Ver. 1.0.0

Research report

Research on the safety re-evaluation of existing additives

FY 2003

Version history:

Ver. 1.0.0 February 9, 2022

This document is the English translation of "*既存添加物の安全性の見直しに関する調査研究（平成15年度調査）*" as a service to a broad international audience/readers. This English version is provided for reference purposes only. In the event of any inconsistency between the Japanese original and English translation, the former shall prevail.

Administrative Information, Ministry of Health, Labour and Welfare

June 24, 2004: Data relating to the Pharmaceutical Affairs and Food Sanitation Council
Research on the safety re-evaluation of existing additives (FY 2003 Survey)

Research on the safety re-evaluation of existing additives

(June 24, 2004, Committee on Food Additives, Pharmaceutical Affairs and Food Sanitation Council)

A report of the "Research on the safety re-evaluation of existing additives" was disclosed in the Committee on Food Additives, Pharmaceutical Affairs and Food Sanitation Council held on June 24, 2004.

FY 2003 food additive safety confirmation grant research

Research report
Research on the safety re-evaluation of existing additives

June 2004

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A. Summary

In the FY 1996 health and welfare science grant research report, “Research on the safety evaluation of existing additives" (Senior Researcher: Hayashi Yuzo) (hereinafter referred as “Hayashi Group Report”) which reviewed existing additives based on international evaluation results, approval status in Europe and the United States, available safety study results, etc., it was concluded that 139 out of 489 additives should be evaluated by the additional safety results including the newly performed studies in the future.

In this study, an investigation was performed on food additives for which safety study results were newly available, including 125 additives out of the 139 additives that had been considered to require further investigation regarding safety in the Hayashi Group Report, excluding 14 additives reported in FY 1999 “Research on the safety evaluation of existing additives” (Senior Researcher: Kurokawa Yuji) (hereinafter, referred to as the “Kurokawa Group Report”). This report collectively lists investigation results for 17 additives: Aureobasidium cultured solution, 5'-Adenylic acid, Alkanet colour, Japanese persimmon colour, Gastric mucin, Kooroo colour, Rice bran oil extract, Artemisia sphaerocephala seed gum, Perilla extract, 5'-Cytidylic acid, Essential oil-removed fennel extract, Bacillus natto gum, Absinth extract, Fukuronori extract, Mastic gum, Yucca foam extract, and Rosin.

The results of a 90-day or longer repeated-dose and a mutagenicity study were available for each of the 17 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. In conclusion, as there is presently no study result that suggests any immediate effects on human health, it is considered that there is no immediate need to perform a new safety study.

B. 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 on the list of existing additives have not been checked individually for safety, therefore, confirmation of their safety is requested in the Diet, etc.

In FY 1996, the basic safety of 489 existing additives was investigated based on international evaluation results, approval status in Europe and the United States, and safety study results, etc., and was then published as the Hayashi Group Report. In the report, it is stated that “Of the 489 additives, 159 have already been evaluated internationally and their basic safety has been confirmed. In addition, it was considered that there is no urgent need to investigate the safety of 41 additives based on the evaluation of available test results and 150 additives based on their origin, method of preparation and definition, at the present stage.” Therefore, the report concluded that the remaining 139 additives are still required further investigation.

In the FY 1999 Kurokawa Group Report, it is stated, “Of the 139 additives reported as requiring

safety confirmation in the Hayashi Group Report, 14 existing additives have no study results suggesting any immediate adverse effects on human health at the present stage, and therefore, there is no urgent need to perform a new safety study."

The present survey aimed to investigate the basic safety of natural additives by collecting domestic and overseas study results and evaluating those results for 125 out of 139 additives that had been considered to require investigation for safety in the FY 1996 Hayashi Group Report, excluding 14 additives that had completed the safety review in the FY 1999 Kurokawa Group Report.

C. Methods

Among 139 existing additives that were considered to require an investigation for safety in the Hayashi Group Report, excluding 14 additives that had completed the safety review in the Kurokawa Group Report, the study of safety evaluation was conducted for remaining 17 additives out of 125 existing additives individually for which both 90-day or longer repeated-dose toxicity test results and mutagenicity test results were available.

D. Results

Regarding the 17 additives for which safety was reviewed in this survey, there is no study results that suggest any immediate effects on human health, at present, for any of these additives (a summary is provided in the Annex).

E. Discussion

Safety study results were collected for the 125 existing additives that had not completed a safety review, among those that had been considered to require an investigation regarding safety in the Hayashi Group Report, and the study results were evaluated for 17 existing additives for which at least both results of a repeated-dose test for 90 days or more and a mutagenicity test were available. As there were no study results that suggest an immediate effect on human health, at present, and for any of these additives, it is therefore considered that there is no immediate need to perform a new safety study for the 17 existing additives that were evaluated. In addition, the Ministry of Health, Labour and Welfare has currently been reorganizing existing additives that are not actually used based on the amendment of the Food Sanitation Act in FY 2003. Specifically, 38 additives (25 out of 38 these additives are considered to require safety confirmation) are notified in the list of additives to be eliminated and are currently being processed. It is necessary to investigate the actual usage status of existing additives continuously, and to efficiently review the additives that require information.

