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Research report

Research on the safety re-evaluation of existing additives

FY 2007

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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19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.

Data relating to the Pharmaceutical Affairs and Food Sanitation Council

September 24, 2008
Department of Food Safety, Pharmaceutical and Food Safety Bureau

Research on the safety re-evaluation of existing additives (FY2007 Survey)

Research on the safety re-evaluation of existing additives
(September 24, 2008, Committee on Food Additives, Food Sanitation Commission, 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, Food Sanitation Commission, Pharmaceutical Affairs and Food Sanitation Council held on September 24, 2008.

Research report

Research on the safety re-evaluation of existing natural additives

March 2008

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Annex

A. Summary

In the FY1996 Health and Welfare Science Grant research report, "Research on the safety evaluation of existing additives" (Senior Researcher: Yuzo Hayashi) (hereinafter referred to as the "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 43 out of the 139 additives that had been considered to require further investigation regarding safety in the Hayashi Group Report, excluding the following additives:

- 13 additives reported in FY1999 "Research on the safety evaluation of existing additives" (Senior Researcher: Yuji Kurokawa) (hereinafter, referred to as the "Kurokawa Group Report")
- 16 additives reported in FY2003 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Tohru Inoue) (hereinafter, referred to as the "FY2003 Inoue Group Report")
- 14 additives reported in FY2004 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Tohru Inoue) (hereinafter, referred to as the "FY2004 Inoue Group Report")
- 7 additives reported in FY2006 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Tohru Inoue) (hereinafter, referred to as the "FY2006 Inoue Group Report")
- The additives that were previously deleted from the list of existing additives (among them, 46 additives are considered to require safety confirmation)

This report collectively lists investigation results for 8 additives, namely: Ellagic acid, Elemi resin, Sclero gum, Tea seed saponin, Tororoaoi, Garden balsam extract, Macrophomopsis gum and Rakanka extract. The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 8 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. In conclusion, as for the 8 additives evaluated, there was no existing study results that suggested any immediate adverse effects on human health, and therefore, it was considered that there was no immediate need to perform a new toxicity 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 included on the list of existing additives have not been checked individually for safety, therefore, confirmation of their safety has been requested in the Diet, etc.

In response to this, in the Hayashi Group Report published in FY 1996, the basic safety of 489 existing additives was reviewed based on the results of international evaluation, the status of approval in Europe and the United States, and the results of safety tests, etc., and 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." (Since one of these additives had no actual distribution, it was excluded from the existing additive list.) Furthermore, the Inoue Group Report in FY 2003 stated that "At present, there are no study results suggesting any immediate adverse effect on human health regarding the 17 additives for which safety was reviewed in this study." (For one of these additives, additional test was conducted just in case.) In addition, the Inoue Group Report published in FY 2004 stated that "regarding the 14 additives for which safety was reviewed, there were no study results that suggested any immediate adverse effect on human health at present." The Inoue Group Report published in FY 2006 also stated that "regarding the 7 additives for which safety was reviewed, there were no study results that suggested any immediate adverse effect on human health at present."

Present research aimed at investigating the basic safety of natural additives by collecting domestic and overseas study results and evaluating those results for 43 out of the 139 additives that had been indicated to require investigation for safety in the FY 1996 Hayashi Group Report, excluding those additives that previously completed the safety review and those additives that have already been deleted from the list of existing additives.

C. Methods

Among 43 of the 139 existing additives that were considered to require an investigation for safety in the Hayashi Group Report, excluding those additives that previously completed the safety review and those additives that have already been deleted from the list of existing additives, this research individually evaluated the safety study results of 8 additives for which the data of both results of a 90-day or longer repeated-dose toxicity study and a mutagenicity study were available.

D. Results

Regarding the 8 additives for which safety was reviewed in this research, there was no study result that suggested an immediate effect on human health at present. A summary is provided in the Annex.

E. Discussion

In this research, the study results for safety evaluation were collected for the 43 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 when the study results were evaluated for 8 existing additives for which both the results of a repeated-dose toxicity study at least 90 days in length and a mutagenicity study were available, there were no study results that suggested an immediate effect on human health at present for any of these additives. Therefore, it was considered that there was no immediate need to perform a new study for safety evaluation for the 8 existing additives that were evaluated.

