ministry of Health and Welfare Research Grant) -, Mutagens & Toxicology, Vol. 12, 82-90, 1980

8. Okumura Masaya, et al.: A Repair Test and a Reverse Mutation Test with Stevioside, Food Hygiene and Safety Science, 19(5), 486-490, 1978

9. Matsui Michiko, et al.: Mutagenicity of Steviol: An Analytical Approach Using the Southern Blotting System, Bulletin of National Institute of Hygienic Sciences, 107, 83-87, 1989

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11. Yoshihira Kunitoshi, et al.: Recent Topics on Stevioside, Toxicology Forum, Vol.10 (3), 281-289, 1987

12. Usami Makoto: Teratogenicity Study of Stevioside in Rats, Bulletin of National Institute of Hygienic Sciences, 113, p.31, 1995

13. G. M. Planas: Contraceptive Properties of Stevia rebaudiana, Science, 162, 1007, 1968

14. Mori Noriko, et al.: Effect of Stevioside on Fertility in Rats, Food Hygiene and Safety Science, 22(5), 409-414, 1981

15. Japan Stevia Association: Pregnancy Suppression by Stevia Dry Leaf Extract in Rats, June

1996

16. L. Xili, et al., Chronic oral toxicity and carcinogenicity study of stevioside in Rats, Fd Chem. Toxic. Vol. 30, No.11, p. 957-965, 1992

17. V. Yodyingyuad, et al., Effect of stevioside on growth and reproduction, Human Reproduction Vol. 6 No. 1 p. 158-165 (1991)

18. Shinpo Kotaro, et al.: Study of Stevia Leaf and Stevioside in Pregnancy, 1978

Spirulina colour

1. Food additive name:
   Spirulina colour

2. Origin, method of preparation, and definition:
   Spirulina colour is obtained from the entire part of alga *Spirulina platensis* (NORD.) GEITLER of the Microcoleaceae family by extraction with water at room temperature. It consists mainly of phycocyanin as a principal coloring component. Spirulina colour is blue.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5,000 mg/kg in mice. [1), 2)]

(2) Repeated-dose study
In a 12-month repeated-dose test in SD rats by dietary administration (1%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 0.5 g/kg/day. [3)]

In a test in which the second litter of the three generation (F_3b) that was obtained in a three-generation test in Wistar rats with dry Spirulina was subjected to a 13-week repeated administration (10, 20, and 30% dry Spirulina), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 15 g/kg/day, as dry Spirulina. [4)]

(3) Teratogenicity study
In a teratogenicity test in CD rats by dietary administration (10, 20, and 30% dry Spirulina), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 15 g/kg/day for dry Spirulina. [10)]

(4) Mutagenicity study
All the results from reverse mutation tests in bacteria, [5), 6)] a chromosomal aberration test in cultured cells, [7)] a DNA repair test in bacteria, [6)] a micronucleus test in mouse bone marrow cells, [8)] and a dominant lethal test in rats [9)] were judged as negative.

(References)

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2. Shimizu Mitsuru, et al.: Acute Oral Toxicity of Food Additives Other Than Synthesized Chemicals in Mice and Rats, Seikatsu Eisei (Journal of Urban Living and Health Association), 37(5), 215, 1993

3. Chronic Toxicity Study of Linablue A, 1979, internal data (unpublished)

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Toxicol. Clim. Exp. 8 (3), 1988

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9. SALAZAR M et al: Study of Lethal Dominant of spirulina maxima in Male Rats, Sci. Aliments 10 (3), 1990

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L-Sorbose

1. Food additive name:
   L-Sorbose

2. Origin, method of preparation, and definition:
   L-Sorbose is derived from the fermentation culture solution of *Gluconobacter* or *Acetobacter* containing D-glucose or its reduction product by separation. It consists mainly of L-Sorbose.

3. Major use:
   Sweetener

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 4,000 mg/kg/day in mice. [1)]

(2) Repeated-dose study
In a 26-week repeated-dose test in SD rats by gavage (1,500, 3,000, and 4,500 mg/kg), salivation and soft stool were observed in administered groups at 3,000 mg/kg or higher, and suppression of body weight gain was observed in the 4,500 mg/kg administered group. The no observed adverse effect level is considered to be 1,500 mg/kg/day. [2)]

In a 2-year subcutaneous (25% aqueous solution, 2 mL/rat, 0.5 mL/mouse, twice per week) administration test in Bethesda black rats and C57BL mice, neither toxicological effect nor tumorigenesis caused by administration with the substance was observed. [3)]

(3) Mutagenicity study
Results from a reverse mutation test in bacteria were judged as negative. [4)]

(References)

1. Sorbose Safety Confirmation Study Report, internal data (unpublished)

2. Report of a 26-Week Repeated Oral Toxicity Study of a Novel Sweetener (Sorbose) in Rats, March 1991, internal data (unpublished)

3. N. C. Hueper: Are Sugars Carcinogens? An Experimental Study, Cancer Research, 25, 440, 1965

4. Mutagenicity Study Report, internal data (unpublished)

Onion colour

1. Food additive name:
   Onion colour

2. Origin, method of preparation, and definition:
   Onion colour is obtained from *Allium cepa* LINNE of the Liliaceae family, by extraction with water or hydrous ethanol or by extraction with an alkaline solution and neutralization. It consists mainly of quercetin. Onion colour is yellow.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 2,000 mg/kg in mice, and over 5,000 mg/kg in rats. [1), 2), 3)]

(2) Repeated-dose study
In a 90-day repeated-dose test in $B6C3F_1$ mice by dietary administration (0.3, 0.6, 1.25, 2.5, and 5%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 10 g/kg/day. [1)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria [4)] and a mouse micronucleus test [5)] were judged as negative.

(References)

1. Toshihiro Kojima T. et al: Acute and Subacute Toxicity Test of Onion Coat, Natural Colorant Extracted from Onion (Allium cepa L.) in (C57BL/6XC3H) F1 Mice, J. Toxi. Envi. Health, 38, 89 (1993)

2. Acute Toxicity Study of Onion Colour, 1991, internal data (unpublished)

3. Single-Dose Toxicity Study, 1988, internal data (unpublished)

4. Mutation Assay in Bacteria, 1986, internal data (unpublished)

5. Micronucleus Test of Onion Colour in Mice, 1997, internal data (unpublished)

Tamarind seed gum

1. Food additive name:
   Tamarind seed gum

2. Origin, method of preparation, and definition:
   Tamarind seed gum is obtained from the endosperms of the seeds of *Tamarindus indica* LINNE of the Fabaceae family, by extraction with water or an alkaline aqueous solution at a warm to hot temperature, or by enzymatic treatment (β-galactosidase). It consists mainly of polysaccharides.

3. Major use:
   Thickening stabilizer

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5.0 g/kg in rats, and over 2.0 g/kg in mice. [1), 2)]

(2) Repeated-dose/carcinogenicity study
In a 78-week repeated-dose test in $B6C3F_1$ mice by dietary administration (1.25 and 5.0%), suppression of body weight gain and increased liver weight were observed in the 5.0% administered group; however, no toxicological effect caused by administration with the substance was observed. No carcinogenicity has been observed. The no observed adverse effect level is considered to be 0.19 g/kg/day. [3)]

In a 24-month repeated-dose test in SD rats by dietary administration (4, 8, and 12%), no toxicological effect caused by administration with the substance was observed. No carcinogenicity has been observed. The no observed adverse effect level is considered to be 6 g/kg/day. [4)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria, [5)] a chromosomal aberration test in mammalian cells, [6)] and a DNA repair test in bacteria [7)] were judged as negative.

(References)

1. Hachiya Noriyuki, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives (1981-1983), Toxicology Forum, 8(1), 91-105, 1985

2. Takizawa Yukio: FY 1993 Study on Reevaluation, etc. of Safety of Food Additives, Study on the Acute Toxicity of Natural Additives, 1994

3. M. Sano et al.: Lack of Carcinogenicity of Tamarind Seed Polysaccharide in $B6C3F_1$Mice, Food and Chemical Toxicology, 34, 463-467, 1996

4. Two Year Feeding Toxicity Study of Tamarind Seed Polysaccharide in Rats, J. Toxic. Sci., 3, 163-192, 1978

5. Miyabe Masaki: FY 1993 Evaluation study of safety of food additives, Mutagenicity (1st)

Ames Test

6. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 6) by FY 1984 Ministry of Health and Welfare Research Grant, Toxicology Forum, 8(6), 705-708, 1985

7. Kurita Toshishiro: FY 1993 Study on Reevaluation, etc. of Safety of Food Additives, Mutagenicity Study, 1st Study; Rec-assay

Tea dry distillate

1. Food additive name:
   Tea dry distillate

2. Origin, method of preparation, and definition:
   Tea dry distillate is obtained from tea prepared from the leaves of *Camellia sinensis* O.KZE. of the Theaceae family by dry distillation. The active ingredient cannot be identified, but it contains amino acids, caffeine, tannin, and catechins.

3. Major use:
   Food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 32 g/kg in rats. [1)]

(2) Repeated-dose study
In a 35-day repeated-dose test in Wistar rats by gavage (2.5, 5.0, and 10.0 g/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 10 g/kg. [2)]

In a 52-week repeated-dose test in Wistar rats by administration in drinking water (1.25, 2.5, and 5%) ad libitum, increased white blood cell count was observed in the 5% administered group. The no observed adverse effect level is considered to be 2.5% (male: 1.3-4.3 g/kg, female: 2.0-4.9 g/kg). [3)]

(3) Reproductive/developmental toxicity study
In a test of administration in SD rats before and early pregnancy by gavage (2.5, 5, and 10 mL/kg as liquids containing 20% plant components), no toxicological effect caused by administration with this additive was observed. The no observed adverse effect level is considered to be 10 mL/kg/day. [5)]

In a study of administration in SD rats during the perinatal and lactation period by gavage (2.5, 5, and 10 mL/kg as liquids containing 20% plant components), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 10 mL/kg/day. [6)]

In a study of administration in SD rats during the period of fetal organogenesis by gavage (2.5, 5, and 10 mL/kg as liquids containing 20% plant components), a significant decrease in the food intake of dams as well as a significant decrease in the conception rate in the next generation F_1 animals were observed in the 10 mL/kg administered group. The no observed adverse effect level is considered to be 5 mL/kg/day. [7)]

(4) Mutagenicity study
Results from a reverse mutation test in bacteria were judged as negative. [4)]

(References)

1. Acute Toxicity Study of FS-500M (Fresh Shiraimatsu)-Investigation in Oral Treatment in Rats-, The Clinical Report, 17(4), 29-31, 1983

2. Subacute Toxicity Study of FS-500M in Rats -35-day Continuous Oral Treatment-, 1982, internal data (unpublished)

3. Chronic Toxicity Study of Fresh Shiraimatsu in Rats -1-Year (52-Week) Continuous Oral Treatment-, 1987, internal data (unpublished)

4. Mutagenicity Study of FS-500M (Fresh Shiraimatsu) in Bacteria, 1980, internal data (unpublished)

5. Reproductive Study of Fresh Shiraimatsu -Treatment in Rats in Pre and Early Pregnancy-, 1986 internal data (unpublished)

6. Reproductive Study of Fresh Shiraimatsu -Treatment in Rats during the Perinatal and Lactation Period-, 1986, internal data (unpublished)

7. Reproductive Study of Fresh Shiraimatsu -Treatment in Rats during the Period of Fetal Organogenesis-, 1986, internal data (unpublished)

Thujaplicin (extract)

1. Food additive name:
   Thujaplicin (extract)

2. Origin, method of preparation, and definition:
   Thujaplicin (extract) is obtained from the trunks, branches or stump roots of the tree *Thujopsis dolabrata* SIEB. et ZUCC. of the Cupressaceae family, by steam distillation followed by removal of essential oil with an alkaline aqueous solution at room temperature, neutralization, recrystallization with hexane, and solvent removal. It consists mainly of thujaplicins.

3. Major use:
   Preservative

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} of hinokitiol is considered to be 399-504 mg/kg in mice. [1), 2)]

(2) Repeated-dose study
In a 6-month repeated-dose test of sodium hinokitiol in mice by gavage (1, 5, 10, and 50 mg/kg), both an increase in lipid granules in the adrenal cortex and mononuclear cell infiltration in hepatic lobules were observed in administered groups at 10 mg/kg and higher. The no observed adverse effect level is considered to be 5 mg/kg/day. [3)]

(3) Mutagenicity study
Results from a reverse mutation test of hinokitiol in bacteria were judged as negative. [4)] DNA repair tests in bacteria produced positive results at 1.0 mg/disk without S9, while the results were negative with S9. [4, 5)] In a chromosomal aberration test in cultured cells, chromosomal aberration was induced without S9 at a dose as low as 0.002 mg/mL, while the results were judged as positive at 0.01 mg/mL with S9. [4)] A mouse micronucleus test was performed at doses of 22.5-90.0 mg/kg, and the results were judged as negative. [6)]

(References)

1. S. Li: Pharmacological Study of Hinokitiol, Niigata Medical Journal 95(2), 1951

2. Yamada Akio: Safety Study of Natural Additives, Acute Toxicity Study of Hinokitiol, Tea Extract and ε-Polylysine, FY 1989 Ministry of Health and Welfare Contract Research Report, Osaka City Institute of Public Health and Environmental Sciences

3. Nakano Satoshi: Experimental and Clinical Study of Unsaturated Seven-Membered Ring Compounds, Niigata Medical Journal, Vol. 73, Suppl.1, 1959

4. Sofuni Toshio, et al.: Mutagenicity Study Results of Food Additives (Part 11), Japanese Journal of Mutagenicity Tests on Chemicals, 2(1), 19-28, 1993

5. Ueno Seiichi and Ishizaki Mutsuo: The DNA-Damaging Activity of Natural Food Additives (VI), Food Hygiene and Safety Science, 33(4), 378-382, 1992

6. Takizawa Yukio: FY 1991 Study on Reevaluation, etc. of Food Safety (Ministry of Health and Welfare Contract Research), Study on the Micronuclei Inducibility of Natural Additives, Akita University

5'-Deaminase

1. Food additive name:
   5'-Deaminase

2. Origin, method of preparation, and definition:
   5'-Deaminase is derived from the culture solution of filamentous fungi (*Aspergillus melleus*, or *Aspergillus oryzae*) by extraction with water at cool to room temperature or by concentration at cool to room temperature, followed by ethanol treatment at a cool temperature.

3. Use
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 25,000 mg/kg in mice and over 15,000 mg/kg in rats. [1)]

(2) Repeated-dose study
In a 35-day repeated-dose test in SD rats by dietary administration (500, 2,000, and 8,000 mg/kg), an increase in submandibular gland weight was observed in the 8,000 mg/kg administered group, while an increase in blood urea nitrogen as well as a decrease in ovary weight were observed in females in the same group. The no observed adverse effect level is considered to be 2,000 mg/kg. [1)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria, [2)] a chromosomal aberration test in mammalian cells, [2)] and a DNA repair test in bacteria [3)] were judged as negative.

(References)

1. Acute and Subacute Toxicity Study of Deamizyme, 1992. 3, internal data (unpublished)

2. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 5), FY 1983 Ministry of Health and Welfare Research Grant, Toxicology Forum, Vol. 7 (6), 634-643, 1984

3. Hachiya Noriyuki, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives, Toxicology Forum, Vol. 8 (1), 91-105, 1985

Dextranase

1. Food additive name:
   Dextranase

2. Origin, method of preparation, and definition:
   Dextranase is derived from the culture solution of filamentous fungi (*Chaetomium erraticum*, *Chaetomium gracile*, or *Penicillium lilacinum*), by extraction with water or an acidic aqueous solution at cool to room temperature, by bacterial elimination followed by concentration at cool to room temperature, or by ethanol treatment at a cool temperature.