F. Conclusion

The basic safety of 17 additives was confirmed by this survey based on the collected study results. It is considered that there is no immediate need to perform additional safety studies at the present stage for any of these additives.

Annex

Aureobasidium cultured solution

1. Food additive name:

Aureobasidium cultured solution (obtained from Aureobasidium cultured solution; contains β-1,3-1,6-glucan as the major component.)

2. Origin, method of preparation, and definition:

Aureobasidium cultured solution is obtained from the culture solution of *Aureobasidium pullulans* by separation. It consists mainly of β-1,3-1,6-glucan.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose oral toxicity test was performed in F344 rats by administration in drinking water, with the additive concentration adjusted to 0.56, 1.67, and 5.0%, respectively. The result showed several significant differences in hematological and blood biochemical tests. However, it was concluded that these changes were not due to the test substance, since they did not show any dose relationship and were mild changes within the normal range. Histopathological test results sporadically showed focal slight microgranuloma and extramedullary hematopoiesis in the liver in females, and mild inflammatory cell infiltration in the myocardium in males; however, these changes are known as naturally occurring lesions in F344 rats, and there were no differences between groups. Therefore, they were considered to have little toxicological significance. Based on these overall results, the no observed adverse effect level was considered to be 5%. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 500 μg/plate and yielded negative results regardless of the presence or absence of metabolic activation. 2)

In a chromosomal aberration test in cultured mammalian cells (CHL/IU), no clastogenicity was observed under feasible experimental conditions (10% v/v). 3)

A micronucleus test in mice was performed up to the technically possible dose (420 mg/kg), and no micronucleus inducibility was observed at any dose. 4)

(References)

1. Wanibuchi Hideki: Health and Labor Sciences Research Grant, Osaka City University Graduate School of Medicine
2. Kojima Akinori: FY 2001 Study on the Preparation of Standards and Criteria, etc. for

Food Additives, Nagoya City Public Health Research Institute

3. Hayashi Makoto: Study on Re-evaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences
4. Ajimi Shozo: Health Sciences Research Grant, Chemicals Evaluation and Research Institute, Japan

5'-Adenylic acid

1. Food additive name:

5'-Adenylic acid

2. Origin, method of preparation, and definition:

5’-Adenylic acid is obtained by enzymatic hydrolysis of nucleic acids that are water-extracted from yeast (*Candida utilis*), followed by isolation. It consists mainly of 5’-adenylic acid.

3. Major use:

Nutrition fortifier

4. Summary of safety study results:

(1) Repeated-dose toxicity study

F344 rats were fed diets containing 5'-Adenylic acid at concentrations of 0, 0.6, 1.26, 2.5, and 5% for 12 weeks. Significant suppression of body weight gain, as well as increased kidney weight were observed in the female 5% group; however, these were very mild changes. In addition, no changes were observed in histopathological test, blood cell count and serological tests. Accordingly, these were not considered as toxicological changes.

It was considered that 5'-Adenylic acid shows no toxicity in F344 rats by oral administration at 5% or less. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA100, TA1535, TA98, TA1537, and WP2*uvrA/*pKM101) was negative, regardless of the presence or absence of S9mix (maximum dose, 5,000 μg/plate). 2)

A chromosomal aberration test in cultured mammalian cells (CHL) was positive, since structural chromosome aberrations were induced at 156 - 1,250 μg/ml, -S9mix, and a 24-hour treatment. 3)

A micronucleus test in bone marrow was performed in mice (ICR, SPF, male, 5 animals per dose) by gavage (500, 1,000, and 2,000 mg/kg×2, suspended in 0.5% CMC). The result was negative, as no significant increase was observed, such as in the frequency of polychromatic erythrocytes with micronuclei, at any dose. 4)

(References)

1. Tofu Yoshiyuki: FY 2000 Study on Reevaluation, etc. of the Safety of Food Additives, Hiroshima University
2. Matsushima Taijiro: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center
3 and 4. Iwamoto Tsuyoshi: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

Alkanet colour

1. Food additive name:

Alkanet colour (obtained from the roots of *Anchusa officinalis* LINNE; contains alkannin as the major component.)

2. Origin, method of preparation, and definition:

Alkanet colour is obtained from the roots of *Anchusa officinalis* LINNE of the Boraginaceae family by extraction with ethanol. It consists mainly of alkaninn as a principal coloring component. It is red to red-purple.

3. Major use:

Color

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with this substance at concentrations of 0.56, 1.67, and 5%. In the test, no animal deaths or changes in general condition, body weight, or food intake were observed.

Hematological tests showed decreased white blood cell count in male groups at 0.56% or higher, with no obvious dose relationship and no change in differential blood count, and no specific histopathological changes in bone marrow, such as decreased cell density. However, values were low in the 5% group, which is considered to be an effect of administration. A trend towards anemia (decreased RBC, Hb, and Ht) was observed in both male and female groups at 0.56% or higher; however, decreased RBC observed in the female 5% group was the only significant difference. Platelet count dose-dependently increased in the female groups at 1.67% or higher, which was considered to be an effect of administration; however, as no histopathological changes were observed, this was considered to be of little toxicological significance.