Prior to this report, the Ministry of Health, Labour and Welfare deleted Madder colour from the list of existing additives and banned its use in July 2004, based on the evaluation of its carcinogenicity performed by the Food Safety Commission and the Pharmaceutical Affairs and Food Sanitation Council. In addition, the Ministry deleted 38 existing additives in December 2004, and 32 additives in September 2007 because of no actual usage.

While the operation of reviewing existing additives is currently undergoing steady progress in this manner, it is considered necessary to continue further investigation, such as the actual usage status of existing additives, and to proceed with reorganization efficiently from the additives that require information.

F. Conclusion

This research revealed that the basic safety of 8 additional natural additives was confirmed. It was considered that there was no immediate need to perform a further safety study at the present stage for any of these additives.

Annex

Ellagic acid

1. Food additive name:

Ellagic acid

2. Origin, method of preparation, and definition:

Ellagic acid is obtained from the gallnuts of sumac (*Rhus javanica* LINNE) of the Anacardiaceae family, the fruits of myrobalan (*Terminalia chebula* RETZ.), the fruits of water chestnut (*Trapa japonica* FLEROV.) of the Trapaceae family, and the leaves of eucalyptus (*Eucalyptus globulus* LABILL.) of the Myrtaceae family by extraction with water or ethanol following defatting. It consists mainly of ellagic acid.

3. Major use:

Antioxidant

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 13-week repeated-dose test was performed in F344 male and female rats by dietary administration (1.25, 2.5 and 5%). It showed no animal deaths, and no changes related to the test substance were observed in general condition or food intake in any groups. All female administered groups showed a significant decrease in body weight gain.

Hematological test showed an increase in MCV in males at a dose of 2.5% and a decrease in MCV in females at a dose of 1.25%; however, it was not considered to be the effect of the test substance because of no dose relationship.

Blood biochemical test showed a decrease in ALP in both sexes at a dose of 1.25%, a decrease in AST in males at a dose of 2.5%, a decrease in ALP in males at a dose of 5%, and a decrease in Ca and P and an increase in Cl in females at a dose of 5%; however, the results were not considered to be the effect of the test substance because the changes occurred sporadically.

As for organ weights, a decreased relative heart weight in males in the 5% group, and an increased relative brain weight in all administered groups of females was observed.

Histopathological test showed no change, probably due to the administration of the test substance.

In conclusion, the no observed effect level was considered to be 5% (3,011 mg/kg/day) for males and less than 1.25% (778 mg/kg/day) for females; as for the no observed adverse effect level, 5% (3,254 mg/kg/day) for both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 10 mg/plate, and the results were negative with and without S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL) to the maximum treatment concentrations of 40 μg/mL (short-term treatment: -S9 mix), 80 μg/mL (short-term treatment: +S9 mix), and 10 μg/mL (continuous treatment). As a result, the induction of chromosomal aberrations was observed only in the case of 40 μg/mL (short-term treatment: -S9 mix); however, the response was not considered definitely positive because of no dose dependency and the slight increase (5%). Based on the above results, the test substance was concluded to be negative. [3)]

A micronucleus test in the bone marrow of mice (BDF1 male) was performed up to the limit dose of 2,000 mg/kg×2, and no significant increase in micronucleus frequency was observed at any dose. In addition, the percentage of polychromatophilic erythrocytes decreased significantly at 2,000 mg/kg, and the mitotic inhibition in bone marrow cells was observed. Based on the above results, the micronucleus induction ability was concluded to be negative. [3)]

Taken together, Ellagic acid was determined to be non-genotoxic.

(References)

1. Nishikawa Akiyoshi: FY 2004 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Division of Pathology, Biological Safety Research Center, National Institute of Health Sciences
2. Asanoma Masaharu: FY 2004 Health and Labor Sciences Research Grant, Studies on Quality Problems in Ensuring Safety of Existing Food Additives in Japan
3. Nakajima Madoka: FY 2004 Health and Labor Sciences Research Grant, Studies on Quality Problems in Ensuring Safety of Existing Food Additives in Japan

Elemi resin

1. Food additive name:

Elemi resin (obtained from the juice of elemi; contains β-amyrin as the major component.)