3. Use
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} using a 1,500,000 units/g bulk powder is 8,260-8,610 mg/kg in mice, and over 4,000 mg/kg in rats. [1)]

The acute oral LD_{50} using a 2,290,000 units/g bulk powder is over 2,000 mg/kg in rats. [2), 3)]

The acute oral LD_{50} using a 63,000 units/mL stock solution is over 20 mL/kg in mice and rats. [2), 4)]

(2) Repeated-dose study
In a 26-week repeated-dose test in Wistar-Imamichi rats by gavage (0.5, 5.0, 50, 500, and 1,000 mg/kg, and 1,500,000 units/g), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be over 1,000 mg/kg. [5)]

In a 90-day repeated-dose test in SD rats by gavage (500, 1,000, and 2,000 mg/kg, and 2,290,000 units/g), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2,000 mg/kg/day. [2), 6)]

(3) Teratogenicity study (Administration during the period of fetal organogenesis)
In a study of administration in Wistar-Imamichi rats during the period of fetal organogenesis by gavage (80, 800, and 2,000 mg/kg, and 1,500,000 units/g), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2,000 mg/kg/day. [10)]

(4) Mutagenicity study
All the results from a DNA repair test in bacteria, a reverse mutation test in bacteria, and a host-mediated assay with bacteria in mice were judged as negative. [7)] All the results from the reverse mutation tests of *Chaetomium erraticum*-derived enzyme (2,290,000 units/g) [8)] and *Chaetomium erraticum*-derived enzyme (liquid) [9)] in bacteria were judged as negative.

(References)

1. Acute Toxicity Study of Dextranase in Mice and Rats, internal data (unpublished)

2. Test Methods for Dextranase

3. Safety Study of Dextranase Produced by *Chaetomium erraticum* (1) Single-Dose Toxicity Study in Rats, October 1990, internal data (unpublished)

4. Acute Toxicity Study of Dextranase L Stock Solution, -Oral Acute Toxicity Study in Mice and Rats, February 1986, internal data (unpublished)

5. Toxicity of Dextranase in Rats, 5-Week Continuous Oral Treatment and 26-Week Continuous Oral Treatment, internal data (unpublished)

6. Safety Study of Dextranase Produced by *Chaetomium erraticum* (4), 90-Day Oral Toxicity Study of Dextranase Bulk in Rats, May 1992, internal data (unpublished)

7. Mutagenicity Study of Dextranase in Bacteria, internal data (unpublished)

8. Safety Study of Dextranase Produced by *Chaetomium erraticum* (2) Mutagenicity Study in Bacteria, December 1990, internal data (unpublished)

9. Safety Study of Dextranase L Produced by *Chaetomium erraticum*, Mutagenicity Study in Bacteria, July 1986, internal data (unpublished)

10. Reproductive Study of Dextranase, Study of Treatment in Rats during the Period of Fetal Organogenesis, internal data (unpublished)

Transglutaminase

1. Food additive name:
   Transglutaminase

2. Origin, method of preparation, and definition:
   Transglutaminase is derived from animal liver or the culture solution of actinomycetes (*Streptomyces, Streptoverticillium mobaraense*) or bacteria (*Bacillus*), by extraction with water at room temperature followed by ethanol treatment at a cool temperature.

3. Major use:
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 2,000 mg/kg in rats. [1)]

(2) Repeated-dose study
In a 3-month repeated-dose test in SD rats by dietary administration (0.2, 1.0, and 5.0%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [2)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria, [3)] a chromosomal aberration test in mammalian cells, [4)] and a mouse micronucleus test [5)] were judged as negative.

(References)

1. Single-Dose Oral Toxicity Study of HBTG in Rats, July 1990, internal data (unpublished)

2. Thirteen-Week Repeated-Dose Toxicity Study of HBTG in Rats by Dietary Treatment and 5-Week Recovery Study, January 1991, internal data (unpublished)

3. Reverse Mutation Study of Enzyme Protein in Bacteria, November 1990, internal data (unpublished)

4. Chromosomal Aberration Test of Enzyme Protein in Cultured Mammalian Cells, March 1991, internal data (unpublished)

5. Micronucleus Test of Enzyme Protein in Rodents, March 1991, internal data (unpublished)

Nystose

1. Food additive name:
   Nystose

2. Origin, method of preparation, and definition:
   Nystose is obtained from sucrose by enzyme treatment (fructosyl transferase) followed by separation. It consists mainly of nytose.

3. Major use:
   Food manufacturing agent

4. Summary of safety study results: (note)

(1) Single-dose study
The acute oral LD_{50} is considered to be over 9,000 mg/kg in rats and mice. [1)]

(2) Repeated-dose/carcinogenicity study
In a 6-week repeated-dose test in Wistar rats by gavage (1,500, 3,000, and 4,500 mg/kg), mild suppression of body weight gain and distension of the cecum were observed in administered groups at 3,000 mg/kg and higher. However, these changes were considered to have occurred because the substance was a scarcely-absorbable carbohydrate. The no observed adverse effect level is considered to be 4,500 mg/kg/day. [1)]

In a 6-week repeated-dose test in Wistar rats by dietary administration (the substance was added to constitute 5% and 10% to the basal diet, from which 5% as sucrose or starch was removed), suppression of body weight gain, decreased serum cholesterol, and distension of the cecum were observed. However, these changes were considered to have occurred because the substance was a scarcely-absorbable carbohydrate. The no observed adverse effect level is considered to be 5,000 mg/kg/day. [1)]

In a 104-week repeated-dose test in F344 rats by dietary administration (0.8, 2, and 5%), neither a toxicological effect caused by administration with the substance nor carcinogenicity was observed. The no observed adverse effect level is considered to be 2,500 mg/kg/day. [2)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria, [3)] unscheduled DNA synthesis assay in cultured mammalian cells, [4)] and a TK locus mutation assay in mouse lymphoma cells [5)] were judged as negative, regardless of the presence or absence of S9 mix.

(Note) The substance subjected to the tests was 39% kestose, 46% nystose, and 10% 1-fructofranosyl nystose.

(References)

1. Takeda Ueto and Niizato Tersutaro: Safety Study of Neosugar, Neosugar Workshop Report, 17-27, 1982

2. Inoue Hiroyuki: Long-Term Safety Study of Neosugar, Neosugar Workshop Report, 45-59,

1988

3. Microbial metabolic activation test to assess the potential mutagenic effect of Neosugar, May 1986, Internal data (unpublished)

4. Autoradiographic assessment of unscheduled DNA repair synthesis in mammalian cells after exposure to Neosuger, Nov. 1986, Internal data (unpublished)

5. An assessment of the mutagenic potential of Neosugar using the mouse lymphoma TK locus assay, Jan. 1987, Internal data (unpublished)

Hyaluronic acid

1. Food additive name:
   Hyaluronic acid

2. Origin, method of preparation, and definition:
   Hyalironic acid is obtained from the chicken combs by extracting it with lukewarm to warm water, alkaline aqueous solution or an acidic aqueous solution, treating the extract with ethanol or hydrous ethanol, or treating it with ethanol or hydrous ethanol after enzymatic treatment, followed by purification. Alternatively, Hyaluronic acid is also obtained from the culture solution of bacteria (*Streptococcus zooepidemicus*), sterilized at cold to warm temperature, treating it with ethanol or hydrous ethanol, followed by purification. It consists mainly of hyaluronic acid.

3. Major use:
   Food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 2,400 mg/kg, over 800 mg/kg, and over 1,000 mg/kg in mice, rats and rabbits, respectively. [1)]

(2) Repeated-dose study
In a 3-month repeated-dose test in SD rats by intraperitoneal administration (15, 30, and 60 mg/kg), decreased total protein was observed in males of the 60 mg/kg administered group, and decreased red blood cells as well as increased MCH and MCV were observed in males of the 30 and 60 mg/kg administered groups, with all animals recovering after withdrawal for 35 days. The no observed adverse effect level for intraperitoneal administration is considered to be 15 mg/kg/day. [2)]

In a 6-month repeated-dose test in beagle dogs, administered twice per week into the knee joint cavity (2, 6, and 12 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level for intraarticular in the knee joint cavity is considered to be 12 mg/kg/day. [3)]

(3) Reproductive/developmental toxicity study
No toxicological effect caused by administration with the substance was observed in any of a study in SD rats by subcutaneous administration (8, 20, and 50 mg/kg) during the period of fetal organogenesis, a study of administration in before and early pregnancy, a study of treatment during the perinatal and lactation period, and a study in New Zealand White rabbits by subcutaneous treatment (8, 20, and 50 mg/kg) during the period of fetal organogenesis. The no observed adverse effect level is considered to be 50 mg/kg/day in all the studies. [6), 7), 8), 9)]

(4) Mutagenicity study
All the results from a reverse mutation test in bacteria, [4)] a chromosomal aberration test in mammalian cells, [4)] and a mouse micronucleus test [5)] were judged as negative.

(5) Other toxicity studies

Both PCA reaction and active systemic anaphylaxis were found to be negative in an antigenicity test in mice and guinea pigs. [10)]

(References)

1. Nagano Kiyoshi, et al.: Acute Toxicity Study of Sodium Hyaluronate (SPH), Japanese Pharmacology & Therapeutics, 2(12), 37-45, 1984

2. Hasegawa Takashi, et al.: Subacute Toxicity Study by 3-Month Intraperitoneal Treatment and Recovery Study of Sodium Hyaluronate (SPH) in Rats, Pharmacometrics, 28(6), 1021-40, 1975

3. Miyoshi Koji, et al.: Chronic Toxicity Study and Recovery Study of Sodium Hyaluronate (SPH) by 6-Month Intraarticular Treatment in the Knee Joint Cavity in Beagle Dogs (1) Systemic Observations, Pharmacometrics, 29(1), 49-81, 1985

4. Onishi Mizuo, et al.: Mutagenicity Study of Sodium Hyaluronate (SH), Japanese Pharmacology & Therapeutics, 20(3), 65-72, 1992

5. Ariga Fumihiko, et al.: Mouse Micronucleus Assay of Sodium Hyaluronate (SH), Japanese Pharmacology & Therapeutics, 20(3), 73-75, 1992

6. Ono Chizuko, et al.: Reproductive and Developmental Toxicity Study of Sodium Hyaluronate (SH) (1) -Study in Rats by Subcutaneous Treatment during the Period of Fetal Organogenesis-, Japanese Pharmacology & Therapeutics, 20(3), 11-26, 1992

7. Ono Chizuko, et al.: Reproductive and Developmental Toxicity Study of Sodium Hyaluronate (SH) (2) -Study in Rats by Subcutaneous Treatment in Pre and Early Pregnancy-, Japanese Pharmacology & Therapeutics, 20(3), 27-35, 1992

8. Ono Chizuko, et al.: Reproductive and Developmental Toxicity Study of Sodium Hyaluronate (SH) (3) -Study in Rats by Subcutaneous Treatment during the Perinatal and Lactation Period-, Japanese Pharmacology & Therapeutics, 20(3), 37-50, 1992

9. Ono Chizuko, et al.: Reproductive and Developmental Toxicity Study of Sodium Hyaluronate (SH) (4) -Study in Rabbits by Subcutaneous Treatment during the Period of Organogenesis-, Japanese Pharmacology & Therapeutics, 20(3), 51-58, 1992

10. Takemoto Minoru, et al.: Antigenicity Study of Sodium Hyaluronate (SH), Japanese Pharmacology & Therapeutics, 20(3), 59-64, 1992

Sunflower seed extract

1. Food additive name:
   Sunflower seed extract

2. Origin, method of preparation, and definition:
   Sunflower seed extract is obtained from the seeds or the pressed seed oil-phase of sunflower (*Helianthus annuus* LINNE) of the Asteraceae family, by extraction with water or hydrous ethanol at high temperature. It contains isochlorogenic acid and chlorogenicacid as active ingredients.

3. Major use:
   Antioxidant

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 2,000 mg/kg in mice. [1)]

(2) Repeated-dose study
In an 8-week repeated-dose test in F344 rats by dietary administration (1%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 0.5 g/kg/day. [2)]

(3) Mutagenicity study
Results from a reverse mutation test in bacteria were judged as negative. [3)]

(References)

1. Acute Toxicity Study of Sunflower Seed Extract, 1990, internal data (unpublished)

2. Subacute Toxicity Study of Sunflower Seed Extract, 1991, internal data (unpublished)

3. Mutagenicity Study of Sunflower Seed Extract, 1990, internal data (unpublished)

Phytic acid

1. Food additive name:
   Phytic acid

2. Origin, method of preparation, and definition:
   Phytic acid is obtained from the seed bran of the rice plant (*Oryza sativa* LINNE) or the seeds of the corn plant (*Zea mays* LINNE) by extraction with water at room temperature or acidic solution, followed by purification. It consists mainly of inositol hexaphosphate.

3. Major use:
   Acid, food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be 0.9 g/kg in mice, and 0.41 g/kg in rats. [1), 2)]

(2) Repeated-dose/carcinogenicity study
In a 12-week repeated-dose test in F344 rats with drinking water (0.6, 1.25, 2.5, 5.0, and 10%), all animals of the 10% administered group and all males and one female of the 5.0% administered group died before the end of the test. Furthermore, suppression of body weight gain was observed in the 1.25 and 2.5% administered groups. The no observed adverse effect level is considered to be 300 mg/kg. [3)]

In a 100- to 108-week carcinogenicity test in F344 rats with drinking water (1.25 and 2.5%), suppression of body weight gain and uric blood were observed in both administered groups. Histopathological test showed hyperplasia in the renal pelvis in males of both administered groups, and renal pelvis papilloma was observed in a small number of animals in the administered groups (2.5% group males, 3/57; 2.5% group females, 4/55; and 1.25% group females, 3/58). This occurrence of renal pelvis papilloma is considered to have occurred because long-term administration with a high dose of chelating substances, such as phytic acid results in calcification in the renal pelvis in rats, and epithelial necrosis and regeneration caused by this stimulation promotes tumor generation. In this test, calcification or papillary necrosis in the kidney was also observed in animals in which renal pelvis papilloma was observed. No histopathological change caused by administration with the substance was observed in any other organs. [4)]

(3) Teratogenicity study
In a teratogenicity test in SD rats from day 7 to 17 of gestation by dietary administration (0.625, 1.25, and 2.5%), no teratogenicity was observed. However, an increase in the frequency of skeletal mutation was observed in the 2.5% administered group, which was considered to be a secondary effect on dams. The no observed adverse effect level is considered to be 750 mg/kg/day.

(4) Mutagenicity study
All the results from a reverse mutation test in bacteria, [5)] a chromosomal aberration test in mammalian cells, [5)] and a mouse micronucleus test [6)] were judged as negative.

(References)

1. Fujitani Tomoko: Acute Toxicity of Phytic Acid and Sodium Phytate to Mice, Annual Report of the Tokyo Metropolitan Research Laboratory of Public Health, 38, 368-370, 1987

2. Acute Study Report, Department of Public Health, Kitasato University (September 1968)

3. Ichikawa Hisatsugu: Studies on Acute Oral Toxicities of Phytic Acid and Sodium Phytate in Rats, Annual Report of the Tokyo Metropolitan Research Laboratory of Public Health, (38), 371-376, 1987

4. Y. Hiasa, Y. Kitahori, J. Morimoto, N. Konishi, S. Nakaoka, and H. Nishioka: Carcinogenicity study in rats of phytic acid 'Daiichi', a natural food additive, Food Chem. Toxicol. 30(2), 117-125, 1992

5. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 2), Mutagens & Toxicology, 4(6), 80-89, 1981

6. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 9), Toxicology Forum, 11(6), 663-669, 1988

7. Matsumoto Nobuo, et al.: FY 1987 Study on Reevaluation, etc. of Safety of Food Additives, Study on the Teratogenicity of Phytic Acid (Ministry of Health and Welfare Contract Research), Jikei University School of Medicine

Pullulanase

1. Food additive name:
   Pullulanase

2. Origin, method of preparation, and definition:
   Pullulanase is derived from the culture solution of bacteria (*Bacillus, Klebsiella, Sulfolobus solfataricus*) by extraction with water at cool to room temperature, followed by bacterial elimination, concentration at cool to room temperature, treatment with ethanol, hydrous ethanol, or acetone at a cool temperature, or fractionation, such as with ammonium sulfate followed by desalting.