In blood biochemical test some test items showed sporadic changes, although no dose relationship was observed, and a histopathological search showed no observations indicating toxicity. The no observed adverse effect level in this test is considered to be 1.67% for males and 0.56% for females. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1637, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate and resulted in negative results regardless of the presence or absence of metabolic activation. 2)

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU). The results showed that the induction of structural chromosome aberrations, which occurred in a dose dependent manner, was observed in the short-term treatment (-S9mix and +S9mix). 3)

A micronucleus test in the mouse bone marrow was performed up to 2,000 mg/kg, which was the accepted limit dose in the guidelines, and micronuclei were not induced at any dose. 4)

Therefore, the clastogenicity observed *in vitro* is considered to cause no particular problems in the body, as it was not observable in the micronucleus test in rodents which had investigated appropriately high doses.

(References)

1. Hirose Yoshinobu: Health and Labor Sciences Research Grant, Gifu University Hospital
2. Kojima Akinori: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Mochizuki Nobuhiko: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Public Interest Incorporated Foundation Biosafety Research Center
4. Noguchi Tadashi: Health Sciences Research Grant, Japan Bioassay Research Center

Japanese persimmon colour

1. Food additive name:

Japanese persimmon colour. It is obtained from the fruits of Japanese persimmon and consists mainly of flavonoids.

2. Origin, method of preparation, and definition:

Japanese persimmon colour is produced from fermented and roasted fruits of Japanese persimmon (*Diospyros kaki* THUNB.) through extraction with hydrous ethanol or through extraction with a weakly alkaline solution and neutralization. It consists mainly of flavonoids as principal coloring components. It is red-brown.

3. Major use:

Color

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose oral toxicity test was performed in SD rats by dietary administration with this substance at concentrations adjusted to 1.26, 2.5, and 5.0%. The result showed that no animal deaths were observed throughout the study period, and no effects due to the test substance were observed regarding body weight, food intake, blood chemical or pathological test.

Regarding general condition, the excretion of black stool was observed in all administered groups, which was considered as colored stool associated with fecal excretion of the test substance and judged to be of no toxicological significance. Hematological test showed a significant decrease in white blood cell count in males in the 5.0% administered group. However, as the decrease was mild and there was no difference in the white blood cell differential count, and pathological test of the lymphoid organs showed no effect of the test substance, thus it was considered to be a fluctuation within the physiological range.

The no observed adverse effect level is estimated to be 5.0% for both males and females. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98 and TA100) was judged as negative because no His+ revertant colonies were induced, regardless of the presence or absence of metabolic activation (maximum dose, 5,000 μg/plate). 2)

In a chromosomal aberration test in cultured mammalian cells (CHL), structural chromosome aberrations were induced. 3)

It was concluded in a micronucleus test in mice (ICR) that there was no micronucleus inducibility at any dose. 4)

(References)

1. and 2. Company data
3. Mutagenicity Study Results of Food Additives (Part 6)
4. Mutagenicity Study Results of Food Additives (Part 9)
5. Datasheet for the evaluation of the mutagenicity of food additives by the Ministry of Health and Welfare, etc.

Gastric mucin

1. Food additive name:

Gastric mucin (obtained from the gastric mucosa of mammals; contains mucopolysaccharides as the major component.)

2. Origin, method of preparation, and definition:

Gastric mucin is obtained from the gastric mucosa of mammals (such as sheep and pigs) by enzymatic treatment followed by ethanol precipitation of the supernatant. It consists mainly of mucopolysaccharides.

3. Major use:

Food manufacturing agent

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with this substance at concentrations of 0.03, 0.126, 0.5, and 2%. The result showed no animal deaths, and no changes in general condition, body weight, or food intake were observed.

Significant differences from the control group were observed sporadically for items in hematological test, blood biochemical test, and organ weight. However, these were considered to be of little toxicological significance, as the changes were mild and showed no dose relationship. No specific changes were observed in either macroscopic observation or histopathological test. Based on these results, it was considered that the 2% group was the dose for which no toxicity was expressed. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) induced His+ revertant colonies not less than 1.5-fold of that of the solvent control in TA1535 in the absence of S9mix, and the reproducibility was also observed. Since a dose-dependent trend was observed in more than on test, it was concluded that the result was suspected to be positive. 2)

A chromosomal aberration test and a micronucleus test were performed in cultured mammalian cells (CHL/IU). Chromosomal aberrations (chromatid gaps, chromatid breaks, and chromatid exchanges) were induced in the absence of S9mix and at 1.25 mg/ml. The micronucleus test was also positive under the same conditions. 3) Neither chromosomal aberrations nor micronucleus induction can be analyzed at doses higher than this level, as cytotoxicity is expressed. All were suppressed by the addition of S9mix.