2. Origin, method of preparation, and definition:

Elemi resin is obtained from the juice of elemi (*Canarium luzonicum* A.GRAY) of the Burseraceae family by drying. It consists mainly of β-amyrin.

3. Major use:

Gum base, thickening stabilizer

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 13-week repeated-dose test was performed in F344/DuCrj male and female rats by gavage (30, 200, and 1,000 mg/kg bw). It showed no animal deaths, and no changes related to the test substance were observed in general condition, body weight, food intake, ophthalmological examination, urinalysis, or autopsy in any groups.

In the results of hematological test, PT and APTT were or tended to be prolonged in males in the 200 mg/kg or higher groups.

The results of blood biochemical test were as follows: in the 30 mg/kg or higher groups, an increase in phospholipids in both sexes, an increase in total cholesterol in females, as well as an increase in α2-globulin ratio and a decrease in albumin level and A/G ratio in males; in the 200 mg/kg or higher groups, an increase in β-globulin ratio in both sexes, an increase in γ-GTP and total protein level and a decrease in albumin level and A/G ratio in females, as well as an increase in α1-globulin ratio in males; in the 1,000 mg/kg group, an increase in α1-globulin ratio in females, and an increase in γ-GTP and total cholesterol in males.

As for organ weights, the increased absolute adrenal weights in both sexes and the increased absolute and relative liver weights in females were observed in the 200 mg/kg or higher groups; the increased relative adrenal weights were observed in both sexes in the 1,000 mg/kg group.

Histopathological test showed fatty degeneration of liver cells at the edge of the lobule in females in the 200 mg/kg or higher groups, and fatty degeneration of cortical cells in the adrenal zona fasciculata in both sexes in the 200 mg/kg or higher groups.

Based on the above results, the effects on the liver and the adrenal glands were observed in both sexes in the 30mg/kg or higher groups and in the 200mg/kg or higher groups, respectively. Considering that all changes in blood biochemical test were within the background data except for phospholipids, and that no changes in liver weights and liver histopathology were observed in the 30 mg/kg group, these changes were considered to

be of poor toxicological significance. Therefore, the no observed effect level is considered to be less than 30 mg/kg/day for both sexes; as for the no observed adverse effect level, 30mg/kg/day for both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria and a chromosomal aberration test in cultured mammalian cells (CHL/IU) were performed, and the results were both negative.[2)] Also, a DNA repair test by using Bacillus subtilis (Rec-assay) showed negative. [3)]

A micronucleus test in the bone marrow of mice (ddY male) was performed up to the limit dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. [4)]

Taken together, Elemi resin was determined to be non-genotoxic.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center
2. Hayashi Makoto: The Datasheet of Mutagenicity Assessment of Food Additives by the Welfare Ministry, etc. (FY 1979 - 1998)
3. Sofuni Toshio: Experimental Results of Mutagenicity of Food Additives (11) (By the aid for experiment and research of the Welfare Ministry in FY 1989)
4. Miyazawa Maki: FY 2004 Studies on Quality Problems in Ensuring Safety of Existing Food Additives in Japan, Kanagawa Prefectural Institute of Public Health

Sclero gum

1. Food additive name:

Sclero gum (derived from the culture media of Sclerotium; contains polysaccharides as the major component.)

2. Origin, method of preparation, and definition:

Sclero gum is derived from the culture media of deuteromycete (*Sclerotium glucanicum*) by separation. It consists mainly of polysaccharides.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in F344/DuCrj SPF rats with the test substance at doses of 100, 300, and 1,000 mg/kg by gavage. It showed one rat in the 1,000 mg/kg group died. On the day before death, there were abnormal general conditions including fur contamination around the mouth and nose, decreased stool volume, hypoactivity, abdominal distention, and abnormal breathing sounds. However, no changes that might pathologically lead to direct cause of death were observed, the rat died early after the exacerbation of the general conditions. Another rat that exhibited similar symptoms in the 1,000 mg/kg group recovered from the abnormal general conditions, with no abnormalities in the results of hematological and blood biochemical test as well as the findings such as histopathological test despite the continuous administration. Therefore, the cause of death was most likely considered to be due to aspiration pneumonia following gavage errors.