3. Major use:
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} of Pullulanase from *Bacillus circulans* is over 3,000 mg/kg in rats. [1)]

The acute oral LD_{50} of Pullulanase from *Bacillus sectorramus* is over 20 mL (approximately 9,000 units)/kg in rats and mice. [2), 3)]

The acute oral LD_{50} of Pullulanase from *Klebsiella pneumoniae* is about 210,000 units/kg in mice, and over 187,000 units/kg in rats. [2), 4)]

(2) Repeated-dose study
In a 13-week repeated-dose test of Pullulanase from *Bacillus circulans* in SD rats by gavage (200, 600, and 2,000 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2,000 mg/kg/day. [5)]

In a 90-day repeated-dose test of Pullulanase from *Bacillus sectorramus* in SD rats by gavage (2.5, 5.0, and 0 mL/kg), increased urine specific gravity, decreased thyroid weight and decreased adrenal gland weight were observed in the 10.0 mL/kg administered group. The no observed adverse effect level is considered to be 5.0 mL (approximately 2,250 units)/kg/day. [6)]

In a 26-week repeated-dose test of Pullulanase from *Klebsiella pneumoniae* in Wistar rats by oral dietary administration (3,750, 7,500, and 15,000 units/kg), suppression of body weight gain was observed in the 15,000 units/kg administered group. The no observed adverse effect level is considered to be 7,500 units/kg/day. [4)]

(3) Mutagenicity study
Both pullulanase from *Bacillus circulans* and from *Bacillus sectorramus* were judged as negative in reverse mutation tests in bacteria. [7), 8)]

(References)

1. Single Oral Gavage Toxicity Study of Amirax in Rats, March 1992, internal data

(unpublished)

2. Test Methods for DB-250

3. Safety Study of the Stock Solution of Debranching Enzyme (DB-1) Produced by *Bacillus sectorramus*, Oral Acute Toxicity Study in Mice and Rats, July 1987, internal data (unpublished)

4. Acute, Subacute and Chronic Toxicity Study of Pullulanase Produced by *Klebsiella pneumoniae*, October 1975, internal data (unpublished)

5. Thirteen-Week Repeated-Dose Oral Toxicity Study of Amirax in Rats, November 1992, internal data (unpublished)

6. Safety Study of the Stock Solution of Debranching Enzyme (DB-1) Produced by *Bacillus sectorramus*, Oral Subacute Toxicity Study in Rats, March 1988, internal data (unpublished)

7. Reverse Mutation Study of Amirax in Bacteria, October 1992, internal data (unpublished)

8. Safety Study of the Stock Solution of Debranching Enzyme (DB-1) Produced by *Bacillus sectorramus*, Mutagenicity Study in Bacteria, May 1987, internal data (unpublished)

Pullulan

1. Food additive name:
   Pullulan

2. Origin, method of preparation, and definition:
   Pullulan is a polysaccharide obtained by isolation from the culture solution of *Aureobasidium pullulans* (DE BARY) ARN. It consists mainly of pullulan.

3. Major use:
   Thickening stabilizer, Food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 14,300-24,100 mg/kg in mice, [1), 2)] and over 5,000 mg/kg in rats. [3)]

(2) Repeated-dose study
Although a 62-week repeated-dose test was performed in SD rats by dietary administration (1, 5, and 10%), the survival rate of the control group was below 50% due to death by pneumonia; accordingly, only a limited number of toxicological observations could be evaluated. Increased cecum weight was observed in the 10% administered group, which was considered to be caused by the administration of scarcely-absorbable carbohydrate. [3)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria, [4), 5)] a DNA repair test in bacteria, [3)] and a mouse micronucleus test [6)] were judged as negative.

(4) Others
No change in the test value was observed in blood biochemical test after a 14-day repeated-dose administration with pullulan with a molecular weight of 50,000 (10 g/day) in 13 healthy adult males. [7)]

(References)

1. Acute Toxicity Study of Pullulan, August 1974, internal data (unpublished)

2. Toxicity Study of Pullulan, June 1974, internal data (unpublished)

3. T. Kimoto, et al.: Safety studies of a novel starch, pullulan. Chronic toxicity in rats and bacterial mutagenicity, Food and Chemical Toxicology, 35, 323-329 (1997)

4. Hachiya Noriyuki, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives, Toxicology Forum, Vol. 8 (1), 91-105, 1985

5. Mutagenicity Study of Pullulan, March 1978, internal data (unpublished)

6. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives (Part 9), by FY 1987 Ministry of Health and Welfare Research Grant, Toxicology Forum, Vol. 11 (6), 663-669,

1988

7. Yoneyama Masaru, et al.: Effects of Pullulan Intake in Humans, Journal of the Japanese Society of Starch Science, 37(3), 123-127, 1990

Hesperidinase

1. Food additive name:
   Hesperidinase

2. Origin, method of preparation, and definition:
   Hesperidinase is derived from the culture solution of filamentous fungi (*Aspergillus, Penicillium decumbens*), by extraction with water at cool to room temperature, followed by concentration at cool to room temperature, and ethanol treatment at a cool temperature.

3. Major use:
   Enzyme

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} of hesperidinase (hesperidinase activity 110 units/g) is over 40 g/kg in mice, and over 24 g/kg in rats. [1)]

(2) Repeated-dose/carcinogenicity study
In a 35-day repeated-dose test of hesperidinase (hesperidinase activity 110 units/g) in ddY mice by dietary administration (0.4, 2, and 10 g/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 10 g/kg. [1)]

In a 35-day repeated-dose test of hesperidinase (hesperidinase activity 110 units/g) in SD rats by dietary administration (0.4, 2, and 10 g/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 10 g/kg. [1)]

(3) Mutagenicity study
Results from a reverse mutation test in bacteria were judged as negative. [2)]

(References)

1. Acute and Subacute Toxicity Study of Hesperidinase, 1982, internal data (unpublished)

2. Mutagenicity Study of Hesperidinase in Bacteria, 1983, internal data (unpublished)

Monascus yellow

1. Food additive name:
   Monascus yellow

2. Origin, method of preparation, and definition:
   Monascus yellow is derived from dried and grinded culture fluids of *Monascus purpureus* WENT. by extraction with lukewarm weakly acidic hydrochloric acid ethanol, followed by neutralization. It consists mainly of xanthomonasins as a principal coloring component. Monascus yellow is yellow.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 60 g/kg in mice. [1)]

(2) Repeated-dose/carcinogenicity study
In a 90-day repeated-dose test in SD rats by gavage (1, 2, and 4 mL/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 4 mL/kg. [2)]

(3) Mutagenicity study
Results from a reverse mutation test of Monascus purpureus extract (major-colored components: xanthomonasins) in bacteria were judged as negative. [3)]

(References)

1. Acute Toxicity Study in Mice, 1982, internal data (unpublished)

2. Subacute Toxicity Study of High-Moon Yellow S in Rats by 90-Day Oral Treatment, 1988, internal data (unpublished)

3. Mutagenicity Study of High-Moon Yellow S in Bacteria, 1984, internal data (unpublished)

Monascus colour

1. Food additive name:
   Monascus colour

2. Origin, method of preparation, and definition:
   Monascus colour is obtained from the fungus body of *Monascus pilosus K.* SATO ex D. HAWKSWORTH et PITT and *Monascus purpreus* WENT. of Ascomycota, by extraction with room temperature to lukewarm hydrous ethanol or hydrous propylene glycol. It consists mainly of monascorubrin, ankaflavin, etc, as principal coloring components. Monascus colour is red.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 14 g/kg in mice, and over 5 g/kg in rats. [1), 2)]

(2) Repeated-dose/carcinogenicity study
In a 13-week repeated-dose test in F344 rats by dietary administration (0.6, 1.25, 2.5, 5, and 7%), suppression of body weight gain and necrosis in the epithelium of the renal tubule were observed in the 7% administered group and in the administered groups at 5% and higher, respectively. The no observed adverse effect level is considered to be 1.25 g/kg. [3)]

In a 108-week carcinogenicity test in F344 rats by dietary administration (0, 1.25, and 2.5%), no tumorigenesis caused by administration with the substance was observed. [4)]

(3) Mutagenicity study
While results from reverse mutation tests in bacteria were judged as negative, [5), 6)] positive results were reported in a test at high doses of 1-200 μL/plate. [7)] All the results from DNA repair tests in bacteria [6), 7), 8)] and a chromosomal aberration test in mammalian cells were judged as negative. [9)] A mouse micronucleus test was performed for doses as high as 5 g/kg, and the results were judged as negative. [10)]

(References)

1. Acute Toxicity of Natural Colour Monascus Red, 1973, internal data (unpublished)

2. Shimizu Mitsuru, et al.: Osaka City Institute of Public Health and Environmental Science, Acute Oral Toxicity of Food Additives Other Than Synthesized Chemicals in Mice and Rats, Seikatsu Eisei (Journal of Urban Living and Health Association), 37, 215-220, 1993

3. Hiasa Yoshio: Subacute Toxicity Study Report of Monascus Colour in F344 Rats, Ministry of Health and Welfare Contract Research (FY 1991)

4. Hiasa Yoshio, et al., Lack of Carcinogenicity of Monascus Colour in Fisher 344 Rats Journal of Toxicological Pathology, 10(4), 187-192, 1997

5. Mutagenicity Study of Anka Red SP500 in Bacteria, 1984, internal data (unpublished)

6. Koizumi Kaio, et al.: Bacteria Mutagenicity of Colour Produced by Monascus, College of Biomedical Technology, Niigata University, Niigata Medical Journal, 95(7), 453, 1981

7. Hachiya Noriyuki: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives, Toxicology Forum, Vol. 8(1), 91-105, 1985

8. Kuroda Koichi, et al.: Rec-Assay of Food Additives (2nd Report) -Results of 49 Natural Additives-, Seikatsu Eisei (Journal of Urban Living and Health Association), 33, 15-23, 1989

9. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives, Mutagens & Toxicology, Vol. 12, 82-90, 1980

10. Hachiya Noriyuki, et al.: Micronucleus test with natural additives, Japanese Journal of Mutagenicity Tests on Chemicals, Vol. 1 (1), 13-17, 1992

Carthamus yellow

1. Food additive name:
   Carthamus yellow

2. Origin, method of preparation, and definition:
   Carthamus yellow is obtained from the flowers of the safflower (*Carthamus tinctorius* LINNE) of the Asteraceae family by extraction with water at room temperature to a slightly warm temperature. It consists mainly of saffor yellows (saflomins) as principal coloring components. Carthamus yellow is yellow.

3. Major use:
   Color

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 20 g/kg in mice [1), 5)] and over 5 g/kg in rats. [2), 3), 4)]

(2) Repeated-dose/carcinogenicity study
In a 6-month repeated-dose test in SD rats by dietary administration (0.1, 1, and 10%, no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 5 g/kg/day. [6)]

In a 108-week carcinogenicity test in F344 rats by dietary administration (2.5 and 5%), no toxicological effect caused by administration with the substance was observed. No carcinogenicity has been observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [7)]

(3) Mutagenicity study
Safflower extract and carthamus yellow (liquid) showed negative results in reverse mutation tests in bacteria, [4), 8)] while positive results were reported for powder at doses of 0.5-200 mg/plate. [4)] Results in a DNA repair test in bacteria for liquid were judged as negative. [4)] All results from both a chromosomal aberration test in cultured mammalian cells and a mouse micronucleus test were judged as negative. [9), 10)]

(References)

1. Carthamus Yellow, WHO Food Additive Series No.12, pp64 (21st), 1985

2. Oral Acute Toxicity Study of Carthamus Yellow in Rats, 1980, internal data (unpublished)

3. Shimizu Mitsuru, et al.: Acute Oral Toxicity of Food Additives Other Than Synthesized Chemicals in Mice and Rats, Seikatsu Eisei (Journal of Urban Living and Health Association), 37(5), 215-220, 1993

4. Hachiya Noriyuki, et al.: Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives, Toxicology Forum, 8(1), 91-105, 1985

5. Acute and Subacute Toxicity Study Report of Safflor Yellow, 1967, internal data (unpublished)

6. Chronic Toxicity Study Report of Yellow Colour Tanacolor Y, 1971, internal data (unpublished)

7. Matsuki Hisashi, et al.: Lack of Carcinogenicity of Commercial Safflower Yellow in Fischer 344 Rats, J. Toxicol. Pathol., 1, 149-155, 1988

8. Bacterial Mutation Study Report, 1986, internal data (unpublished)

9. Ishidate Motoi, et al.: Mutagenicity Study Results of Food Additives, Mutagens & Toxicology, Vol. 12, 82-90, 1980

10. Sofuni Toshio, et al.: Mutagenicity Study Results of Food Additives (Part 11), Japanese Journal of Mutagenicity Tests on Chemicals, 2(1), 19-28, 1993

Peptidase

1. Food additive name:
   Peptidase

2. Origin, method of preparation, and definition:
   Peptidase is derived from the culture solution of filamentous fungi (*Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus sojae*, and *Rhizopus oryzae*) or bacteria (*Bacillus* and *Lactococcus lactis*), by extraction with water at cool to room temperature, by bacterial elimination with or without ethanol treatment at a cool temperature, or by solid-liquid separation of the culture solution followed by concentration and filtration.

3. Major use:
   Enzyme

4. Summary of safety study results:

(1) Repeated-dose study
   In a 90-day repeated-dose test of Peptidase from *Rhizopus oryzae* (135,000 units/g) in SD rats by gavage (500, 1,000, and 2,000 mg/kg), suppression of body weight gain, decreased food and water intake, and decreased urine volume, along with an associated increased urine osmolality and urine creatinine were observed in males in the 2,000 mg/kg administered group. The no observed adverse effect level is considered to be 1,000 mg/kg/day. [1)]

(2) Mutagenicity study
   Results from mouse micronucleus tests with peptidase from *Rhizopus oryzae* (135,000 units/g) were judged as negative. [2), 3)]

(References)

1. Safety Study of Peptidase R from *Rhizopus oryzae*, 90-Day Repeated Oral Toxicity Study in Rats, 1990.10, internal data (unpublished)

2. Test Methods for Peptidase R

3. Safety Study of Peptidase R from *Rhizopus oryzae* No. 3545 (V), Mouse Micronucleus Test, October 1990, internal data (unpublished)

ε-Polylysine

1. Food additive name:
   ε-Polylysine

2. Origin, method of preparation, and definition:
   ε-Polylysine is obtained from the culture solution of actinomycete (*Streptomyces albulus*) by adsorption and isolation using ion-exchange resin. It consists mainly of ε-polylysine.

3. Major use:
   Preservative

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5,000 mg/kg in both rats and mice. [1), 2)]

(2) Repeated-dose/carcinogenicity study
In a 3-month repeated-dose test in SD rats by dietary administration (0.2, 1, and 5%), decreased food intake, suppression of body weight gain, decreased blood glucose, blood albumin, blood triglyceride, and blood phospholipid, and decreased liver weight and decreased thyroid weight were observed in the 5% administered group. In addition, decreased white blood cell count associated with decreased lymphocytes was observed. The no observed adverse effect level is considered to be 0.5 g/kg/day. [3)] In a 104-week combined chronic toxicity/carcinogenicity study in SD rats by dietary administration (0.2, 0.65, and 2%), suppression of body weight gain was observed in the 2% administered group at the early stage of administration, although the animals recovered to normal afterwards. No carcinogenicity has been observed. The no observed adverse effect level is considered to be 1 g/kg/day. [4)]

(3) Mutagenicity study
All the results from reverse mutation tests in bacteria, [5), 6)] a DNA repair test in bacteria, a chromosomal aberration test in mammalian cells, [6)] and a mouse micronucleus test [7)] were judged as negative.