A bone marrow micronucleus test was performed in mice (ddy, female, 5 animals per dose) by gavage, 2 times with water solution at doses of 1 and 2 g/kg. The results were negative, because no significant increase was observed, such as in the frequency of

polychromatic erythrocytes with micronuclei, at any dose. 4)

(References)

1. Tofu Yoshiyuki: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Hiroshima University
2. Kojima Akinori: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Hayashi Makoto: FY 1999 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, Center for Biological Safety and Research, National Institute of Hygienic Sciences
4. Hachiya Noriyuki: FY 1999 Study on Reevaluation of the Safety of Food Additives, Akita University

Kooroo colour

1. Food additive name:

Kooroo colour (obtained by extraction from the roots of *Dioscorea matsudai* HAYATA)

2. Origin, method of preparation, and definition:

Kooroo colour is obtained from the roots of *Dioscorea matsudai* HAYATA of the Dioscoreaceae family by extraction with water, weakly alkaline aqueous solution, or propylene glycol at a high temperature, or with hydrous ethanol at room temperature. It is red-purple.

3. Major use:

Color

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344/DuCrj rats with Kooroo colour (color valency, 30; containing 79.9, 5.8, 0.8, 1.62, and 0.06% of propylene glycol, potassium chloride, chlorine, potassium and sodium, respectively) by dietary administration (0, Vehicle, 0.5, 1.5, and 5.0%). The results including histopathological tests showed that the administration with the substance did not induce any changes in any of the groups. The no observed adverse effect level is considered to be 5.0% (male: 2,993 mg/kg/day, female: 3,376 mg/kg/day). 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) resulted in the induction of His+ revertant colonies (-S9mix, 200 μL/plate) at 4.8-fold the level of the TA1537 strain, and concentration dependency was also observed. This test was therefore judged as positive. 2) However, mutagenicity was abolished by the addition of S9mix.

The results of a chromosomal aberration test in cultured mammalian cells (CHL) showed no significant increase in the frequency of cells with structural chromosome aberrations and aberrant polyploid cells, regardless of the presence or absence of S9mix. This test result was judged as negative. 3)

A bone marrow micronucleus test was performed in mice (ICR, SPF, male, 6 animals per dose) by gavage 2 times at 500, 1,000, and 2,000 mg/kg (suspended in 0.5% CMC solution in water). The results were judged as negative. 4)

(References)

1. Inoue Tohru: FY 1997 Study on Food Additives, Biological Safety Research Center, National Institute of Health Sciences
2. Miyabe Masaki: FY 1995 Study on Reevaluation, etc. of the Safety of Food Additives, Nagoya City Public Health Research Institute

3 and 4. Kurita Toshishiro: FY 1995 Study on Reevaluation, etc. of the Safety of Food Additives, The Institute of Environmental Toxicology

Rice bran oil extract

1. Food additive name:

Rice bran oil extract (obtained from rice bran oil; contains ferulic acid as the major component.)

2. Origin, method of preparation, and definition:

Rice bran oil extract is obtained from the unsaponifiable matter of rice bran oil obtained from the seed of rice (*Oryza sativa* LINNE) of the Poaceae family via extraction with ethanol. Its active ingredient is ferulic acid.

3. Major use:

Antioxidant

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration at concentrations of 0.06, 0.25, 1, and 4%. The result showed that no animal deaths were observed, and no changes were observed in general condition, body weight, or food intake. Hematological and blood chemical test showed sporadic significant differences in test items; however, these changes were considered to be of little toxicological significance, as the range of variation was very mild, remaining in the biologically normal range, and showed no dose relationship. In addition, macroscopic observation and histopathological test by autopsy showed no toxicologically significant changes. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and all were negative regardless of the presence or absence of metabolic activation. 2)

In a chromosomal aberration test in cultured mammalian cells (CHL/IU), chromosomal aberrations were not observed up to 5,000 μg/plate, regardless of the presence or absence of the metabolic activation system. 3)

A bone marrow micronucleus test in mice was performed up to 2,000 mg/kg, and no micronucleus inducibility was observed at any dose. 4)

(References)

1. Kamiya Kenji: Health and Labor Sciences Research Grant, Hiroshima University Research Institute for Radiation Biology Medicine
2. Noguchi Tadashi: Health Sciences Research Grant, Japan Bioassay Research Center
3 and 4. Honma Masamitsu Health Sciences Research Grant, Division of Genetics and Mutagenesis, National Institute of Health Sciences

Artemisia sphaerocephala seed gum

1. Food additive name:

Artemisia sphaerocephala seed gum (obtained from the seed coats of Artemisia sphaerocephala; contains polysaccharides as major components.)

2. Origin, method of preparation, and definition:

Artemisia sphaerocephala seed gum is obtained from the seed coats of Artemisia sphaerocephala (*Artemisia halodendron* TURCZ. ex BESS., *Artemisia ordosica* KRASCHEN., *Artemisia sphaerocephala* KRASCH.) of the Asteraceae family by defatting and drying. It consists mainly of neutral polysaccharides and acidic polysaccharides with α-cellulose as the basic structure.

3. Major use:

Food manufacturing agent, Thickening stabilizer

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in male and female F344 rats by dietary administration at concentrations of 0.5, 1.5, and 5%, and no changes that were suggestive of toxicity were observed. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) resulted in negative results, for all. 2)

In a chromosomal aberration test in cultured mammalian cells (CHL/IU), it was considered that no chromosomal aberrations were observed, regardless of the presence or absence of the metabolic activation system. 3)

Mice (ddy, male, 6 mice per group) were gavaged twice with aqueous solutions of Artemisia sphaerocephala seed gum. The test was performed up to 760 mg/kg as the maximum dose (administration at higher concentrations was not possible, as the solution became paste-like), and no micronucleus inducibility was observed in the bone marrow at any dose. 4)

(References)

1. Inoue Tohru: FY 1998 Study on Reevaluation, etc. of the Safety of Food Additives, Biological Safety Research Center, National Institute of Health Sciences
2. Miyabe Masaki: FY 1997 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Sofuni Toshio: FY 1997 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences

4. Hachiya Noriyuki: FY 1997 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Akita University

Perilla extract

1. Food additive name:

Perilla extract (obtained from the seeds or the leaves of Perilla crispa; contains terpenoids as major components.)