In contrast, no other animals showed any abnormalities of general condition, trajectory of body weight, or food intake, and no specific changes probably due to the administration of sclero gum were observed in urinalysis, ophthalmological examination, the results of hematological and blood biochemical test, organ weight, as well as findings of autopsy and histopathological test.

In conclusion, the no observed adverse effect level is considered to be 1,000 mg/kg/day in both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and *Escherichia coli* (WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative with and without S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the maximum treatment concentration of 5,000 μg/mL, resulting in no induction of

chromosomal aberrations under any treatment condition. [3)]

A micronucleus test in the bone marrow of mice (BDF1 male) was performed up to the limit dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. [4)]

Taken together, Sclero gum was determined to be non-genotoxic.

(References)

1. Takashima Hiromasa: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center
2. Shimada Sawako: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Biosafety Research Center
3. Shimada Sawako: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Biosafety Research Center
4. Shimada Sawako: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Biosafety Research Center

Tea seed saponin

1. Food additive name:

Tea seed saponin (obtained from the seeds of tea; contains saponin as the major component.)

2. Origin, method of preparation, and definition:

Tea seed saponin is obtained from the seeds of tea (*Camellia sinensis* O.KZE.) of the Theaceae family by reflux extraction with ethanol following defatting with hexane. It consists mainly of saponins (such as tea saponin).

3. Major use:

Emulsifier

4. Summary of safety study results:

(1) 90-day repeated-dose study

In a 90-day repeated-dose test in F344/DuCrj SPF rats by gavage (30, 100, 300.1,000 mg/kg), abnormal respiratory sounds were observed in a small number of both male and female animals in the 1,000 mg/kg administered group, and deaths were found (2 cases each of males and females). In these fatal cases, pulmonary edema and congestion were observed in the lungs at autopsy and, moreover, these pathological changes in the lungs could be found only in the fatal cases. Transient edema as acute toxicity is most likely to have developed in other animals. However, diffuse hypertrophy of adrenal zona fasciculata was found in both the survival and dead animals, in which decreased total cholesterol in males as well as increased adrenal weights and decreased blood glucose levels in females were observed. Also, low body weight, the decrease in food intake, and tendency toward anemia were observed.

From the observations on the general condition, loose stool, spilled bait, as well as transient salivation and decreased stool volume following the administration were seen in the 1,000mg/kg administered group; the results of blood biochemical test showed decreased levels of globulin, total protein, urea nitrogen, and triglyceride, as well as increased levels of ALT and γ-GTP activities and Na. In this group, decreased thymus weights in males and increased liver weights in females were also seen. In the 300 mg/kg administered group, there were diffuse hypertrophy of adrenal zona fasciculata cells in both sexes, decreased total cholesterol, as well as loose stool and spilled bait from the observations on the general condition in males, increased A/G ratio and decreased triglyceride levels, as well as decreased α1- and γ-globulin fractions in males.

In conclusion, the no observed adverse effect level (NOAEL) is considered to be 100 mg/kg/day in both sexes. [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 the results were negative

with and without S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL) to the maximum treatment concentration of 120 μg/mL (-S9 mix) and 80 μg/mL (+S9 mix), resulting in no induction of chromosomal aberrations under any treatment condition. [3)]

A micronucleus test in the bone marrow of mice (ICR (Crj:CD-1), male, 5 animals per dose) was performed up to the maximum tolerated dose of 500 mg/kg×2, and no micronucleus induction was observed at any dose. [3)]

Taken together, Tea seed saponin was determined to be non-genotoxic.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center
2. Matsushima Taijiro: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center
3. Iwamoto Tsuyoshi: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

Tororoaoi

1. Food additive name:

Tororoaoi (obtained from the roots of Hibiscus manihot; contains polysaccharides as the major component.)