(References)

1. Acute Oral Toxicity Study of Polylysine Preparation in Rats, 1989, internal data (unpublished)

2. Yamada Akio: Safety Study of Natural Additives, Acute Toxicity Study of Hinokitiol, Tea Extract and ε-Polylysine, FY 1989 Ministry of Health and Welfare Contract Research, Osaka City Institute of Public Health and Environmental Sciences

3. Ishii Minoru, et al.: Three-Month Repeated Oral Toxicity Study of Polylysine Powder in Rats by Dietary Treatment, The Clinical Report, 27, 6, 2013-2033, 1993

4. Fukutome Akira, et al.: Combined Oral Chronic Toxicity/Carcinogenicity Study of Polylysine Powder in Rats by Dietary Treatment, The Clinical Report, 29, 6, 1416-1434, 1995

5. Mutagenicity Study Report No. 2008, 1988, internal data (unpublished)

6. Sofuni Toshio, et al.: Mutagenicity Study Results of Food Additives (Part 12), Japanese Journal of Mutagenicity Tests on Chemicals, 3(4), 206-215, 1994

7. Takizawa Yukio: FY 1991 Study on Reevaluation, etc. of Food Safety (Ministry of Health and Welfare Contract Research), Study on the Micronuclei Inducibility of Natural Additives, Akita University

Menaquinone (extract)

1. Food additive name:
   Menaquinone (extract)

2. Origin, method of preparation, and definition:
   Menaquinone (extract) is obtained from the culture solution of bacteria (*Arthrobacter nicotianae*) by extraction with butanol at room temperature, followed by extraction with hexane at room temperature and purification. It consists mainly of menaquinone-4.

3. Major use:
   Nutrition fortifier

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 5,000 mg/kg in mice. [1)]

(2) Repeated-dose/carcinogenicity study
In a 6-month repeated-dose test of menaquinone (synthetic) in Wistar rats by gavage (8, 40, and 200 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 200 mg/kg/day. [2)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria and a DNA repair test in bacteria with menaquinone (synthetic) were judged as negative. [3)]

(References)

1. Acute Toxicity Study Results of Menaquinone in Mice, 1991, internal data (unpublished)

2. Ogawa Tadashi, et al.: Toxicity Study of Menaquinone-4, (I) Acute, Subacute and Chronic Toxicity Study in Mice, Rats and Dogs, Pharmacometrics, 5(3), 445-459, 1971

3. Mochida Hisatoshi, et al.: Mutagenicity Study of Menatetrenone (K2), Pharmacotherapy 14(2), 55-58, 1981

Chinese bayberry extract

1. Food additive name:
   Chinese bayberry extract

2. Origin, method of preparation, and definition:
   Chinese bayberry extract is obtained from the fruits, bark or leaves of Chinese bayberry (*Myrica rubra* SIEBOLD) of the Myricaceae family by extraction with water, ethanol, or methanol. Chinese bayberry extract contains myricitrin as a component.

3. Major use:
   Antioxidant

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be over 2,000 mg/kg in rats. [1)]

(2) Repeated-dose study
In a 3-month repeated-dose test in SD rats by dietary administration (1.25, 2.5, and 5%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [2)]

(3) Mutagenicity study
Results from a reverse mutation test in bacteria were judged as negative. [3)]

(References)

1. Acute Toxicity Study of Chinese Bayberry Extract in Rats by Single Oral Treatment, 1993, internal data (unpublished)

2. Subacute Toxicity Study of Chinese Bayberry Extract in Rats by 3-Month Oral Treatment, 1994, internal data (unpublished)

3. Reverse Mutation Study of Chinese Bayberry Extract in Bacteria, 1993, internal data (unpublished)

Rutin (extract)

1. Food additive name:
   Rutin (extract)

2. Origin, method of preparation, and definition:
   Rutin (extract) is obtained from the entire part of azuki, the buds or flowers of enju, or the entire part of the buckwheat. It consists mainly of rutin.

3. Major use:
   Antioxidant, nutrition fortifier, color

4. Summary of safety study results:

(1) Repeated-dose/carcinogenicity study
   No carcinogenicity was observed in ACI rats due to either a 540-day dietary administration (5%) or an 850-day dietary administration (10%), or in golden hamsters due to a 735-day dietary administration (10%). [1), 2)]

(2) Mutagenicity study
   Results from a reverse mutation test in bacteria were judged as positive. [3)] All the results from a DNA repair test in bacteria, a chromosomal aberration test in mammalian cells, and a mouse micronucleus test were judged as negative. [3), 4), 5), 6)]

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3. Hachiya Noriyuki, et al.: II. Summary of Results from Acute Toxicity and Mutagenicity Studies with Natural Additives (FY 1981-1983), Toxicology Forum, 8(1), 91-105, 1985

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5. Ishidate Motoi, et al.: I. Mutagenicity Study Results of Food Additives (Part 8) by FY 1986 Ministry of Health and Welfare Research Grant, Toxicology Forum, 10(6), 649-654, 1987

6. Ministry of Health and Welfare FY 1991 Study on Reevaluation, etc. of Safety of Food Additives, Report on Micronucleus Test (2nd Study)

Wasabi extract

1. Food additive name:
   Wasabi extract

2. Origin, method of preparation, and definition:
   Wasabi extract is obtained from the rhizomes or leaves of *Wasabia japonica* MATSUM. of the Brassicaceae family by extraction with ethanol. It consists mainly of isothiocyanates.

3. Major use:
   Food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} of allyl isothiocyanate is considered to be 339 mg/kg in rats. [1)]

(2) Repeated-dose/carcinogenicity study
Allyl isothiocyanate (purity 93% and higher) was dissolved in corn oil, and 6 studies by gavage were performed.

In a 14-day repeated-dose test in $B6C3F_1$ mice by gavage (3, 6, 12, 25, and 50 mg/kg), hyperplasia in the mucosal epithelium of the forestomach and the mucosal epithelium of the urinary bladder were observed in the 50 mg/kg administered group. [2)]

In a 14-day repeated-dose test in F344/N rats by gavage (25, 50, 100, 200, and 400 mg/kg), hyperplasia in the mucosal epithelium of the stomach was observed in administered groups at 50 mg/kg and higher. [2)]

In a 13-week repeated-dose test in $B6C3F_1$ mice by gavage (1.5, 3, 6, 12, and 25 mg/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 25 mg/kg/day. [2)]

In a 13-week repeated-dose test in F344/N rats by gavage (1.5, 3, 6, 12, and 25 mg/kg), no toxicological effect caused by administration with the substance was observed. [2)]

In a 103-week carcinogenicity test in $B6C3F_1$ mice by gavage (12 and 25 mg/kg), an increasing trend in the vacuolation of hepatocytes was observed in the substance administered groups. No carcinogenicity has been observed. [2)]

In a 103-week carcinogenicity test in F344/N rats by gavage (12 and 25 mg/kg), the occurrence of transitional cell papilloma in the urinary bladder (control group, 0/49; 12 mg/kg group, 2/49; and, 25 mg/kg group, 4/49) and hyperplasia in the urinary bladder epithelium (control group, 0/49; 12 mg/kg group, 1/49; and, 25 mg/kg group, 6/49) were observed in males in the substance administered groups, albeit at low rates. Regarding the occurrence of transitional cell papilloma in the urinary bladder due to high-dose long-term treatment with allyl isothiocyanate, the United States NTP (National Toxicology Program) suggested the possibility that the substance acts as a promotor, rather than an as an initiator in the tumorigenesis in the urinary bladder. [2)]

In addition, allyl isothiocyanate is in Group 3 (not classifiable as to its carcinogenicity to humans) in the monograph from the WHO International Agency for Research on Cancer (IARC). [1)]

(3) Teratogenicity study

In the teratogenicity test of allyl isothiocyanate in CD-1 mice during day 6 to 15 of gestation by gavage (0.3, 1.3, 6.0, and 28.0 mg/kg), an increase in the number of dead/absorbed fetuses was observed in the 28.0 mg/kg administered group. The no observed adverse effect level is considered to be 6.0 mg/kg/day. [1)]

No toxicological effect caused by administration with the substance was observed in any of a teratogenicity test of allyl isothiocyanate in Wistar rats during day 6 to 15 of gestation by gavage (0.2, 0.85, 4.0, and 18.5 mg/kg), a teratogenicity test in golden hamsters during day 6 to 10 of gestation by gavage (0.2, 1.1, 5.1, and 23.8 mg/kg), and a teratogenicity test in Dutch-belted rabbits during day 6 to 18 of gestation by gavage (0.123, 0.6, 2.8, and 12.3 mg/kg). No teratogenicity was observed. The corresponding no observed adverse effect levels are considered to be 18.5, 23.8, and 12.3 mg/kg/day, respectively. [1)]

(4) Mutagenicity study

Allyl isothiocyanate was reported as negative in a DNA repair test in *Bacillus subtilis*, [1)] while a positive result was reported in a DNA repair test in *Escherichia coli*. [3)] In a reverse mutation test using bacteria, the plate method yielded negative results regardless of the presence or absence of S9 mix. Meanwhile, the pre-incubation method yielded positive results, however, there are a report that the addition of S9 mix does not affect the activity and a report that the activity disappears when the treatment method was changed to one in which S9 mix was added. [1)] Positive results were confirmed in a reverse mutation test in Salmonella. [3)] In a reverse mutation test in *E. coli*, positive results have been obtained only in the presence of S9 mix. [1)]

Positive results were obtained for allyl isothiocyanate in a chromosomal aberration test in plant root tip cells and a sex-linked recessive lethal test in drosophila, while there has also been a report of negative results for the latter. [1)] Positive results were obtained in a chromosomal aberration test in mammalian cells at 5 nM, and the addition of S9 mix did not affect the activity. [1)] On the other hand, a dominant lethal test in mice was performed at doses up to 19 mg/kg, and the results were judged as negative. [1)]

For mustard extract containing allyl isothiocyanate at 90% or higher, negative results were obtained in a host-mediated assay with yeast using mice treated with up to 130 mg/kg. [1)] In addition, all the results from a reverse mutation test in bacteria, [4)] a chromosomal aberration test in cultured human cells and rat bone marrow cells (treated with up to 130 mg/kg), and a dominant lethal test in rats (treated with up to 100 mg/kg) [1)] were negative. On the other hand, all the results from a chromosomal aberration test in wheat root tip cells, [1)] a sex-linked recessive lethal test in drosophila, [1)] and a DNA repair test in *Bacillus subtilis* [4)] were positive. In a chromosomal aberration test in cultured Chinese hamster cells, the induction of structural aberration was extremely weak, and was judged as a false-positive, while the induction of polyploid cells was clearly observed. [5)]

In addition, grated wasabi was negative in both a DNA repair test in *E. coli* and a reverse mutation test in Salmonella. [3)] Anti-mutagenicity has been observed for the water extract of wasabi. [6)]

(References)

1. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vo1. 36, 55-68, 1985

2. Carcinogenesis bioassay of Allyl Isothiocyanate (CAS No. 57-06-7) in F344/N Rats and B6C3F, mice (gavage study): National Toxicology Program Technical Report Series No. 234, 1-142 (1982)

3. Yoshihiro Nishiyama, et al.: Mutagenicity and Its Mechanism of Allyl Isothiocyanate and Wasabi and Mustard Products, Program and Abstracts of 24th Annual Meeting of the Japanese Environmental Mutagen Society, Program No. P-82, 1995

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6. Takemura Hitomi, et al.: Mutagenicity Suppression by Wasabi and Its Mechanism, Program and Abstracts of 24th Annual Meeting of the Japanese Environmental Mutagen Society, Program No. P-74, 1995

Annex 2. Reference

Curdlan

1. Food additive name:
   Curdlan

2. Origin, method of preparation, and definition:
   Curdlan is derived from the culture solution of a gram-negative bacterium (*Agrobacterium, Alcaligenes facecalis* CAST) by separation. It consists mainly of β-1,3-glucan.

3. Major use:
   Thickening stabilizer

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is over 10,000 mg/kg/g in rats and mice. [1), 2)]

(2) Repeated-dose/carcinogenicity study
In a 1-year repeated-dose test in beagle dogs by dietary administration (1, 5, and 15%, and 10% gel mixed to constitute 40% of feed), soft stool was observed in the 15% administered group and the 10% gel-40% administered group. Mucous stool or bloody stool was observed until 2 months after the start of administration rarely in the 1% administered group, frequently in the 5% administered group, and very commonly in the 15% administered group and the 10% gel-40% administered group, while the occurrence decreased after 2 months in the 1 and 5% administered groups and the 10% gel-40% administered group. A slight suppression of body weight gain was observed in males in the 1, 5%, and 15% administered groups. Acute mild inflammation in the small intestine associated with petechial hemorrhage and erosion was observed in the 1%, 15% and 10% gel-40% administered groups; however, this observation is considered to be temporary, and to have been caused by irritation of the mucosa by the particles of the test substance. In addition, an increase in cecum weight (with content) and empty cecum weight was observed in the 15% administered group, which is considered to be observed when a scarcely digestive substance is administered in large quantities. [3)]

In a 24-month repeated-dose test in CD rats by dietary administration (1, 5, and 15%, and 10% gel mixed to constitute 40% of feed), decreased feed intake, suppression of body weight gain, and an increase in the weight of the cecum and the empty cecum were observed in the 15% administered group. No carcinogenicity was observed. The no observed adverse effect level is considered to be 2.5 g/kg/day. [4)]

In an 18-month carcinogenicity test in CD mice by dietary administration (1, 5, and 15%, and 10% gel mixed to constitute 40% of feed), neither a toxicological effect caused by administration with the substance nor carcinogenicity was observed. The no observed adverse effect level is considered to be 7.5 g/kg/day. [5)]

(3) Reproductive toxicity study
In a 3-generation administration test in CD rats by dietary administration (1, 5, 15%, and 10% gel mixed to constitute 40% of feed), a slight suppression of body weight gain in males of the 15% administered group, decreased feed intake in the 15% administered group, and generally lower body weight in offspring of the 15% administered group were observed.

The no observed adverse effect level is considered to be 2.5 g/kg/day. No reproductive toxicity was observed. In addition, regarding the change in empty cecum weight, both generations that showed an increase and a decrease were observed. [1), 3)]

(4) Teratogenicity study
In a teratogenicity test in rabbits by gavage (1, 2, and 5 g/kg, and 20 g (10% gel)/kg), 3, 1, 3, and 16 parent animals died in the 1, 2, 5 g/kg, and 20 g (10% gel)/kg administered group, respectively. The high mortality of the 20 g (10% gel)/kg administered group was caused by suffocation due to the intake of gel in large quantity and was unlikely to represent toxicity. No teratogenicity was observed. The no observed adverse effect level is considered to be 5 g/kg/day. [14)]

(5) Mutagenicity study
All the results from a reverse mutation test in bacteria, [6), 7)] a DNA repair test in bacteria, [8)] chromosomal aberration tests in mammalian cells, [9), 10)] a TK locus mutation assay in mouse lymphoma cells, [11)] and a mouse micronucleus test [12)] were judged as negative.

(References)

1. Takizawa Yukio: FY 1992 Study on Reevaluation, etc. of Safety of Food Additives, Study on the Acute Toxicity of Natural Additives, Akita University

2. Preliminary Acute Toxicity of Polysaccharide 13140 in Mice and Rats, 1968.12, Internal data (unpublished)

3. One Year Feeding Study in Dogs - PS 13140, 1975.1, Internal data (unpublished)

4. Two Year Feeding Study in Rats - PS 13140, 1976.8, Internal data (unpublished)

5. Lifetime carcinogenic Study in Mice - PS 13140, 1976.8, Internal data (unpublished)

6. Miyabe Masaki: FY 1992 Evaluation study of safety of food additives, Mutagenicity (1st Study)

7. Curdlan testing for mutagenic activity with *Salmonella* Typhimurium, 1994.5, Internal data (unpublished)

8. Ishizaki Mutsuo: FY 1992 Study on Reevaluation, etc. of Safety of Food Additives, Ibaraki Prefectural Institute of Public Health

9. Sofuni Toshio: FY 1992 Report, Study on Reevaluation, etc. of Safety of Food Additives, Chromosomal Aberration Test of Natural Additives in Cultured Mammalian Cells, National Institute of Hygienic Sciences, 1993

10. Curdlan Chromosomal aberrations assay with Chinese hamster ovary cell in vitro (OECD PROTOCOL), 1994.6, Internal data (unpublished)

11. Curdlan Mouse lymphoma mutation assay, 1994.5, Internal data (unpublished)

12. Curdlan Micronucleus test in bone marrow of CD-1 mice, 1994.6, Internal data

(unpublished)

13. Multigeneration Reproduction Study in Rats - PS 13140, 1976.5, Internal data (unpublished)

14. Teratology Study in Rabbits - PS 13140, 1974.9, Internal data (unpublished)

Enzymatically decomposed lecithin

1. Food additive name:
   Enzymatically decomposed lecithin

2. Origin, method of preparation, and definition:
   Enzymatically decomposed lecithin is obtained from vegetable lecithin or egg yolk lecithin by pH adjustment with water or an alkaline aqueous solution, followed by enzymatic decomposition at either room or warm temperature, with or without subsequent extraction with ethanol, isopropyl alcohol, or acetone. In addition, enzymatically decomposed lecithin may also be manufactured continuously from egg yolk. It consists mainly of lysolectithin and phosphatidic acid.