2. Origin, method of preparation, and definition:

Perilla extract is obtained from an extract of the seeds or leaves of (*Perilla crispa* TANAKA) of the Lamiaceae family extracted with an acidic aqueous solution or at a warm temperature with hydrous ethanol. It consists mainly of terpenoids.

3. Major use:

Food manufacturing agent

4. Summary of safety study results:

(1) Repeated-dose study

In a 90-day repeated-dose test in F344 rats at concentrations of 2.5, 5, and 10% by administration in drinking water, water intake increased in the administered groups at 5% and higher, which was considered as a possible effect of the sucrose contained in Perilla extract. In addition, gain suppression was observed in the male 10% group. Hematological test showed increased WBC in male groups at 5% and higher, and decreased Seg and increased Lymph in all male administered groups; however, these changes were not considered to be toxicologically significant.

Serum chemistry showed increased TC, Alb, TP, etc.; however, these changes were not considered to be caused by administration, because they were within the background data range for untreated rats, and no notable pathological changes were observed. Regarding organ weight, the relative weights of the liver and heart increased in the male 10% group, the absolute and relative weights of the liver increased in the female 5% group and higher, and the absolute and relative weights of the heart increased in all female administered groups. However, none of these changes were considered as toxicity, as no pathological changes indicating organ toxicity were observed. Based on these results, as no toxicological changes caused by administration were observed in this study, the no observed adverse effect level is considered to be 10% (109.6 mg/kg/day) for males, and 10% (86.7 mg/kg/day) for females. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1635, TA1537, and TA1538) was negative, regardless of the presence or absence of S9mix (Maximum dose, 200 µl/plate). 2)

A chromosomal aberration test in cultured mammalian cells (CHL) was negative, regardless of the presence or absence of S9mix, showing no inducibility for structural chromosome aberrations or polyploid abnormalities up to a concentration of 5 mg/ml. 3)

Mice (ICR SPF, male, 6 mice in each concentration) were gavaged twice with 500, 1,000, and 2,000 mg/kg (0.5% CMC solution). A micronucleus test in the bone marrow was negative, as no significant increase was observed, such as in the frequency of polychromatic erythrocytes with micronuclei, at any dose. 4)

(References)

1. Hirose Masao: FY 1996 Study on the Establishment of Standards and Criteria, etc. for Food Additives, Division of Pathology, National Institute of Health Sciences
2. Miyabe Masaki: FY 1996 Study on Reevaluation, etc. of the Safety of Food Additives, Nagoya City Public Health Research Institute

3 and 4. Kurita Toshishiro: FY 1996 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

5'-Cytidylic acid

1. Food additive name:

5'-Cytidylic acid

2. Origin, method of preparation, and definition:

5'-Cytidylic acid is obtained by enzymatic hydrolysis of the nucleic acids that are water-extracted from cells of the yeasts (*Candida utilis*) in the presence of salt, followed by isolation. It consists of 5'-Cytidylic acid.

3. Major use:

Nutrition fortifier

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with the substance at concentrations of 0.06, 0.25, 1, and 4%. The result showed that no animal deaths were observed, and no changes were observed in general condition, body weight, or food intake. Significant differences were sporadically observed for items in hematological test, serum biochemical test, and organ weight changes; however, these changes were considered to be of little toxicological significance, because they were very mild and within the biologically normal value range, no dose relationship observed, and no corresponding changes were observed in histopathological test. Based on these results, the toxicity of 5'-Cytidylic acid was considered to be extremely low. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and resulted in negative results for all, regardless of the presence or absence of metabolic activation. 2)

A chromosomal aberration test in cultured mammalian cells [CHL/IU] showed no structural aberrations or polyploid abnormalities by short-term treatment, regardless of the presence or absence of the metabolic activation system. Long-term treatment in the absence of S9mix was also negative (Maximum dose, 3,232 μg/ ml (10 mmol)). 3)

Mice (ICR SPF, Crj-CD-1, 5 mice per group) were gavaged twice with water suspended 5'-Cytidylic acid. Bone marrow was collected 24 hours after the second administration in order to perform a micronucleus test. The test was performed up to 2,000 mg/kg x 2 times (the limit dose) and yielded negative results, as no significant increase was observed, such as in the frequency of polychromatic erythrocytes with micronuclei, at any dose. 4)

(References)

1. Kamiya Kenji: FY 2000 Study on Reevaluation, etc. of the Safety of Food Additives, Hiroshima University

2. Kojima Akinori: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Masumori Shoji: Biosafety Research Center
4. Iwamoto Tsuyoshi: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

Essential oil-removed fennel extract

1. Food additive name:

Essential oil-removed fennel extract (glucosyl sinapyl alcohol obtained from the seed of fennel; contains glucosyl sinapyl alcohol as the principal component.)