2. Origin, method of preparation, and definition:

Tororoaoi is obtained from the roots of Hibiscus manihot (*Abelmoschus manihot* MED.) of the Malvaceae family by drying and crushing. It consists mainly of polysaccharides.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration (0.5, 1.5, and 5.0%). It showed no animal deaths, and no marked changes were observed in general condition. In addition, no toxicological effects probably due to the administration of the test substance were observed in body weight, food intake, water consumption, urinalysis, ophthalmological examination, hematological test, blood biochemical test, gross pathological finding, organ weight, or histopathological test.

In conclusion, the no observed adverse effect level was considered to be 5.0% (male: 2,939 mg/kg/day; female: 3,325 mg/kg/day) under the conditions of this test. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria and a chromosomal aberration test in cultured mammalian cells (CHL/IU) were performed, and both results were negative. [2)] Also, a DNA repair test by using Bacillus subtilis (Rec-assay) showed negative. [2)]

A micronucleus test in the bone marrow of mice (ICR male) was performed up to the limit dose of 2,000 mg/kg×2, and no micronucleus was judged to be induced at any dose. [3)]

Taken together, Tororoaoi was determined to be non-genotoxic.

(References)

1. Tamano Seiko: FY 2005 Study on Standards and Criteria for Food and Food Additives, etc., DIMS Institute of Medical Science, Inc.
2. Hayashi Makoto: The Datasheet of Mutagenicity Assessment of Food Additives by the Welfare Ministry, etc. (FY 1979 - 1998)
3. Ono Hiroshi: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Hatano Research Institute, Food and Drug Safety Center

Garden balsam extract

1. Food additive name:

Garden balsam extract (obtained from the whole plant of garden balsam by extraction.)

2. Origin, method of preparation, and definition:

Garden balsam extract is obtained from the whole plant of garden balsam (*Impatiens balsamina* LINNE) of the Balsaminaceae family by extraction with hydrous ethanol at room temperature.

3. Major use:

Antioxidant

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in F344/DuCrj rats by ad libitum of water mixtures with test substance concentrations at 1.25, 2.5, and 5.0%, respectively. It showed no deaths in the test substance administered groups, and no obvious changes related to the test substance were observed in general condition, body weight, food intake, water consumption, hematological test, organ weight, or histopathological test.

Serum biochemical test showed dose-related changes in chlorine, sodium, potassium, and inorganic phosphorus levels. However, these changes were considered to be of little toxicological significance.

In conclusion, the no observed adverse effect level was considered to be 5.0% in both sexes (male: 3,997 mg/kg/day; female: 4,577 mg/kg/day). [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 100 mg/plate, and the results were negative with and without metabolic activation. [2)]

When a chromosomal aberration test was performed in cultured mammalian cells (CHL) to the maximum treatment concentration of 5,000 μg/mL, the result was concluded to be negative, because of no induction of chromosomal aberrations under any treatment condition with and without metabolic activation. [3)]

When a micronucleus test in the bone marrow of mice (ICR male) was performed up to the limit dose of 2,000 mg/kg×2, no significant increase in micronucleus frequency was observed at any dose, and therefore, the result was concluded to be negative. [4)]

Taken together, Garden balsam extract was determined to be non-genotoxic.

(References)

1. Hirose Masao: FY 2004 Study on the Preparation of Standards and Criteria, etc. for Food Additives, National Institute of Health Sciences
2. Asanoma Masaharu: Studies on Quality Problems in Ensuring Safety of Existing Food Additives in Japan, Nagoya City Public Health Research Institute
3. Matsumoto Kyomu: FY 2004 Studies on Quality Problems in Ensuring Safety of Existing Food Additives in Japan (A Chromosomal Aberration Test in Cultured Mammalian Cells for Garden Balsam Extract), The Institute of Environmental Toxicology
4. Matsumoto Kyomu: FY 2004 Studies on Quality Problems in Ensuring Safety of Existing Food Additives in Japan (A Mouse Micronucleus Test for Garden Balsam Extract), The Institute of Environmental Toxicology

Macrophomopsis gum

1. Food additive name:

Macrophomopsis gum (obtained from the culture fluid of Macrophomopsis; contains polysaccharides as the major component.)

2. Origin, method of preparation, and definition:

Macrophomopsis gum is derived from the culture fluid of *Macrophomopsis* by separation. It consists mainly of polysaccharides.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in F344/DuCrj SPF rats by gavage (100, 300, and 1,000 mg/kg). As a result, low levels of blood albumin and total cholesterol, although a slight significant difference, were observed in males in the 1,000 mg/kg administered group. In contrast, there were no abnormalities of general condition, trajectory of body weight, or food intake probably due to the test substance toxicity, and no specific changes were observed in urinalysis, ophthalmological examination, the results of hematological test, organ weight, as well as findings of autopsy and histopathological test.
In conclusion, the no observed adverse effect level (NOAEL) is considered to be 300 mg/kg/day in both sexes. [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 the results were negative with and without S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL) to the maximum treatment concentration of 5,000 μg/mL, resulting in no induction of chromosomal aberrations under any treatment condition over a short and long time course with and without S9 mix. [3)]

A micronucleus test in the bone marrow of mice (ICR (Crj:CD-1), 5 males per dose) was performed up to the limit dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. 3)

Taken together, Macrophomopsis gum was determined to be non-genotoxic.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center

2. Matsushima Taijiro: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center
3. Iwamoto Tsuyoshi: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

Rakanka extract

1. Food additive name:

Rakanka extract (obtained from the fruits of rakanka; contains mogrosides as the major component.)

2. Origin, method of preparation, and definition:

Rakanka extract is obtained from the fruits of rakanka (*Momordica grosvenori* SWINGLE) of the Cucurbitaceae family by extraction with water, hydrous methanol or ethanol, or by extraction with hydrous methanol from room to elevated temperatures and removal of the oil soluble components with vegetable oils. It consists mainly of mogrosides as sweetening components.

3. Major use:

Sweetener

4. Summary of safety study results:

(1) Acute toxicity study

An acute toxicity study was performed in ICR mice by gavage (2,000 mg/kg). No abnormalities or deaths were observed in the test animals, and the lethal dose was found to be 2,000 mg/kg or higher in both sexes. [1)]

(2) 90-day repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by mixing the test substance in the drinking water at concentrations of 0.25, 0.5, 1.0, and 2.0%. It showed no toxicity such as body weight loss or death was observed at all. In addition, there were no lesions in major organs or abnormal serum biochemical findings. [2)]

A 90-day repeated-dose test was performed in Wistar Hannover (GALAS) rats by mixing the test substance into the diet at the concentrations of 0.04, 0.2, 1, and 5%. It showed no animal deaths, and no changes due to the administration of test substance were observed in body weight gain, hematological test, serum biochemical test, organ weight, or histopathological test in any groups.

In conclusion, the no observed adverse effect level is considered to be 5% or higher in both sexes (male: 2,523.5 mg/kg/day; female: 3,202.7 mg/kg/day). [3)]

(3) 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 the results were negative with or without S9 mix. [4)]Also a reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative with and without S9 mix. [5)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the microscopically observable maximum dose of 625 μg/mL. As a result, the clastogenicity was judged to be positive, because the induction of chromosomal aberrations (11.5%) was observed in the continuous treatment, and there was also induction determined false-positive (5.0 - 7.0%) in the short-term treatment (both -S9 and +S9). The minimum D20 that is the relative comparison value of mutagenicity potency was calculated as 1.27 mg/mL. [6)]

A micronucleus test in the bone marrow of mice (BDF1, male, 5 animals per dose) was performed up to the limit dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. [6)]

Taken together, Rakanka extract was determined to be non-genotoxic.

(References)

1. In-house data
2. Hirose Ikuo: FY 1999 Study on Reevaluation, etc. of the Safety of Food Additives, Hiroshima University
3. Mitsumori Kunitoshi: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Tokyo University of Agriculture and Technology
4. In-house data
5. Matsushima Taijiro: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center
6. Kikuchi Masanori: FY 2003 Study on Standards and Criteria for Food and Food Additives, etc., Biosafety Research Center

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Review on the Safety of Existing Additives (as of August 2008):