3. Major use:
   Emulsifier

4. Summary of safety study results:

(1) Single-dose study
The acute oral LD_{50} is considered to be 5,000 mg/kg in mice, and over 4,000 mg/kg in rats. [1), 2), 3)]

(2) Repeated-dose/carcinogenicity study
In a 3-month repeated-dose test of enzymatically decomposed lecithin (soybean) in Wistar rats by gavage (0.5, 1, and 2 g/kg), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 2 g/kg/day. [4)]

In a 28-day repeated-dose test of enzymatically decomposed lecithin (soybean) in SD rats by dietary administration (0.2, 1.0, and 5.0%), no toxicological effect caused by administration with the substance was observed. The no observed adverse effect level is considered to be 481 mg/kg/day. [5)]

In a 3-week repeated-dose test by dietary administration (5, 10, 20, 30, and 40%) and a 13-week repeated-dose test by dietary administration (1, 2.5, 5, 10, and 20%) in Wistar rats with enzymatically decomposed lecithin (soybean), no toxicological difference from lecithin (soybean), which was used as the control was observed. [6)]

In a 4-week repeated-dose test of enzymatically decomposed lecithin (egg yolk) in Wistar rats by dietary administration (5, 10, 15, and 20%), no toxicological difference from lecithin (egg yolk), which was used as the control was observed. [6)]

(3) Mutagenicity study
All the results from a reverse mutation test in bacteria [7)] and a DNA repair test in bacteria [8)] were judged as negative.

(References)

1. Study on the Safety of Enzymatically Decomposed Lecithin -Acute and Simplified Subacute Toxicity Study in Rats, 1986, internal data (unpublished)

2. Oral Acute and Simple Subacute Toxicity Study of Enzymatically (Phospholipase-D) Decomposed Soybean Lecithin in Male Rats, 1988, internal data (unpublished)

3. Ministry of Health and Welfare FY 1993 Study on Reevaluation, etc. of Safety of Food Additives, Study on the Acute Toxicity of Natural Additives, Akita University Faculty of Medicine

4. Study on the Safety of Enzymatically Decomposed Lecithin -Subacute Oral Toxicity Study in Rats, 1986, internal data (unpublished)

5. Phospholipase D-hydrolysed Soya Lecithine: 4-week Subacute Oral Toxicity Study in Rats, 1991, Internal data (unpublished)

6. Christian E. Dutilh et al.: Improvement of Product Attributes of Mayonnaise by Enzymic Hydrolysis of Egg Yolk with Phospholipase A2, J. Sci. Food Agric., 32, 451-458, 1981

7. Phospholipase D-hydrolysed Soya Lecithine: Testing for Mutagenic Activity with *Salmonella* Typhimurium TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2*uvr*A, 1989, Internal data (unpublished)

8. Ministry of Health and Welfare FY 1993 Study on Reevaluation, etc. of Safety of Food Additives, Mutagenicity Study, 1st Study, Rec-Assay Report, Institute of Environmental Toxicology

Annex 3. Table 1~6

The following tables does not exist in the published original Japanese version.
They are provided for reference purposes only.

Table 1 Natural additives of which safety has been evaluated by JECFA

(Note 1. The number in the Remarks column represents the number of JECFA meeting at which the ADI was established.)

Name in the list of existing additives	Evaluation by JECFA		
	Name	ADI (mg/kg b.w.)	Remarks
アナトー色素	Annatto extracts	0.065 (As bixin)	26
α－アミラーゼ	α-Amylase from *Bac.subtilis*,	Not specified	31
	α-Amylase from *Asp. oryzae*, var.;etc.	Acceptable under current use conditions	31
β－アミラーゼ	Malt Carbohydrases	Not limited	15
アラビアガム	Gum arabic (Acacia gum)	Not specified	35
アルギン酸	Alginic acid	Not specified	39
アルミニウム	Aluminium	PTWI 7 (Temporary tolerable daily intake)	33
イモカロテン	Carotene (vegetable)	Acceptable under current use conditions	41
ウコン色素	Curcumin	1.0 (Temporary)	39
カオリン	Aluminium silicate	Not specified	29
カタラーゼ	Catalase from bovine liver	Not limited	15
活性炭	Activated carbon	Not limited	31
カラギナン	Carrageenan	Not specified	28
カラメルⅠ	Caramel color I	Not specified	29
カラメルⅢ	Caramel color III	200 (150 on a solid basis)	29
カラメルⅣ	Caramel color IV	200 (150 on a solid basis)	29
カラヤガム	Karaya gum	Not specified	33
カルナウバロウ	Carnauba wax	7	39
カロブビーンガム	Carob bean gum (Locust bean gum)	Not specified	25
カンデリラロウ	Candelilla wax	Acceptable under current use conditions	39
キサンタンガム	Xanthan gum	Not specified	30
キラヤ抽出物	Quillaja extract	5	29
金	Gold	Acceptable under current use conditions	21

Name in the list of existing additives	Evaluation by JECFA		
	Name	ADI (mg/kg b.w.)	Remarks
グァーガム	Guar gum	Not specified	19
グアヤク脂	Guaiac resin	2.5	17
グアヤク樹脂	Guaiac resin	2.5	17
グルカナーゼ	β-Glucanase from *Asp. niger* var.	Not specified	35
	β-Glucanase from *Tricho. harzianum* ;etc.	Not specified	39
グルコアミラーゼ	Amyloglucosidase from *Asp. Niger,* var.	Not specified	35
グルコースイソメラーゼ	Glucose isomerase from *Bac. coagulans,* (immobilized)	Not specified	29
	Glucose isomerase from *Str. rubiginous* (immobilized); etc.	Not specified	29
グルコースオキシダーゼ	Glucose oxidase from *Asp. niger*	Not specified	18
クロロフィル	Chlorophylls	Not limited	13
くん液	Smoke flavourings	Temporarily acceptable	31
シェラック	Shellac	Acceptable under current use conditions	39
ジェランガム	Gellan gum	Not specified	37
シクロデキストリン	β-Cyclodextrin	6 (Temporary)	41
植物レシチン	Lecithin	Not limited	17
生石灰	Calcium oxide	Not limited	9
セルラーゼ	Cellulase from *Tricho. Longibrachiatum*	Not specified	39
タウマチン	Thaumatin	Not specified	29
タラガム	Tara gum	Not specified	30
タルク	Talc	Not specified	30
胆汁末	Cholic acid	1.25	17
タンニン(抽出物)	Tannic acid	Not specified	35
窒素	Nitrogen	Acceptable under current use conditions	24

Name in the list of existing additives	Evaluation by JECFA		
	Name	ADI (mg/kg b.w.)	Remarks
デュナリエラカロテン	Carotene (alagae)	20 (Temporary)	44
トウガラシ色素	Paprika oleoresins	Acceptable under current use conditions	35
d-α-トコフェロール	d-α-Tocopherol, concetrate	0.15-2	30
トラガントガム	Tragacanth gum	Not specified	29
トリプシン	Trypsin	Not limited	15
ニンジンカロテン	Carotene (vegetable)	Acceptable under current use conditions	41
パパイン	Papain	Not limited	15
パーム油カロテン	Carotene (vegetable)	Not limited	41
微結晶セルロース	Microcyrstalline cellulose	Not specified	19
ビートレッド	Beet red	Not specified	31
ファーセレラン	Furcellaran	Not specified	28
ブドウ果皮色素	Grapeskin extract	25	26
プロテアーゼ	Protease from *Asp. niger,* Protease from *Asp. oryzae,* var.; etc.	Not specified Acceptable under current use conditions	35 31
ブロメライン	Bromelain	Not limited	15
分別レシチン	Lecithin	Not limited	17
粉末セルロース	Powdered cellulose	Not specified	20
ヘキサン	Hexane	GMP	14
ペクチナーゼ	Pectinase from *Asp. niger*	Not specified	35
ペクチン	Pectins	Not specified	25
ペプシン	Pepsin (hog stomach)	Not limited	15
ヘプタン	Heptanes	GMP	14
ヘミセルラーゼ	Hemicellulase from *Asp.niger,* var.	Not specified	35
マイクロクリスタリンワックス	Micrcrystalline wax	Not specified	39
未焼成カルシウム	Bone phosphate	MTDI 70 mg/kg b.w. (maximum tolerable daily intake for phosphorus from all sources, expressed as P)	29

Name in the list of existing additives	Evaluation by JECFA		
	Name	ADI (mg/kg b.w.)	Remarks
ミックストコフェロール	Mixed tocopherol concentrate	2	17
ミツロウ	Bees wax, white and yellow	Acceptable under current use conditions	39
卵黄レシチン	Lecithin	Not specified	17
リゾチーム	Lysozyme hydrochloride	Acceptable under current use conditions	39
リパーゼ	Lipase from *Asp. oryzae,* var.	Not specified	18
流動パラフィン	Mineral oil	20 (High-viscosity) 1 (Medium-viscosity) 0.1 (Low-viscousity)	44
レンネット	Rennet bovine, Rennet from *Mucor species,* etc.	Not limited Not specified	15 18

Table 2 Natural additives of which distribution has been confirmed to be authorized in the United States

(Note) The CFR Section Number for the article is shown in parenthesis in the Name in the United States column.
The FDA or FASEB evaluation columns indicate if the safety evaluation results by the FDA or the FASEB were available, and the year of evaluation is shown in parenthesis ("19" is omitted from the year).

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
Lーアスパラギン	L-Asparagine (172.320)		Available (92)
Lーアスバラギン酸	L-Aspartic acid (172.320)		Available (92)
アナトー色素	Annatto extract (73.30)		
アミノペプチターゼ	Aminopeptidase, enzyme preparation derived from *lactococcus lactis* (184.1985)	Available (96)	
αーアミラーゼ	Carbohydrase and cellulase derived from *Asp. niger* (173.120) *Rhizopus oryzae* (173.130)		
βーアミラーゼ	Carbohydrase and cellulase derived from *Asp. niger* (173.120) Carbohydrase from *Rhizopus oryzae* (173.130)		
Lーアラニン	L-Alanine (172.320)		Available (92)
アラビアガム	Acacia (gum arabic) (184.1330)		Available (73)
Lーアルギニン	L-Arginine (172.320)		Available (92)
アルギン酸	Alginic acid (184.1011)		Available (74)
イソアルファー苦味酸	Hops/Essential oils, oleoresins (solvent- free), and natural extractives including distillates (182.20)		
イノシトール	Inositol (184.1370)		Available (76)
イモカロテン	β-Carotene (73.95)		Available (80)
ウコン色素	Turmeric oleoresin (73.615)		
ウレアーゼ	Urease (184.1924)	Available (92)	
オゾン	Ozone (184.1563)		

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
オレガノ抽出物	Origanum/Essential oils, oleoresins (solvent-free), and natural extractives including distillates(182.20)		
カオリン	Kaolin (184.1256)		
カタラーゼ	Catalase (bovine liver) (184.1034, etc.)	Available (95)	
ガティガム	Gum ghatti (184.1333)		Available (73)
カードラン	Curdlan (172.809)	Available (96)	
カフェイン(抽出物)	Caffeine (182.1180)		Available (78)
カラギナン	Carrageenan (172.620)		Available (73)
α-ガラクトシダーゼ	α-Galactosidase (173.145)		
β-ガラクトシダーゼ	Lactase enzyme preparation from *Kluyveromyces lactis* (184.1388)		
カラシ抽出物	Mustard/Essential oils, oleoresins (solvent- free), and natural extractives including distillates (182.20)		Available (75)
カラメルⅠ	Caramel (73.85)		Available (73)
カラメルⅡ	Caramel (73.85)		Available (73)
カラメルⅢ	Caramel (73.85)		Available (73)
カラメルⅣ	Caramel (73.85)		Available (73)
カラヤガム	Karaya gum (184.1349)		
カルナウバロウ	Carnauba wax (184.1978)		Available (76)
カロブビーンガム	Locust (carob) bean gum (184.1343)		Available (72)
カンゾウ抽出物	Licorice and licorice derivatives (184.1408)		Available (75)
カンデリラロウ	Candelilla wax (184.1976)		Available (81)
キサンタンガム	Xanthan gum (172.695)		
グァーガム	Guar gum (184.1339)		Available (73)

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
グッタハンカン	Chewing gum base, Gutta hang kang (172.615)		
グルカナーゼ	Carbohydrase and Cellulase derived from *Asp. niger* (173.120)		
グルコアミラーゼ	Amyloglucosidase derived from *Rhizopus niveus* (173.110)		
グルコースイソメラーゼ	Insoluble glucose isomerase enzyme preparations (184.1372)		
L-グルタミン	L-Glutamine (172.320)		Available (92)
クローブ抽出物	Clove and its derivatives (184.1257)		
くん液	Smoke flavorings solutions		Available (82)
高級脂肪酸	Fatty acid (172.860)		
香辛料抽出物	Spices and other natural seasonings and flavorings (182.10): etc.		
酵素分解レシチン	Enzyme-modified lecithin (184.1063)	Available (96)	
コチニール色素	Cochineal extract. carmine (73.100)		
ゴム	Chewing gum base, *Euphorbiaceae*: Natural rubber (172.615)		
ゴム分解樹脂	Chewing gum base, *Euphorbiaceae*: Natural rubber (172.615)		
コメヌカロウ	Rice bran wax (172.890)		
酸性白土	Bentonite (184.1155)		Available (77)
サイリウムシードガム	Psylium seed husk gum		Available (82)
シアノコバラミン(抽出物)	Vitamin B_{12} (184.1945)		Available (78)
シェラック	Shellac (184.1705 proposal)	Available (89)	Available (82)
シェラックロウ	Shellac wax (184.1706 proposal)	Available (89)	Available (82)

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
ジェランガム	Gellan gum (172.665)	Available (90)	
ジェルトン	Chewing gum base, *Apocynaceae*: Jelutong (172.615)		
L-シスチン	L-Cystine (72.320)		Available (92)
ショウガ抽出物	Zinger/Essential oils, oleoresins (solvent- free), and natural extractives including distillates (182.20)		
焼成カルシウム	Calcium oxide (184.1210)		Available (75)
植物レシチン	Lecithin (184.1400)		Available (79)
生石灰	Calcium oxide (184.1210)		Available (75)
ゼイン	Zein (184.1984)		
セージ抽出物	Sage/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		
L-セリン	L-Serine (172.320)		Available (92)
セルラーゼ	Carbohydrase and cellulase derived from *Asp. niger* (173.120)		
ソルバ	Chewing gum base, *Apocynaceae*, Lechecaspi (sorva) (172.615)		
ソルビンハ	Chewing gum base, *Apocynaceae*, Pendare, Perillo (172.615)		
胆汁末	Ox bile extract (184.1560)		Available (75)
タンニン(抽出物)	Tannic acid (184.1097)	Available (85)	Available (77)
チクル	Chewing gum base, *Sapotaceae*, Chicle (172.615)		
窒素	Nitrogen (184.1540)		
チャ抽出物	Tea/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
チルテ	Chewing gum base, *Euphorbiaceae*, Chilte (172.615)		
L-チロシン	L-Tyrosine (172.320)		Available (92)
ツヌー	Chewing gum base, *Moraceae*, Tunu (tuno) (172.615)		
低分子ゴム	Chewing gum base, *Euphorbiaceae*: natural rubber (172.615)		
鉄	Iron, elemental (184.1375)		Available (80)
デュナリエラカロテン	β-Carotene (73.95)		Available (80)
トウガラシ色素	Paprika oleoresins (73.345)		
トウガラシ水性抽出物	Capsicum/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		
d-α-トコフェロール	a-Tocopherols (184.1890)		Available (76)
トラガントガム	Gum tragacanth (184.1351)		Available (73)
トリプシン	Trypsin (184.1914)	Available (95)	
ナフサ	Petroleum naphtha (172.250)		
生コーヒー豆抽出物	Coffee/Essential oils, oleoresins (solvent- free), and natural extractive (including distillates) (182.20)		
ナリンジン	Naringin/Essential oils, oleoresins (solvent- free), and natural extractive (including distillates) (182.20)		Available (82)
ニガーグッタ	Chewing gum base, *Moraceae*, nigergutta (172.615)		