2. Origin, method of preparation, and definition:

Essential oil-removed fennel extract is obtained from the residue of the seed of fennel (*Foeniculum vulgare* LINNE) of the Apiaceae family, after steam distillation by extraction with water at a high temperature, followed by concentration. It consists mainly of 4-O-α-D-glucosyl sinapyl alcohol.

3. Major use:

Antioxidant

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose oral toxicity test was performed in F344 rats by dietary administration with this substance adjusted at concentrations of 0.06, 0.25, 1, and 4%. The result showed no deaths during the test, and no suppression of body weight gain or toxicological observations were histologically apparent in any administered group. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) was performed up to 20 mg/plate and judged as positive, as His+ revertant colonies not less than 2-fold of that of the solvent control were induced in TA98 under +S9mix conditions, and showed both dose-dependency and reproducibility. 2)

In a chromosomal aberration test in cultured mammalian cells (CHL/IU), no clear induction of chromosomal aberrations caused by administration with the test substance was observed up to the maximum dose of 5 mg/ml. 3)

A micronucleus test in mice was performed up to the maximum dose (3 g/kg), and it was concluded that there was no micronucleus inducibility at any dose. 4)

It was reported that a reverse mutation test in bacteria was positive, with a reverse mutation frequency of not less than 2-fold of that of the control group only in TA98, under +S9mix conditions at a dose of not less than 10 mg/plate. It is therefore considered that mutagenicity cannot be denied. However, the reaction is above the accepted limit dose in the guidelines, and there was a misinterpretation in the results of the report. Thus, it is considered that there is no mutagenicity or a very weak mutagenicity, at worst, and that no genotoxicity causing a problem in the body will be expressed in normal use.

(References)

1. Kamiya Kenji: FY 1999 Study on Reevaluation, etc. of the Safety of Food Additives, Hiroshima University
2. Miyabe Masaki: FY 1998 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Sofuni Toshio: FY 1998 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Health Sciences
4. Hachiya Noriyuki: FY 1998 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Akita University

Bacillus natto gum

1. Food additive name:

Bacillus natto gum (obtained from the culture solution of Bacillus natto; contains polyglutamic acid as the principal component.)

2. Origin, method of preparation, and definition:

Bacillus natto gum is obtained from the culture solution of Bacillus natto (*Bacillus subtilis*) by separation. Its major component is polyglutamic acid.

3. Major use:

Thickening stabilizer, Food manufacturing agent

4. Summary of safety study results:

(1) Repeated-dose study

In a 90-day repeated-dose test in F334/DuCrj rats by dietary administration (0.18, 0.55, 1.66, and 5%), there were no serious changes that were considered to be caused by the test substance, and no histopathological toxicological changes even at 5%, which was the maximum dose. Accordingly, the no observed adverse effect level is considered to be 5% (2,616.4 mg/kg/day for males, and 2,727.6 mg/kg/day for females). 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) was performed up to 20 mg/plate, and was judged as negative, regardless of the presence or absence of metabolic activation. 2)

In a chromosomal aberration test in cultured mammalian cells (CHL/IU), abnormalities were observed in 6% of cells in short-term treatment in the presence of the metabolic activation system by treatment at 2.5 mg/ml, which was the limit dose; however, no chromosomal aberrations were induced by any other treatment condition. 3)

A bone marrow micronucleus test in mice (ddy, male, 6 animals per dose) was performed up to the maximum dose (3,000 mg/kg x 2), and no micronucleus inducibility was observed at any dose. 4)

Therefore, it is considered that there are no concerns regarding a toxicological effect in the body, taking into consideration that the positive result in the chromosomal aberration test in cultured mammalian cells was a reaction which occurred only at a very high dose, that the frequency of abnormalities was not high, and that the micronucleus test, which investigated appropriately high doses, was negative.

(References)

1. Hirose Masao: FY 1996 Study on Reevaluation of the Safety of Food Additives, Division of Pathology, National Institute of Health Sciences

2. Miyabe Masaki: FY 1996 Study on Reevaluation, etc. of the Safety of Food Additives, Nagoya City Public Health Research Institute
3. Sofuni Toshio: FY 1996 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Health
4. Hachiya Noriyuki: FY 1996 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Akita University Faculty of Medicine

Absinth extract

1. Food additive name:

Absinth extract (obtained from the whole plant of absinth; contains sesquiterpenes as principal components.)

2. Origin, method of preparation, and definition:

Absinth extract is obtained from the whole plant of absinth (*Artemisia absinthium* L.) of the Asteraceae family by extraction with water or at room temperature with ethanol. It consists mainly of sesquiterpenes (such as absinthin).

3. Major use:

Bitterant, etc.

4. Summary of safety study results:

(1) Repeated-dose study

In a 13-week repeated-dose test in Wistar rats by dietary administration (0.125, 0.5, and 2%), no animal deaths were observed in any administered group, and no changes caused by administration with the test substance was observed in body weight gain, hematological test, serological test, organ weight, or histopathological test. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and resulted in negative results, regardless of the presence or absence of metabolic activation. 2)

A chromosomal aberration test in cultured mammalian cells (CHL/IU) was performed up to 5,000 μg/plate and showed the dose-dependent induction of chromosomal aberrations by short-term treatment, under conditions without metabolic activation. 3)

A micronucleus test in mice was performed up to 2,000 mg/kg, and was judged as negative, as no micronucleus inducibility was observed at any dose. 4)

Positive results are reported for the chromosomal aberration test in cultured mammalian cells. However, it is unlikely that the clastogenicity observed *in vitro* will be expressed in the body, considering that the micronucleus test in rodents, which was performed for appropriately high doses, was negative. It is therefore considered that Absinth extract does not express genotoxicity which causes a problem in the body.

(References)

1. Mitsumori Kunitoshi: Health and Labor Sciences Research Grant, Faculty of Agriculture, Tokyo University of Agriculture and Technology
2. Matsushima Taijiro: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center

3 and 4. Mochizuki Nobuhiko: Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center

Fukuronori extract

1. Food additive name:

Fukuronori extract (obtained from the whole plant of Fukuronori; contains polysaccharides as principal components.)

2. Origin, method of preparation, and definition:

Fukuronori extract is obtained from the whole plant of Fukuronori (*Gloiopeltis furcata* POSTEL et RUPR) of the Endocladiaceae family by extraction with water at a high temperature. It consists mainly of polysaccharides.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) Repeated-dose study

In a 90-day repeated-dose test in F344/DuCrj rats by dietary administration (0.5, 1.5, and 5.0%), no clear differences were observed between groups regarding general condition and body weight for either males or females, throughout the study period. An increasing trend was observed in food intake for both males and females in the 1.5 and 5.0% groups; however, this change was not considered to be of toxicological significance. Hematological test showed no clear differences between groups. Blood biochemical test showed significant decreases in TP, T-Cho, and ALP in the male 6.0% group, and a significant increase in GPT in the female 1.5 and 5.0% groups. In addition, absolute and relative liver weights decreased significantly in males. However, although these changes were statistically significant, they were within the range of the background data. Moreover, histopathological test did not show the induction of lesions due to administration in the 5.0% administered group. Based on these results, the no observed adverse effect level of Fukuronori extract is considered to be 5.0% (male: 3,362 mg/kg/day, female: 3,594 mg/kg/day). 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) showed no induction of revertant colony count in test strains, regardless of the presence or absence of S9mix, and mutagenicity was judged as negative (Maximum dose, 20 mg/plate). 2)

In a chromosomal aberration test in cultured mammalian cells (CHL/IU), there was no significant increase of cells with structural chromosome aberrations and polyploid abnormalities, regardless of the presence or absence of S9mix, and it was judged that Fukuronori extract displayed no clastogenicity. (Slight precipitation occurred at the maximum dose of 250 μg/ml.) 3)
In a micronucleus test in mice (ICR SPF, male, 6 animals per group) by gavage 2 times at 500, 1,000, and 2,000 mg (dissolved in olive oil), micronucleus inducibility was judged

as negative, as no significant increase was observed in the frequency of polychromatic erythrocytes with micronuclei, at any dose. 4)

(References)

1. Inoue Tohru: FY 1996 Study on Food Additives, Biological Safety Research Center, National Institute of Health Sciences
2. Miyabe Masaki: FY 1996 Study on Reevaluation, etc. of the Safety of Food Additives, Nagoya City Public Health Research Institute

3 and 4. Kurita Toshishiro: FY 1996 Study on Reevaluation, etc. of the Safety of Food Additives, The Institute of Environmental Toxicology

Mastic gum

1. Food additive name:

Mastic gum (obtained from the secretion of *Pistacia lentiscus*; contains masticadienonic acid as the principal component.)

2. Origin, method of preparation, and definition:

Mastic gum is obtained from the secretion of *Pistacia lentiscus* LINNE of the Anacardiaceae family by removal of the low-boiling point components by distillation, extraction with ethanol at a high temperature, and removal of ethanol by distillation. It consists mainly of masticadienonic acid.

3. Major use:

Gum base

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with this additive at concentrations of 0.22, 0.67, and 2%. The result showed no animal deaths, and no changes were observed in either general condition or food intake. Suppression of body weight gain was observed in the male 2% group and the female 0.67% and higher groups.

Hematological test showed increased white blood cell count and increased platelet count in the male 2% group. Blood biochemical test showed increased TP and ALB, as well as decreased CRN in the male 0.67% and higher groups, and increased Ca and ALP, and decreased TG in the male 2% group. Increased TP, increased BUN, and increased γ-GT were observed in the female 2% group, increased TC was observed in the 0.67% and higher groups, and decreased P was observed in the 0.22% and higher groups. Regarding organ weight, the absolute weight of the liver increased in both males and females of the 0.67% and higher groups, and the relative weight increased in the male 0.22% and higher groups, and in the female 0.67% and higher groups. A histopathological search showed microgranuloma and extramedullary hematopoiesis in the liver, basophilic renal tubules and hyaline droplet precipitation in the kidney, and mild inflammatory cellular infiltration in the myocardium in males, as well as microgranuloma and extramedullary hematopoiesis in the liver, mineralization in the kidney, and mild inflammatory cellular infiltration in the myocardium. All of these were sporadic and showed no differences between groups.