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
ニッケル	Nickel (184.1537)		Available (80)
ニンジンカロテン	β-Carotene (73.95) Carrot oil (73.300)		Available (80)
ニンニク抽出物	Garlic and its derivatives (184.1317)		Available (73)
パパイン	Papain (184.1585)		Available (77)
パーム油カロテン	P-Carotene (73.95)		
パラフィンワックス	Paraffin wax (172.886)		
パンクレアチン	Pancreatin (184.1583)	Available (95)	
L-ヒスチジン	L-Histidine (172.320)		Available (92)
ビートレッド	Dehydrated beets (73.40)		
ピメンタ抽出物	Pimennta/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		
ファーセレラン	Furcellaran (172.655)		
フィシン	Ficin (184.1316)	Available (95)	
ブタン	n-Butane and isobutane (184.1165)		Available (80)
ブドウ果皮色素	Grape skin extract (enocianina) (73.170)		
プロテアーゼ	Mixed carbohydrase protease enzyme product (184.1027)		
プロパン	Propane (184.1655)		Available (80)
ブロメライン	Bromelain (184.1024)	Available (95)	
L-プロリン	L-Proline (172.320)		Available (92)
分別レシチン	Lecithin (184.1400)		Available (79)
ヘキサン	Hexane (173.270)		
ペクチナーゼ	Carbohydrase and cellulase derived from *Asp. niger* (173.120) Carbohydrase derived from *Rhizopus oryzae* (173.130)		

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
ペクチン	Pectins (184.1588)		
ヘスペリジン*	Hesperidine		Available (82)
ベネズエラチクル	Chewing gum base, *Sapotaceae*, Venezuela chicle (172.615)		
ペパー抽出物	Pepper, black; Pepper, white/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		
ペプシン	Pepsin (184.1595)	Available (95)	
ヘミセルラーゼ	Carbohydrase and cellulase derived from Asp. niger (173.120) Carbohydrase derived from *Rhizopus oryzae* (173.130)		
ヘリウム	Helium (184.1355)		
ベントナイト	Bentonite (184.1155)		
マイクロクリスタリンワックス	Petroleum wax (172.886)		
マッサランドバチョコレート	Chewing gum base, *Sapotaceae*, Massaranduba chocolate (172.615)		
マッサランドババラタ	Chewing gum base, *Sapotaceae*, Massaranduba balata (172.615)		
マリーゴールド色素	Tagetes (Aztec marigold) meal and extract (73.295)		
ミックストコフェロール	Tocopherols (182.8890)		Available (76)
ミツロウ	Beeswax (yellow and white) (184.1973)		Available (76)

* Not described in the CFR but the evaluation result by the FASEB was available.

Name in the list of existing additives	Status in the United States		
	Name	FDA evaluation	FASEB evaluation
モクロウ*	Japan wax		Available (75)
ラノリン	Chewing gum base, plasticizing materials, Lanolin (172.615)		
卵黄レシチン	Lecithins (184.1400)		Available (79)
L-リシン	L-Lysine (172.320)		Available (92)
リパーゼ	Animal lipase (184.1415) Esterase-lipase derived from *Mucor miehei* (173.140)		
流動パラフィン	White mineral oil (172.878)		
レッチュデバカ	Chewing gum base, Leche de vaca (172.615)		
レモン果皮抽出物	Citrus peel/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		
レンネット	Rennet (184.1685) Milk-clotting enzymes, microbial (173.150)	Available (93)	Available (77)
L-ロイシン	L-Leucine (172.320)		Available (92)
ロシディンハ	Chewing gum base, *Sapotaceae*, Roshidinha (rosadinha) (172.615)		
ローズマリー抽出物	Rosemary/Essential oils, oleoresins (solvent-free), and natural extractives including distillates (182.20)		

* Not described in the CFR but the evaluation result by the FASEB was available.

Table 3 Natural additives of which distribution has been confirmed to be authorized in the EU

(Note) The number for a food additive in the EU is shown in parenthesis in the Name in the EU column. The SCF evaluation column indicates if the safety evaluation results by the SCF (Scientific Committee for Food) were available, and the year of evaluation is shown in parenthesis (year of publication of the report; “19” is omitted from the year).

Name in the list of existing additives	Status in the EU	
	Name	SCF evaluation
アナトー色素	Annatto, bixin, norbixin (E160(b))	Available (79)
アラビアガム	Acacia gum (gum arabic)(E414)	Available (78)
アルギン酸	Alginic acid (E400)	Available (78)
アルミニウム	Alminium (E173)	Available (75)
イモカロテン	Carotenes (E160(a))	
ウコン色素	Curcumin (E100)	Available (75)
カラギナン	Carrageenan (E407) Processed euchema seaweed (E407a)	Available (78)
カラメルⅠ	Plain caramel (E150(a))	Available (89)
カラメルⅡ	Caustic caramel (E150(b))	Available (89)
カラメルⅢ	Ammonia caramel (E150(c))	Available (89)
カラメルⅣ	Sulfite ammonia caramel (E150(d))	Available (89)
カラヤガム	Karaya gum (E416)	Available (84)
カルナウバロウ	Carnauba wax (E903)	Available (92)
カロブビーンガム	Locust bean gum (E410)	Available (78)
カンデリラロウ	Candelilla wax (E902)	Available (92)
キサンタンガム	Xanthan gum (E415)	Available (92)
キラヤ抽出物	Quillaja extract (E999)	Available (78)
金	Gold (E175)	Available (75)
銀	Silver (E174)	Available (75)
グァーガム	Guar gum (E412)	Available (78)
クロロフィル	Chlorophylls and chlorophyllins (E140)	Available (75)
コチニール色素	Cochineal Carminic acid, Carmines (E120)	Available (79)

Name in the list of existing additives	Status in the EU	
	Name	SCF evaluation
シェラック	Shellac (E904)	Available (92)
ジェランガム	Gellan gum (E418)	Available (92)
植物炭末色素	Vegetable Carbon (E153)	Available (77)
植物レシチン	Lecithins (E322)	Available (78)
タウマチン	Thaumatin (E957)	Available (89)
タラガム	Taragum (E417)	Available (92)
タルク	Talc (E553(b))	
窒素	Nitrogen (E941)	
トウガラシ色素	Paprika extract, capsanthin, capsorbin (E160 (c))	
トマト色素	Lycopene (E160(d))	Available (89)
トラガントガム	Tragacanth gum (E413)	Available (84)
ニンジンカロテン	Carotenes (E160(a))	
パーム油カロテン	Carotenes (E160(a))	
微結晶セルロース	Cellulose (E460)	
ビートレッド	Beet red (E162)	Available (75)
ファーセレラン	Furcellaran (E407)	Available (78)
ブタン	Butane (extraction solvent)	
ブドウ果皮色素	Anthocyanins (E163)	Available (75)
プロパン	Propane (extraction solvent)	
分別レシチン	Lecithin (E322)	Available (78)
粉末セルロース	Cellulose (E460)	Available (78)
ヘキサン	Hexane (extraction solvent)	
ペクチン	Pectin (E440)	Available (78)
ミックストコフェロール	Tocopherol-rich extract	Available (89)
ミツロウ	Bees wax (E901)	Available (92)
ムラサキイモ色素	Anthocyanins (E163)	Available (75)
ムラサキトウモロコシ色素	Anthocyanins (E163)	
ムラサキヤマイモ色素	Anthocyanins (E163)	Available (75)
卵黄レシチン	Lecithins (E322)	Available (78)
リゾチーム	Lysozyme (E1105)	

Table 4 Availability status of safety study results

(Note) Additives shown in the table are those of which safety studies commissioned by the Ministry of Health and Welfare were performed or safety study results were available with the cooperation of the Japan Food Additives Association among those other than ones listed in Table 1, Table 2 and Table 3 above.
Circles indicate that the study results for the test item were available.

Name in the list of existing additives	Acute toxicity	28-day or longer repeated-dose toxicity	Mutagenicity	Other safety studies
Aureobasidium cultured solution	■	■	■	■
Aeromonas gum	■		■	
Madder colour	■	■	■	
Acylase	■	■	■	
N-Acetylglucosamine	■		■	
Azotobacter vinelandii gum	■		■	
Alginate lyase	■		■	
Isoamylase	■			
Itaconic acid	■		■	
Polyfructan (inulin type)				■
Elastase	■			
Elemi resin			■	
Barley husk extract	■		■	
Krill colour	■	■	■	
Oligo-N-acetylglucosamine	■			
Oligoglucosamine	■			
γ-Oryzanol	■	■	■	■
Oregano extract	■		■	
Cacao colour	■	■	■	
Japanese persimmon colour	■		■	
Carob germ colour	■		■	
Rumput roman extract			■	

Name in the list of existing additives	Acute toxicity	28-day or longer repeated-dose toxicity	Mutagenicity	Other safety studies
Licorice oil extract	■		■	
Xylose			■	
Aloe extract	■		■	■
Chitosan	■		■	
Enzymatically hydrolyzed guar gum	■	■	■	
Quercetin	■	■	■	■
Gardenia blue	■	■	■	■
Gardenia red	■	■	■	
Gardenia yellow	■	■	■	
Gutta percha	■		■	
α-Glucosyltransferase treated stevia	■	■	■	
Glutaminase	■		■	■
Kooroo colour	■		■	
Diatomaceous earth			■	
Gentian root extract			■	
Kojic acid			■	
Enzymatically modified isoquercitrin	■		■	
Enzymatically modified rutin	■		■	
Enzymatically decomposed apple extract	■			
Kaoliang colour	■	■	■	
Enzymatically decomposed rice bran	■		■	
Shea nut colour	■		■	
Cyclodextrin glucanotransferase	■	■	■	

Name in the list of existing additives	Acute toxicity	28-day or longer repeated-dose toxicity	Mutagenicity	Other safety studies
Perilla extract			■	
Sandalwood red		■	■	
Milt protein extract	■		■	
Stevia extract	■	■	■	■
Spirulina colour	■	■	■	■
Essential oil-removed fennel extract			■	
Sesamol		■		■
Sepiolite	■			
Sorbose	■	■	■	
Taurine (extract)			■	
Onion colour	■	■	■	
Tamarind colour	■		■	
Tamarind seed gum	■	■	■	■
Amino acid-sugar reaction product			■	
Dammar resin			■	
Tea dry distillate	■	■	■	■
Thujaplicin (extract)	■	■ (Sodium salt)	■	
5'-Deaminase	■	■	■	
Tourmaline	■		■	
Dextranase	■	■	■	■
Tocotrienol	■		■	
Transglucosidase	■		■	
Transglutaminase	■	■	■	■
Trehalose	■		■	
Trehalose phosphorylase	■		■	
Naringinase			■	

Name in the list of existing additives	Acute toxicity	28-day or longer repeated-dose toxicity	Mutagenicity	Other safety studies
Absinth extract				■
Nystose	■	■	■	■
Roasted rice bran extract	■			■
Roasted soybean extract	■		■	
Hyaluronic acid	■	■	■	■
Hydroxyproline	■			
Sunflower seed extract	■	■	■	
Xanthomonas campestris protein		■		
Phytic acid	■	■	■	■
Ferulic acid	■		■	
Pullulanase	■	■	■	■
Pullulan	■	■	■	
Propolis extract	■			
Pectin digests	■		■	
Hesperidinase	■	■	■	
Monascus yellow	■	■	■	■
Monascus colour	■	■	■	■
Carthamus red	■		■	■
Carthamus yellow	■	■	■	■
Peptidase	■	■	■	■
Heme iron	■		■	■
Phosphodiesterase	■	■		
Phospholipase	■		■	
Gallic acid	■		■	
ε-Polylysine	■	■	■	
Maltose phosphorylase	■		■	

Name in the list of existing additives	Acute toxicity	28-day or longer repeated-dose toxicity	Mutagenicity	Other safety studies
Maltotriohydrolase				■
Citrus seed extract			■	
Methylthioadenosine	■	■		
Menaquinone (extract)	■	■	■	
Mousouchiku dry distillate	■		■	■
Morin			■	
Chinese bayberry extract	■	■	■	
Vegetable oil soot colour	■			
Rakanka extract	■		■	
Lactoferrin concentrates	■		■	
Lac colour	■		■	■
Enzymatically decomposed rutin			■	
Rutin (extract)		■	■	
Levan		■		
Wasabi extract		■ (Allyl isothiocyanate)	■	

Table 5 Safety confirmation status of existing additives

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Aureobasidium cultured solution	Thickening stabilizer				■	
Aeromonas gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
Hollyhock flower extract	Antioxidant					
Madder colour	Color				■	
Agarase	Enzyme					Enzyme
Actinidine	Enzyme					Enzyme
Agrobacterium succinoglycan	Thickening stabilizer					
Achromopeptidase	Enzyme					Enzyme
Acylase	Enzyme				■	
Ascorbate oxidase	Enzyme					Enzyme
L-Asparagine	Flavoring agent, nutrition fortifier		■			
L-Aspartic acid	Flavoring agent, nutrition fortifier		■			
Aspergillus terreus extract	Antioxidant					
Aspergillus terreus glycoprotein	Food manufacturing agent					
N-Acetylglucosamine	Sweetener					Monosaccharide
α-Acetolactate decarboxylase	Enzyme					Enzyme
Azotobacter vinelandii gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
5'-Adenylic acid	Food manufacturing agent					
Annatto extract	Color	■	■	■		
Linseed gum	Thickening stabilizer					
Aminopeptidase	Enzyme		■			
α-Amylase	Enzyme	■	■			
β-Amylase	Enzyme	■	■			
Almond gum	Thickening stabilizer					
L-Alanine	Flavoring agent, nutrition fortifier		■			
Gum Arabic	Thickening stabilizer	■	■	■		
Arabinogalactan	Thickening stabilizer					Consists of monosaccharides arabinose and galactose
L-Arabinose	Sweetener					Monosaccharide
Alkanet colour	Color					
L-Arginine	Flavoring agent, nutrition fortifier		■			
Alginic acid	Thickening stabilizer	■	■	■		
Alginate lyase	Enzyme					Enzyme
Aluminium	Color	■		■		
Aloe vera extract	Thickening stabilizer					
Anthocyanase	Enzyme					Enzyme
Isoamylase	Enzyme					Enzyme
Iso-α-bitter acid	Bitterant, etc.		■			

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Isomaltodextranase	Enzyme					Enzyme
Itaconic acid	Acid					
Fig leaf extract	Food manufacturing agent					
Rice straw ash extract	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Inulinase	Enzyme					Enzyme
Polyfructan (inulin type)	Food manufacturing agent					
Inositol	Nutrition fortifier		■			
Sweet potato carotene	Nutrition fortifier, colour	■	■	■		
Invertase	Enzyme					Enzyme
Welan gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
Turmeric colour	Color	■	■	■		
Udo extract	Preservative					
Urushi wax	Gum base, glazing agent					
Urease	Enzyme		■			
Exomaltotetraohydrolase	Enzyme					Enzyme
Japanese styrax benzoin extract	Preservative					Consists mainly of benzoic acid
Esterase	Enzyme					Enzyme
Shrimp colour	Color					similar to krill colour
Ellagic acid	Antioxidant					
Elastase	Enzyme					Enzyme
Erwinia mitsuensis gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
Elemi resin	Thickening stabilizer, gum base					
Enju saponin	Emulsifier					
Sodium chloride-decreased brine (saline lake)	Flavoring agent					Alkali metals, etc.
Enterobacter gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
Enterobacter simanus gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
Endomaltohexaohydrolase	Enzyme					Enzyme
Endomaltopehtaohydrolase	Enzyme					Enzyme
Urucury wax	Gum base, glazing agent					
Barley husk extract	Emulsifier, food manufacturing agent					Similar to microcrystalline cellulose and powdered cellulose
Krill colour	Color				■	
Ozokerite	Gum base					
Ozone	Food manufacturing agent		■			
Opopanax resin	Gum base					
Oligo-N-acetylglucosamine	Sweetener					Oligosaccharide
Oligogalacturonic acid	Food manufacturing agent					Oligosaccharide
Oligoglucosamine	Thickening stabilizer					Oligosaccharide
γ-Oryzanol	Antioxidant				■	
Oregano extract	Food manufacturing agent		■			

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Orange colour	Color					
Seaweed ash extract	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Kauri gum	Gum base					
Kaolin	Food manufacturing agent	■	■			
Cacao colour	Color			■		
Cacao carbon black	Color					Similar to vegetable carbon black
Japanese persimmon colour	Color					
Granite porphyry	Food manufacturing agent					Mineral
Cassia gum	Thickening stabilizer					One of polysaccharides such as gum arabic obtained from legume seeds
Gastric mucin	Food manufacturing agent					
Catalase	Enzyme	■	■			
Active carbon	Food manufacturing agent	■				
Activated acid clay	Food manufacturing agent					Mineral
Gum ghatti	Thickening stabilizer		■			
Catechin	Antioxidant					
Curdlan	Thickening stabilizer, food manufacturing agent		■			
Crayfish colour	Color					Similar to krill colour
Caffeine (extract)	Bitterant, etc.		■			
Carrageenan	Thickening stabilizer	■	■	■		
α-Galactosidase	Enzyme		■			
β-Galactosidase	Enzyme		■			
Mustard extract	Food manufacturing agent		■			
Caramel I	Color, food manufacturing agent	■	■	■		
Caramel II	Color, food manufacturing agent		■	■		
Caramel III	Color, food manufacturing agent	■	■	■		
Caramel IV	Color, food manufacturing agent	■	■	■		
Karaya gum	Thickening stabilizer	■	■	■		
Carnauba wax	Gum base, glazing agent	■	■	■		
Carboxypeptidase	Enzyme					Enzyme
Carob germ colour	Color					
Carob bean gum	Thickening stabilizer	■	■	■		
Kawaratake extract	Bitterant, etc.					
Rumput roman extract	Preservative					
Licorice extract	Sweetener		■			
Licorice oil extract	Antioxidant					
Candelilla wax	Gum base, glazing agent	■	■	■		
Xanthan gum	Thickening stabilizer	■	■	■		
Xylanase	Enzyme					Enzyme
D-Xylose	Sweetener					Monosaccharide

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Aloe extract	Thickening stabilizer					
Chitinase	Enzyme					Enzyme
Chitin	Thickening stabilizer					
Chitosanase	Enzyme					Enzyme
Chitosan	Thickening stabilizer, food manufacturing agent					Approved as a Food for Specified Health Uses
Redbark cinchona extract	Bitterant, etc.					Consists of a drug quinine, etc.
Phellodendron bark extract	Bitterant, etc.					Consists of a drug berberine, etc.
Fish scale foil	Color					
Quillaia extract	Emulsifier	■		■		
Gold	Color, food manufacturing agent	■		■		
Silver	Color			■		
Guar gum	Thickening stabilizer	■	■	■		
Enzymatically hydrolyzed guar gum	Thickening stabilizer				■	
Guaiac resin	Antioxidant	■				
Guaiac resin (extract)	Gum base	■				
Guayule	Gum base					One of those that mainly consists of isoprene such as chicle
Quercetin	Antioxidant				■	
Kusagi colour	Color					
Gardenia blue	Color				■	
Gardenia red	Color				■	
Gardenia yellow	Color				■	
Gutta katiau	Gum base					Similar to gutta hang kang
Gutta hang kang	Gum base		■			
Gutta percha	Gum base					Similar to gutta hang kang
Cristobalite	Food manufacturing agent					Mineral
Green tuff	Food manufacturing agent					Mineral
Glucanase	Enzyme	■	■			
Curculin	Sweetener					
Glucoamylase	Enzyme	■	■			
Glucosamine	Thickening stabilizer					Monosaccharide
α-Glucosidase	Enzyme					Enzyme
β-Glucosidase	Enzyme					Enzyme
α-Glucosyltransferase	Enzyme					Enzyme
α-Glucosyltransferase treated stevia	Sweetener				■	
Glucose isomerase	Enzyme	■	■			
Glucose oxidase	Enzyme	■				
Glutaminase	Enzyme					Enzyme
L-Glutamine	Flavoring agent, nutrition fortifier		■			
Grapefruit seed extract	Food manufacturing agent					

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Kooroo colour	Color					
Clove extract	Antioxidant		■			
Chlorophylline	Color					Similar to chlorophyll
Chlorophyll	Color	■		■		
Mulberry bark extract	Food manufacturing agent					
Smoke flavourings	Food manufacturing agent	■	■			
Diatomaceous earth	Food manufacturing agent					Mineral
Spermaceti wax	Gum base, glazing agent					
α-Ketoglutaric acid (extract)	Acid					
Gentian root extract	Bitterant, etc.					Listed in the JP as Gentian
Higher fatty acid	Food manufacturing agent		■			
Kojic acid	Food manufacturing agent					
Spice extracts	Bitterant, etc.		■			
Enzymatically modified isoquercitrin	Antioxidant					
Enzymatically modified licorice extract	Sweetener					Similar to licorice extract
Enzymatically modified soybean saponin	Emulsifier					
Enzymatically modified tea extract	Food manufacturing agent					Similar to tea extract
Enzymatically modified naringin	Bitterant, etc.					Similar to naringin
Enzymatically modified hesperidin	Nutrition fortifier					Similar to hesperidin that is one of vitamin P
Enzymatically modified rutin (extract)	Antioxidant					Similar to rutin (extract)
Enzymatically modified lecithin	Emulsifier					Similar to lecithin
Enzymatically hydrolyzed licorice extract	Sweetener					Similar to licorice extract
Enzymatically hydrolyzed coix extract	Preservative					
Enzymatically decomposed apple extract	Antioxidant					Enzymatically decomposed apple fruit
Enzymatically decomposed lecithin	Emulsifier		■			
Yeast cell wall	Thickening stabilizer, food manufacturing agent					Part of yeast
Kaoliang colour	Color				■	
Cochineal extract	Color		■	■		
Bone charcoal	Food manufacturing agent					Similar to vegetable carbon black
Bone carbon black	Color					Similar to vegetable carbon black
Copaiba balsam	Gum base					
Copal resin	Gum base					
Cobalt	Food manufacturing agent					Metal
Sesame seed oil unsaponified matter	Antioxidant					
Sesame straw ash extract	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Rubber	Gum base		■			
Resin of depolymerized natural rubber	Gum base		■			
Rice bran oil extract	Antioxidant					
Enzymatically decomposed rice bran	Antioxidant					

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Rice bran wax	Gum base, glazing agent		■			
Psyllium seed gum	Thickening stabilizer		■			
Bamboo grass colour	Color					Similar to chlorophyll
Cane wax	Gum base, glazing agent					Similar to bees wax
Artemisia sphaerocephala seed gum	Food manufacturing agent, thickening stabilizer					
Acid clay	Food manufacturing agent		■			
Acid phosphatase	Enzyme					Enzyme
Oxygen	Food manufacturing agent					Listed in the JP as a drug
Sandarac resin	Gum base					
Shea nut colour	Color					
Cyanocobalamin	Nutrition fortifier		■			
Shellac	Gum base, glazing agent	■	■	■		
Shellac wax	Gum base, glazing agent		■			
Gellan gum	Thickening stabilizer	■	■	■		
Jelutong	Gum base		■			
Cyclodextrin	Food manufacturing agent	■				
Cyclodextrin glucanotransferase	Enzyme				■	
Shikon colour	Color					
L-Cystine	Flavoring agent, nutrition fortifier		■			
Perilla extract	Food manufacturing agent					
Sandalwood red	Color				■	
5'-Cytidylic acid	Nutrition fortifier					
Jamaica quassia extract	Bitterant, etc.					
Ginger extract	Food manufacturing agent		■			
Calcinated calcium	Nutrition fortifier, food manufacturing agent		■			
Vegetable sterol	Emulsifier					Similar to cholesterol
Vegetable carbon black	Color			■		
Vegetable lecithin	Emulsifier	■	■	■		
Edible canna extract	Antioxidant					
Milt protein	Preservative					
Hydrogen	Food manufacturing agent					Reducing agent for oils, etc.
Sappan colour	Color					
Sclero gum	Thickening stabilizer					
Stevia extract	Sweetener				■	
Powdered stevia	Sweetener					Similar to stevia extract
Superoxide dismutase	Enzyme					Enzyme
Spirulina colour	Color				■	
Sphingolipid	Emulsifier					
Quicklime	Food manufacturing agent	■	■			

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Essential oil-removed fennel extract	Antioxidant					
Horseradish extract	Antioxidant, food manufacturing agent					
Zein	Food manufacturing agent		■			
Zeolite	Food manufacturing agent					Mineral
Sesamolin	Antioxidant					
Sesamol	Antioxidant					
Sage extract	Antioxidant		■			
Sesbania gum	Thickening stabilizer					One of polysaccharides such as gum arabic obtained from legume seeds
Sepiolite	Food manufacturing agent					Mineral
Dropwort extract	Antioxidant					
L-Serine	Flavoring agent, nutrition fortifier		■			
Cellulase	Enzyme	■	■			
Crude potassium chloride (sea water)	Flavoring agent					Consists mainly of potassium chloride
Crude magnesium chloride (sea water)	Food manufacturing agent					Bittern
Buckwheat ash extract	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Sorva	Gum base		■			
Sorvinha	Gum base		■			
L-Sorbose	Sweetener				■	
Soybean saponin	Emulsifier					
Soybean ash extract	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Thaumatin	Sweetener	■		■		
Taurine (extract)	Flavoring agent					Commonly used as a drug (nutrient)
Water pepper extract	Food manufacturing agent					
Onion colour	Color				■	
Tamarind colour	Color					
Tamarind seed gum	Thickening stabilizer				■	
Tara gum	Thickening stabilizer	■		■		
Talc	Gum base, food manufacturing agent	■		■		
Powdered bile	Emulsifier	■	■			
Amino acid-sugar reaction product	Antioxidant					Complex of monosaccharides and amino acids
Tannase	Enzyme					Enzyme
Tannin (extract)	Food manufacturing agent	■	■			
Dammar resin	Thickening stabilizer, gum base					
Chicle	Gum base		■			
Nitrogen	Food manufacturing agent	■	■	■		
Tea dry distillate	Food manufacturing agent				■	
Tea seed saponin	Emulsifier					
Tea extract	Antioxidant, food manufacturing agent		■			
Chilte	Gum base		■			

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
L-Tyrosine	Flavoring agent, nutrition fortifier		■			
Tunu	Gum base		■			
Thujaplicin (extract)	Preservative				■	
5'-Deaminase	Enzyme				■	
Depolymerized natural rubber	Gum base		■			
Theobromine	Bitterant, etc.					Similar to caffeine
Dextranase	Enzyme				■	
Dextran	Thickening stabilizer					Listed in the JP as a phermaceutical excipient
Iron	Nutrition fortifier, food manufacturing agent		■			
Dunaliella carotene	Nutrition fortifier, colour	■	■			
Tourmaline	Food manufacturing agent					Mineral
Tempeh extract	Antioxidant					
Tenryocha extract	Sweetener					
Copper	Food manufacturing agent					Metal
Paprika colour	Color	■	■	■		
Capsicum water-soluble extract	Food manufacturing agent		■			
Cholesterol	Emulsifier					Listed in the JP as a phermaceutical excipient
Corn colour	Color					
Dokudami extract	Antioxidant					
Tocotrienol	Antioxidant					
d-α-Tocopherol	Antioxidant, nutrition fortifier	■	■			
d-β-Tocopherol	Antioxidant, nutrition fortifier					Similar to d-α-tocopherol
d-γ-Tocopherol	Antioxidant, nutrition fortifier					Similar to d-α-tocopherol
Tomato colour	Color			■		
Tomato glucolipid	Emulsifier					
Tragacanth gum	Thickening stabilizer	■	■	■		
Transglucosidase	Enzyme					Enzyme
Transglutaminase	Enzyme				■	
Triacanthos gum	Thickening stabilizer					One of polysaccharides such as gum arabic obtained from legume seeds
Triacylglycerol lipase	Enzyme					Enzyme
Trypsin	Enzyme	■	■			
Trehalose	Food manufacturing agent					Disaccharide
Trehalose phosphorylase	Enzyme					Enzyme
Tororoaoi	Thickening stabilizer					
Monellin	Sweetener					
Rapeseed oil extract	Antioxidant					
Bacillus natto gum	Thickening stabilizer, food manufacturing agent					
Petroleum naphtha	Food manufacturing agent		■			
Coffee bean extract	Antioxidant		■			

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Soybean extract	Food manufacturing agent					Derived from soy milk
Naringinase	Enzyme					Enzyme
Naringin	Bitterant, etc.		■			
Quassia extract	Bitterant, etc.					
Niger gutta	Gum base		■			
Absinth extract	Bitterant, etc.					
Nystose	Food manufacturing agent				■	
Nickel	Food manufacturing agent		■			
Nitrilase	Enzyme					Enzyme
Olibanum	Gum base					
Carrot carotene	Nutrition fortifier, colour	■	■	■		
Garlic extract	Food manufacturing agent		■			
Neuraminidase	Enzyme					Enzyme
Nordihydroguaiaretic acid	Antioxidant					
Roasted rice bran extract	Food manufacturing agent					
Roasted soybean extract	Food manufacturing agent					
Peroxidase	Enzyme					Enzyme
Hachiku extract	Food manufacturing agent					
Platinum	Food manufacturing agent					Metal
Papain	Enzyme	■	■			
Paffia extract	Food manufacturing agent					
Palm oil carotene	Nutrition fortifier, colour	■	■	■		
Perlite	Food manufacturing agent					Mineral
Palladium	Food manufacturing agent					Metal
Balata	Gum base					One of those that mainly consists of isoprene such as chicle
Paraffin wax	Gum base, glazing agent		■			
Pancreatin	Enzyme		■			
Hyaluronic acid	Food manufacturing agent				■	
Isodonis extract	Bitterant, etc.					
Microcrystalline cellulose	Food manufacturing agent	■		■		
Microfibrillated cellulose	Thickening stabilizer, food manufacturing agent					Similar to microcrystalline cellulose and powdered cellulose
L-Histidine	Flavoring agent, nutrition fortifier		■			
Beet saponin	Emulsifier					
Beet red	Color	■	■	■		
L-Hydroxyproline	Flavoring agent, nutrition fortifier					Evaluated by FASEB as one of protein components
Peanut colour	Color					
Sunflower seed extract	Antioxidant				■	
Himematsutake extract	Bitterant, etc.					
Pimenta extract	Antioxidant		■			

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Xanthomonas campestris protein	Food manufacturing agent					
Vermiculite	Food manufacturing agent					Mineral
Betel nut extract	Food manufacturing agent					
Furcellaran	Thickening stabilizer	■	■	■		
Fir balsam	Gum base					
Phaffia colour	Color					
Ficin	Enzyme		■			
Phytase	Enzyme					Enzyme
Phytic acid	Acid, food manufacturing agent				■	
Phytin (extract)	Food manufacturing agent					Similar to phytic acid
Ferritin	Nutrition fortifier					Similar to heme iron
Ferulic acid	Antioxidant					
Fukuronori extract	Thickening stabilizer					
L-Fucose	Sweetener					Monosaccharide
Butane	Food manufacturing agent		■	■		
Grape skin colour	Color	■	■	■		
Grape skin-derived substance	Food manufacturing agent					
Grape seed extract	Antioxidant, food manufacturing agent					
Brazilian licorice extract	Sweetener					Similar to licorice extract
Fructosyl transferase	Enzyme					Enzyme
Fructosyl transferase-treated stevia	Sweetener					Similar to stevia extract
Blueberry leaf extract	Antioxidant					
Pullulanase	Enzyme				■	
Pullulan	Thickening stabilizer, food manufacturing agent				■	
Protease	Enzyme	■	■			
Propane	Food manufacturing agent		■	■		
Propolis extract	Antioxidant					
Bromelain	Enzyme	■	■			
L-Proline	Flavoring agent, nutrition fortifier		■			
Fractionated lecithin	Emulsifier	■	■	■		
Powdered cellulose	Food manufacturing agent	■		■		
Powdered pulp	Gum base					Similar to microcrystalline cellulose and powdered cellulose
Powdered rice hulls	Gum base					Similar to microcrystalline cellulose and powdered cellulose
Pecan nut colour	Color					
Hexane	Food manufacturing agent	■	■	■		
Pectinase	Enzyme	■	■			
Pectin	Thickening stabilizer	■	■	■		
Pectin digests	Preservative					Similar to pectin
Hego-ginkgo leaf extract	Antioxidant					

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Hesperidinase	Enzyme				■	
Hesperidin	Nutrition fortifier		■			One of vitamin P
Hesperetin	Antioxidant					
Betaine	Flavoring agent					
Monascus yellow	Color				■	
Monascus colour	Color				■	
Powdered annatto	Color					Similar to annatto extract
Carthamus red	Color					
Carthamus yellow	Color				■	
Venezuelan chicle	Gum base		■			
Pepper extract	Antioxidant		■			
Pepsin	Enzyme	■	■			
Heptane	Food manufacturing agent	■				
Peptidase	Enzyme				■	
Haematococcus algae colour	Color					
Hemicellulase	Enzyme	■	■			
Heme iron	Nutrition fortifier					Approved as a Food for Specified Health Uses
Helium	Food manufacturing agent		■			
Benzoin gum	Gum base					
Bentonite	Food manufacturing agent		■			
Garden balsam extract	Antioxidant					
Magnolia obovata extract	Preservative					
Hokosshi extract	Food manufacturing agent					
Phosphodiesterase	Enzyme					Enzyme
Phospholipase	Enzyme					Enzyme
Gallic acid	Antioxidant					
Jojoba wax	Gum base					
Borapet	Bitterant, etc.					
Polyphenol oxidase	Enzyme					Enzyme
ε-Polylysine	Preservative				■	
Microcrystalline wax	Gum base, glazing agent	■	■			
Macrophomopsis gum	Thickening stabilizer					
Mastic gum	Gum base					
Madake extract	Food manufacturing agent					
Massaranduba chocolate	Gum base		■			
Massaranduba balata	Gum base		■			
Marigold colour	Color		■			
Maltose phosphorylase	Enzyme					Enzyme
Maltotriohydrolase	Enzyme					Enzyme

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
Non-calcinated calcium	Nutrition fortifier	■				
Citrus seed extract	Food manufacturing agent					
Mixed tocopherols	Antioxidant, nutrition fortifier	■	■	■		
Bees wax	Gum base, glazing agent	■	■	■		
Miracle fruit extract	Sweetener					
Myrrh	Gum base					
Purple sweet potato colour	Color			■		
Purple corn colour	Color			■		
Purple yam colour	Color			■		
Muramidase	Enzyme					Enzyme
Methylthioadenosine	Bitterant, etc.					
Menaquinone (extract)	Nutrition fortifier				■	
Mevalonic acid	Food manufacturing agent					
Melaleuca oil	Antioxidant					Essential oil
Mousouchiku dry distillate	Food manufacturing agent					Similar to smoke flavourings
Mousouchiku charcoal extract	Food manufacturing agent					Similar to vegetable carbon black
Mousouchiku extract	Food manufacturing agent					
Wood chip	Food manufacturing agent					Removed after use as absorbent
Charcoal	Food manufacturing agent					Similar to active carbon
Japan wax	Gum base, glazing agent		■			
Timber ash	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Timber ash extract	Food manufacturing agent					Consists of alkali metals and alkaline earth metals
Rice hull extract	Food manufacturing agent					
Peach gum	Thickening stabilizer					
Morin	Antioxidant					
Montan wax	Gum base, glazing agent					
Chinese bayberry extract	Antioxidant				■	
Vegetable oil soot colour	Colour					Similar to vegetable carbon black
Eucalyptus leaf extract	Antioxidant					
Yucca foam extract	Emulsifier, food manufacturing agent					
Oil stuff seed wax	Gum base, glazing agent					
Rakanka extract	Sweetener					
Lactoperoxidase	Enzyme					Enzyme
Lactoferrin concentrates	Food manufacturing agent					An ingredient of modified dry milk for special purposes
Lac colour	Colour					
Lanolin	Gum base, glazing agent		■			
Rhamsan gum	Thickening stabilizer					One of polysaccharides such as xanthan gum produced by gram-negative bacteria
L-Rhamnose	Sweetener					Monosaccharide
Yolk lecithin	Emulsifier	■	■	■		

Name	Use	JECFA	US	EU	Safety studies 28-day or longer repeated dose + mutagenicity	Remarks
L-Lysine	Flavouring agent, nutrition fortifier		■			
Lysozyme	Enzyme	■		■		
Lipase	Enzyme	■	■			
Lipoxygenase	Enzyme					Enzyme
D-Ribose	Sweetener					Monosaccharide
Liquid paraffin	Food manufacturing agent	■	■			
Linter cellulose	Food manufacturing agent					Similar to microcrystalline cellulose and powdered cellulose
Gentian root extract	Antioxidant					
Enzymatically decomposed rutin	Antioxidant					
Rutin (extract)	Antioxidant				■	
Ruthenium	Food manufacturing agent					Metal
Mannentake extract	Bitterant, etc.					
Leche de vaca	Gum base		■			
Levan	Thickening stabilizer					
Lemon peel extract	Food manufacturing agent		■			
Forsythia extract	Preservative					
Rennet	Enzyme	■	■			
L-Leucine	Flavouring agent, nutrition fortifier		■			
Logwood colour	Colour					
Rosidinha	Gum base		■			
Rosin	Gum base					
Rosemary extract	Antioxidant		■			
Wasabi extract	Food manufacturing agent				■	

Table 6 Existing additives of which safety study results, etc., are required

Large classification	Small classification	Name	Use
Amino acids/ Proteins		Aspergillus terreus glycoprotein	Food manufacturing agent
		Milt protein	Preservative
		Bacillus natto gum	Thickening stabilizer, food manufacturing agent
		Xanthomonas campestris protein	Food manufacturing agent
Nucleic acids		5'-Adenylic acid	Nutrition fortifier
		5'-Cytidylic acid	Nutrition fortifier
		Methylthioadenosine	Bitterant, etc.
Carboxylic acids		Itaconic acid	Acid
		α-Ketoglutaric acid (extract)	Acid
		Mevalonic acid	Food manufacturing agent
Carotenoids		Orange colour	Colour
		Corn colour	Colour
		Phaffia colour	Colour
		Haematococcus algae colour	Colour
Saponins		Enju saponin	Emulsifier
		Enzymatically modified soybean saponin	Emulsifier
		Soybean saponin	Emulsifier
		Tea seed saponin	Emulsifier
		Paffia extract	Food manufacturing agent
		Beet saponin	Emulsifier
		Yucca foam extract	Emulsifier, food manufacturing agent
Polysaccharides	Consists mainly of glucans	Agrobacterium succinoglycan	Thickening stabilizer
	Extracted from aloe	Aloe vera extract	Thickening stabilizer
		Aloe extract	Thickening stabilizer
	Produced by imperfect fungi	Sclero gum	Thickening stabilizer
		Macrophomopsis gum	Thickening stabilizer
	Others	Levan	Thickening stabilizer
		Linseed gum	Thickening stabilizer
		Almond gum	Thickening stabilizer
		Gastric mucin	Food manufacturing agent
		Chitin	Thickening stabilizer
		Artemisia sphaerocephala seed gum	Food manufacturing agent, thickening stabilizer
		Dammar resin	Thickening stabilizer, gum base
		Tororoaoi	Thickening stabilizer
		Fukuronori extract	Thickening stabilizer

Large classification	Small classification	Name	Use
		Peach gum	Thickening stabilizer
Terpenoids		Perilla extract	Food manufacturing agent
		Absinth extract	Bitterant, etc.
		Isodonis extract	Bitterant, etc.
Flavonoids	Extracted from citrus seeds	Grapefruit seed extract	Food manufacturing agent
		Citrus seed extract	Food manufacturing agent
	Others	Japanese persimmon colour	Colour
		Carob germ colour	Colour
		Licorice oil extract	Antioxidant
		Kooroo colour	Colour
		Mulberry bark extract	Food manufacturing agent
		Shea nut colour	Colour
		Edible canna extract	Antioxidant
		Sappan colour	Colour
		Tamarind colour	Colour
		Rapeseed oil extract	Antioxidant
		Peanut colour	Colour
		Grape seed extract	Antioxidant, food manufacturing agent
		Blueberry leaf extract	Antioxidant
		Propolis extract	Antioxidant
		Pecan nut colour	Colour
		Carthamus red	Colour
Others	Quassin	Quassia extract	Bitterant, etc.
		Jamaica quassia extract	Bitterant, etc.
	Phytic acid	Enzymatically decomposed rice bran	Antioxidant
	Ferulic acid	Rice bran oil extract	Antioxidant
		Ferulic acid	Antioxidant
	Maltol	Roasted rice bran extract	Food manufacturing agent
		Roasted soybean extract	Food manufacturing agent
	Rutin-related compounds	Enzymatically modified isoquercitrin	Antioxidant
		Dokudami extract	Antioxidant
		Enzymatically decomposed rutin	Antioxidant
	Plant secretion used as gum base	Elemi resin	Thickening stabilizer, gum base
		Opopanax resin	Gum base
		Kauri gum	Gum base
		Copaiba balsam	Gum base
		Copal resin	Gum base
		Sandarac resin	Gum base

Large classification	Small classification	Name	Use
		Olibanum	Gum base
		Fir balsam	Gum base
		Benzoin gum	Gum base
		Mastic gum	Gum base
		Myrrh	Gum base
		Rosin	Gum base
Others	Wax used as gum base, etc.	Urushi wax	Gum base, glazing agent
		Urucury wax	Gum base, glazing agent
		Ozokerite	Gum base
		Spermaceti wax	Gum base, glazing agent
		Jojoba wax	Gum base
		Montan wax	Gum base, glazing agent
		Oil stuff seed wax	Gum base, glazing agent
	Extracted from mushrooms	Kawaratake extract	Bitterant, etc.
		Himematsutake extract	Bitterant, etc.
		Mannentake extract	Bitterant, etc.
	Sesame oil extracts	Sesame seed oil unsaponified matter	Antioxidant
		Sesamolin	Antioxidant
		Sesamol	Antioxidant
	Extracted from bamboo	Hachiku extract	Food manufacturing agent
		Madake extract	Food manufacturing agent
		Mousouchiku extract	Food manufacturing agent
	Extracted from wasabi	Horseradish extract	Antioxidant, food manufacturing agent
	Extracted from other plants	Hollyhock flower extract	Antioxidant
		Alkanet colour	Colour
		Fig leaf extract	Food manufacturing agent
		Udo extract	Preservative
		Rumput roman extract	Preservative
		Kusagi colour	Colour
		Curculin	Sweetener
		Enzymatically hydrolyzed coix extract	Preservative
		Shikon colour	Colour
		Essential oil-removed fennel extract	Antioxidant
		Dropwort extract	Antioxidant
		Water pepper extract	Food manufacturing agent
		Tenryocha extract	Sweetener
		Tomato glucolipid	Emulsifier
		Monellin	Sweetener

Large classification	Small classification	Name	Use
		Betel nut extract	Food manufacturing agent
		Grape skin-derived substance	Food manufacturing agent
		Hego-ginkgo leaf extract	Antioxidant
		Garden balsam extract	Antioxidant
		Magnolia obovata extract	Preservative
		Hokosshi extract	Food manufacturing agent
		Miracle fruit extract	Sweetener
		Rice hull extract	Food manufacturing agent
		Eucalyptus leaf extract	Antioxidant
		Rakanka extract	Sweetener
		Gentian root extract	Antioxidant
		Forsythia extract	Preservative
		Logwood colour	Colour
Others	Others	Aspergillus terreus extract	Antioxidant
		Polyfructan (inulin type)	Food manufacturing agent
		Ellagic acid	Antioxidant
		Catechin	Antioxidant
		Fish scale foil	Colour
		Kojic acid	Food manufacturing agent
		Sphingolipid	Emulsifier
		Tempeh extract	Antioxidant
		Tocotrienol	Antioxidant
		Nordihydroguaiaretic acid	Antioxidant
		Betaine	Flavouring agent
		Borapet	Bitterant, etc.
		Gallic acid	Antioxidant
		Morin	Antioxidant
		Lac colour	Colour

Research on the safety evaluation of existing natural additives

– FY 1996 health and welfare science grant research report –

Hayashi Yuzo, Senior Researcher

Supervised by Food Chemistry Division, Environmental Health Bureau, Ministry of Health and Welfare

Issued by Japan Food Additives Association

1-3-9 Horidome-cho, Nihonbashi, Chuo-ku, Tokyo 103-0012 Japan

Phone: 03-3667-8311

Fax: 03-3667-2860

Transfer Account: 00140-4-65875

Price: ¥1,429 + Tax

Appendix

List of errata

"Research on the safety evaluation of existing natural additives, FY 1996 health and welfare science grant research report"

Location	Correction	Error
Page 18, Table 3, Line 3	Vegetable carbon black	Vegetable carbon black
Page 20, Table 4, Line 1	Aspergillus terreus glycoprotein	Aureobasidium cultured solution
Page 25, Table 5, Line 1 (Aureobasidium cultured solution)	(Columns for 28-day or longer repeated-dose toxicity +Mutagenicity	(Same as the left)
	Delete the circle mark	Circle mark
Line 14 (Aspergillus terreus glycoprotein)	Add the circle mark	Blank
Page 38, Table 6, Line 1 (Amino acids/proteins)	(Columns for Name and Use) Delete	(Same as the left) Aspergillus terreus glycoprotein, Food manufacturing agent
Line 22 (Glucans)	Insert Aureobasidium cultured solution, Food manufacturing agent	–
	(Attachment 1. Summary of safety studies)	(Same as the left)
Page 45, Line 1 (Title)	Aspergillus terreus glycoprotein	Aureobasidium cultured solution
Page 45, Line 3 (Food additive name)	Aspergillus terreus glycoprotein	Aureobasidium cultured solution
Page 45, Lines 5-6 (Origin, method of preparation, and definition)	Aspergillus terreus glycoprotein is obtained from the fermentation culture solution of glucose, starch, and soybean meal by a filamentous fungus (Aspergillus terreus) by bacterial elimination, followed by fractionation with ammonium sulfate and desalting. Its major component is glycoprotein.	Aureobasidium cultured solution is obtained from cultured Aureobasidium pullulans by separation. Its major component is β-1,3-1,6-glucan.
Page 45, Line 8 (Major use)	Food manufacturing agent	Thickening stabilizer
Page 72, Line 17 (Repeated-dose/ carcinogenicity study)	(Attachment 1. Summary of safety studies, Tamarind seed gum)	
	1.9 g/kg/day	0.19 g/kg/day
Page 87, Line 24 (Repeated-dose/ carcinogenicity study)	(Attachment 1. Summary of safety studies, Pullulanase)	
	10 mL/kg	0 mL/kg