The no observed adverse effect level was considered to be not more than 0.22% for males, and 0.22% for females. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A) was

performed up to 5,000 μg/plate and resulted in negative results regardless of the presence or absence of metabolic activation. 2)

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the concentration where cytotoxicity was observed, and resulted in the statistically significant induction of chromosomal aberrations only in the presence of the metabolic activation system. However, the frequency was as low as 6%, and the reaction was observed only at the dose at which cytotoxicity was observed. 3)

A micronucleus test in the mouse bone marrow was performed up to 2,000 mg/kg, which was the limit dose, and micronucleus induction was not observed, at any dose. 4)

Therefore, the clastogenicity observed *in vitro* was not observed in the *in vivo* test system and is not considered to cause any particular problems in the body.

(References)

1. Wanibuchi Hideki: Health and Labor Sciences Research Grant, Osaka City University Graduate School of Medicine
2. Ajimi Shozo: Health Sciences Research Grant, Chemicals Evaluation and Research Institute

3 and 4. Tanaka Noriho: Health Sciences Research Grant, Food and Drug Safety Center

Yucca foam extract

1. Food additive name:

Yucca foam extract (obtained from the whole plant of *Yucca arborescens* TREL. or *Yucca schidigera* ROEZL ex Orlgies; contains saponins as principal components.)

2. Origin, method of preparation, and definition:

Yucca foam extract is obtained from the whole plant of *Yucca arborescens* TREL. of the Liliaceae family or *Yucca schidigera* ROEZL ex Orlgies of the Liliaceae family, by extraction with water at a high temperature, or with hydrous ethanol or hydrous isopropyl alcohol at from room temperature to a slightly warm temperature. It consists mainly of saponins (such as sarsasaponin).

3. Major use:

Emulsifier, Food manufacturing agent

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with this additive at concentrations of 0.1, 0.3, 1, and 3%. The result showed no animal deaths, and no changes were observed in general condition, body weight, or food intake.
Significant differences from the control group were observed in various items of hematological test, blood biochemical test, and organ weight; however, these changes were considered to be of little toxicological significance, as they were very mild and within the biologically normal value range, and no dose relationship was observed. Histopathological test showed no toxicologically notable changes. 3)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A), a chromosomal aberration test in cultured mammalian cells (CHL/LU), and a micronucleus test in mouse bone marrow were performed, and none of these showed any problem in methods, results, etc. 1)

Moreover, an *in vivo/in vitro* unscheduled DNA synthesis assay was performed in rats by gavage with 2,000 mg/kg Yucca foam extract, and negative results were reported. It is therefore considered that this extract has no genotoxicity. 2)

(References)

1. Hayashi Makoto: Datasheet for the evaluation of mutagenicity of food additives by the Ministry of Health and Welfare, etc. (FY 1979-1998)
2. Mochizuki Nobuhiko: FY 2000 Study on Standards and Criteria, etc. for Food Additives, Biosafety Research Center
3. Nishimura Takahiro: FY 1997 Study on Reevaluation, etc. of Food Additives, Hiroshima University

Rosin

1. Food additive name:

Rosin (obtained from the secretion of pine; contains abietic acid as the principal component.)

2. Origin, method of preparation, and definition:

Rosin is obtained from the secretion of the bark of pine (*Pinus palustris* MILL.) of the Pinaceae family by removal of low-boiling point components by distillation. It consists mainly of abietic acid.

3. Major use:

Gum base

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with this additive at concentrations of 0.03, 0.125, 0.5, and 2.0%. Although no deaths or histopathological toxicity changes were observed, suppression of body weight gain and decreased food intake were observed in both males and females of the 2.0% group. In addition, significant increases and decreases were observed for many items in hematological test, blood chemical test, and organ weight measurement. Some of these changes were considered to be caused by the avoidance of food due to the terpene odor of Rosin. In the 0.5% group, males showed suppression of body weight gain, and the same changes observed in the 2.0% in some items in hematological test, blood chemical test and tissue weight measurement. In addition, an increase in the relative weight of the liver was observed in both males and females, suggesting a relationship with administration with the test substance. In the 0.125% and lower groups, significant differences were observed in several items; however, these changes were small, and were not considered to represent the toxicity of the test substance. The no observed adverse effect level was estimated to be 0.125% for both males and females. 1)

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) was performed up to 5,000 μg/plate and resulted in negative results regardless of the presence or absence of metabolic activation. 2)

A chromosomal aberration test in cultured mammalian cells (CHL/IU) was performed up to the maximum dose (5,000 mg/ml) and showed no clear induction of chromosomal aberrations caused by administration with the test substance, regardless of the presence or absence of metabolic activation. 3)

A micronucleus test in mice was performed up to 2,000 mg/kg, and was concluded as negative, as no micronucleus inducibility was observed at any dose. 4)

Therefore, it is considered that Rosin has no genotoxicity.

(References)

1. Watanabe Hiromitsu: FY 1998 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Hiroshima University
2. Miyabe Masaki: FY 1997 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. and 4. Kurita Toshishiro: FY 1997 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology