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Research report

Research on the safety re-evaluation of existing additives

FY 2009

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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21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

June 23, 2010
Committee on Food Additives, Food Sanitation Commission, Pharmaceutical Affairs and Food Sanitation Council

Research on the safety re-evaluation of existing natural additives (FY 2009 Survey)

Research on the safety re-evaluation of existing natural additives
(June 23, 2010, 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 June 23, 2010.

Research report

Research on the safety re-evaluation of existing natural additives

March 2010

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Table of contents

A. Summary
B. Objective
C. Methods
D. Results
E. Discussion
F. Conclusion

Attachment

Copal resin
Sesame seed oil unsaponified matter
Olibanum
Roasted soybean extract
Peach gum
Enzymatically decomposed rutin

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.
Among the 139 items for which further study was deemed necessary in the Hayashi Group Report, of the 28 items other than those listed below that have already been reviewed for safety or those that have been excluded from the list of existing additives, this research examined 6 items for which new safety test results could be collected: Copal resin, Sesame seed oil unsaponified matter, Olibanum, Roasted soybean extract, Peach gum, and Enzymatically decomposed rutin.

- 13 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”)
- 16 additives reported in FY 2003 “Research on the safety re-evaluation of existing natural additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY 2003 Inoue Group Report”)
- 14 additives reported in FY 2004 “Research on safety re-evaluation of existing natural additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY 2004 Inoue Group Report”)
- 7 additives reported in FY 2006 “Research on the safety re-evaluation of existing natural additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY 2006 Inoue Group Report”)
- 8 additives reported in FY 2007 “Research on the safety re-evaluation of existing natural additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY 2007 Inoue Group Report”)
- 7 additives reported in FY 2008 “Research on the safety re-evaluation of existing natural additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY 2008 Inoue Group Report”)
- The additives that were previously deleted from the list of existing additives (including 46 additives considered to require safety confirmation)

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 6 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. In the results, the 6 investigated items were concluded to have no toxicity that is immediately detrimental to human health in the range of current use as additives.

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 so that confirmation of their safety is 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, in the Inoue Group Reports published in FY 2004, 2006, 2007 and 2008, there are 14, 7, 8 and 7 additives, respectively, which are not considered to have any toxicity may immediately affect human health to the extent that they are currently used as additives.

The present research aimed to investigate the basic safety of natural additives by collecting domestic and overseas study results and evaluating those results for 6 among the 28 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 28 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 deleted from the list of existing additives, this research individually evaluated the safety study results of 6 additives for which the required results of a 90-day or longer repeated-dose toxicity study and a mutagenicity study were available.

D. Results

Each study result for 6 additives whose safety has been reviewed in this research is summarized in Annex.
Regarding Olibanum and Peach gum, there was no study result that suggested an immediate effect on human health at present. Since actual use of Olibanum as an additive cannot be confirmed, the third deletion procedure is under way as a food to be deleted.

Regarding Copal resin, no findings suggesting transition to liver tumor or such were observed in a 90-day repeated dose study. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health in the range of use as an additive.

Regarding Roasted soybean extract, no toxicity findings based in genotoxicity were observed in the 1-year repeated dose toxicity study that was conducted based on the results of the genotoxicity test. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health in the range of use as an additive.

Regarding Enzymatically decomposed rutin, no findings suggesting toxicity to the hematopoietic system were observed in the 1-year repeated dose toxicity study conducted based on the observation of anemia in a 90-day repeated dose study. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health in the range of use as an additive.

E. Discussion

In this research, out of 28 items which are existing additives requiring safety confirmation in the Hayashi Team Report and have not been reviewed, regarding 6 items for which both repeated dose test results and mutagenicity test results of at least 90 days or more were available, as a result of evaluating these test results, there was thought to be no toxicity with immediate harmful effect on human health in any of these in their range of current use as additives.

The Ministry of Health, Labor and Welfare is currently conducting a third deletion procedure for existing additives that are not in actual use, following December 2004 and September 2007.

While the operation of reviewing existing additives is steadily proceeding in this manner, it is considered necessary to continue further investigation such as the actual usage status of existing additives, and to proceed with review efficiently from the required additives.

F. Conclusion

This research showed that the basic safety of 6 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.

Review on the Safety of Existing Additives (as of May 2010):

Annex

Copal resin

1. Food additive name:

Copal resin (obtained from secretion of copal, containing agatendicarboxylic acid as main component)

2. Origin, method of preparation, and definition:

Parts of low boiling point are removed by distillation from the stem secretions of Araucariaceae (*Agathis loranthifolia* SALISB), followed by extraction with ethanol at room temperature, and ethanol is distilled off from the filtrate. It consists mainly of agatendicarboxylic acid.

3. Major use:

Gum base

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in male and female F344 rats by dietary administration with at concentrations of 0.625, 1.25, 2.5, and 5%. It showed no animal deaths, and no changes were observed in general condition, body weight or food intake.

In hematological and the blood chemical test, significant difference was observed in sporadic test items, but toxicological significance was considered to be scant because dose correlation was not observed.

In organ weight, the absolute and relative weights of the liver increased in males of all groups and females at 1.25% or higher groups, and the absolute weight of the kidneys decreased in females of the 5% group, but no histopathological changes were observed in the liver or kidneys. In addition, effects on the weight of the brain, spleen, testes, heart, and ovaries were observed, but they were not accompanied by histopathological changes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, WP2*uvr*A/pKM101) was negative within the tested dose range, with or without metabolic activation. [2)]

A chromosomal aberration test in cultured mammalian cells (CHL) was considered not to show structural or numerical chromosomal aberrations, regardless of the presence or absence of metabolic activation. [3)]

In a micronucleus test using mice, micronucleus induction was not observed at any dose. [4)]

Therefore, it was concluded that genotoxicity is not observed.

(3) One-year repeated-dose toxicity study

In a 1-year repeated-dose toxicity test by dietary administration (0.2, 1.0, 5.0%) using F334 rats, there were no deaths or changes in general condition, body weight or food consumption related to administration of the test substance.

In hematological test, high platelet count, low values for Hb, Ht, MCV and MCH, and prolongation of APTT in both sexes, and low value for MCHC and prolongation of PT in males were observed in the 5.0% group, suggesting an anemic tendency.

In blood chemical test, high values for Ca in males and high values for TP in females at 0.2% or higher groups, high values for T-Cho and Alb in both sexes, high values for TP and low values for TG in males, and high values for Ca in females at 1.0% or higher groups, and high values for BUN, low values for Cl in both sexes, high values for T-Bil and low values for ALP in males, high values for γ-GPT and low values for blood glucose in females of the 5.0% group were observed. In protein fractions, in the 5.0% group, high values for β-globulin ratio in both sexes, and high values for α_1- and α_2-globulin ratios and low values for albumin ratio, γ-globulin ratio and A/G ratio in females were observed. The high values for Ca, K, TP and Alb and low values for Cl and ALP that were observed at 0.2% or higher groups were not considered to be a toxic effect, because no corresponding changes were observed histopathologically. In addition, the low TG values, high Ca values, and changes in protein fractions that were observed at 1.0% or higher groups were considered to have little toxicological significance because no related histopathological changes were observed.

In the organ weight of males, increased absolute/relative weight of the liver and absolute weight of the epididymis at 1.0% or higher groups, and decreased absolute weight of heart, and increased absolute/relative weight of the kidneys and testes and relative weight of the epididymis and seminal vesicle at 5.0% group were observed. In females, increased relative weight of the liver at 0.2% or higher groups, increased absolute weight of the liver at 1.0% or higher groups, and decreased absolute/relative weight of the adrenal gland were observed at 5.0% group. At necropsy, dark brown coloration and hypertrophy of the liver and dark brown coloration of the kidneys were observed in both sexes at 5.0% group. The changes in organ weight in the epididymis, heart, kidney, testis, seminal vesicle, and adrenal gland were judged not to be changes due to administration of the test substance because they did not involve histological changes. In addition, the change in the relative liver weight in females of the 0.2% group was not regarded as a toxic effect because there was no abnormality in the indices related to liver disorders.

In histopathological test, increases in centrilobular hepatocyte hypertrophy in the liver and brown pigmentation of the renal tubules in both sexes, increases in basophilic renal tubules and hyaline droplets in the kidneys in males, and intracellular brown pigment in the liver and mucosal necrosis of the fundus gland of the glandular stomach in females were observed in the 5.0% group. The brown pigment in the liver and kidney was confirmed by special staining to be due to deposition of lipofuscin.

In conclusion, the no observed adverse effect level is considered to be 0.2% in both sexes

(male: 96 mg/kg/day; female: 122 mg/kg/day). [5)]

(References)

1. Tanaka Takuji: Health and Labor Sciences Research Grant, Pathology I, Kanazawa Medical University
2. Matsushima Taijiro: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center
3. Iwamoto Tsuyoshi: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology
4. Ajimi Shozo: Health and Labour Sciences Research Grant, Chemicals Evaluation and Research Institute
5. Shiraishi Keiji: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Chemicals Evaluation and Research Institute

Sesame seed oil unsaponified matter

1. Food additive name:

Sesame seed oil unsaponified matter (with sesamolin obtained from sesame seeds as main component)

2 Origin, method of preparation, and definition:

It is obtained by extraction from the seeds or pressed oil cakes of the seeds of sesame (*Sesamum indicum* LINNE) of the Pedaliaceae family with ethanol. It consists mainly of sesamolin.

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 gavage (100, 300, and 1,000 mg/kg bw). It showed no animal death in any group, no abnormalities were found in general condition, weight changes or food consumption, and no changes considered to be attributable to the test substance were observed in urinalysis, ophthalmological test, hematological or histopathological test.

In blood chemical test in the 1,000 mg/kg group, the total cholesterol concentration and A/G ratio increased in males and γ-GTP activity increased in females. In organ weight, increases in absolute and relative liver weight were observed in both sexes of the 1,000 mg/kg group. Relative liver weight increased in females of the 300 mg/kg group, but because there were no changes in blood chemical or histopathological test, the toxicological significance was considered scant.

In conclusion, the no observed adverse effect level is considered to be 300 mg/kg/day in both sexes. [1)]

(2) Genotoxicity study

In the reverse mutation test using bacteria (TA98, TA100, TA1535, TA1537, WP2*uvrA*), increase in the number of revertant colonies with dose dependency in the presence of S9 mix was observed in the TA98 and TA1537 strains, and in TA100, although not more than twice that of the negative control, increase in the number of revertant colonies of 1.5-fold or more was observed. From the above, it was judged that induction of gene mutations (frame shift type) was positive. [2)]

A chromosomal aberration test using mammalian cultured cells (CHL/IU) was judged to be positive because dose-dependent induction of chromosome structural abnormalities was observed in short-term treatment regardless of the presence or absence of S9 mix. [3)]
A micronucleus test in mice (BDF_1, male) was performed up to the limit dose of 2,000 mg/kg×2, and was judged to be negative because the frequency of micronuclei showed no significant increase at any dose compared with the negative control group. [4)]

Using transgenic mice (Big Blue®), a genetic mutation test was performed by gavage at doses of 500 and 1,000 mg/kg repeatedly for 28 days. In the results, induction of genetic mutation in the liver and kidney was not observed at any dose. [5)]

Genetic mutation tests using human cultured cells (TK6) up to the maximum treatment concentrations of 0.070 mg/ml in the absence of S9 mix and 0.095 mg/ml in the presence of S9 mix, and no significant increase in the mutation rate was observed in either 4-hour treatment or 24-hour treatment. [6)]

From the above results, although positive results were obtained in the reverse mutation and chromosomal aberration tests, considering the results of *in vivo* micronucleus, mouse and gene mutation tests, it was concluded that there is no genotoxicity which is a particular problem *in vivo*.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center
2. Mochizuki Nobuhiko: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc. (Reverse Mutation Test of Sesame Seed Oil Unsaponified Matter using Bacteria), Biosafety Research Center
3. Mochizuki Nobuhiko: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc. (Chromosomal Aberration Test of Sesame Seed Oil Unsaponified Matter using Mammalian Cultured Cells), Biosafety Research Center
4. Mochizuki Nobuhiko: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc. (Micronucleus Test of Sesame Seed Oil Unsaponified Matter using Mice), Biosafety Research Center
5. Mochizuki Nobuhiko: FY 2007 Study on the Safety of Existing Additives (Genetic Mutation Test of Sesame Seed Oil Unsaponified Matter Using Transgenic Mice), Biosafety Research Center
6. Kojima Koichi: FY 2007 Study on the Safety of Existing Additives (Genetic Mutation Test Using Human Cultured Cells), Food and Drug Safety Center

Olibanum

1. Food additive name:

Olibanum (Containing α-boswellic acid and β-boswellic acid as main components, obtained from the secretions of frankincense.)

2. Origin, method of preparation, and definition:

Oleoresin which has been separated from the secretions of frankincense (*Boswellia frereana* BIRDW.) of Burseraceae family is extracted with hot ethanol, and the ethanol is distilled away. It consists mainly of α- and β-boswellic acid.

3. Major use:

Gum base

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in SD rats by dietary administration (0.2%, 0.5%, 1%). It showed no animal deaths, and no changes related to the test substance were observed in general condition, ophthalmological examination, or urinalysis in any group. However, suppressed body weight gain and decreased food consumption were observed in females in the 1% group.

In the results of hematological test, PLT was high and PT and APTT were prolonged in males, and APTT was prolonged in females in the 0.5% or higher groups.

Blood biochemical test revealed high T-Cho in males and females, high γ-GTP in males and high globulin fraction α2 in females in the 0.5% or higher groups, and high AST, ALT, Ca, and high globulin fraction α2 and β in males and high γ-GTP and low globulin fraction γ in females in the 1% group.

In organ weight, the relative weight of the liver was high in males and females in the 0.5% or higher groups, and the relative weight of the brain, thyroid, adrenal gland, and uterus was high in females in the 1% group.

Histopathological test revealed hypertrophy of the adrenal glomerulosa cells and vacuolation of the hepatic marginal zone hepatocytes in males and females, and multinucleation of hepatocytes and slight eosinophilic bodies in the renal tubular epithelial cells in males in the 1% group. In addition, slight vacuolation of proximal tubular epithelial cells was observed in females in 0.5% or higher groups, but since no other organic changes were observed, it was considered to be of little pathological significance.

In conclusion, the no observed adverse effect level was estimated to be 0.2% in both sexes (male: 113 mg/kg/day; female: 124 mg/kg/day). [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvrA*) was performed up to 500 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 concentrations of 2500 μg/mL (short-term treatment: +S9 mix), 1250 μg/mL (short-term treatment: -S9 mix), 625 μg/mL (continuous treatment, 24-hour

treatment) and 313 μg/mL (continuous treatment, 48-hour treatment). As a result, no induction of chromosomal aberration was observed under any treatment conditions. [3)]
A micronucleus test in mice (ICR, male) was performed up to 1,000 mg/kg × 2, and was judged to be negative because the frequency of micronuclei showed no significant increase at any dose compared with the negative control group. [4)]
Therefore, it was concluded that genotoxicity is not observed.

(References)

1. Murata Nozomu: FY 2007 Safety study of existing additives (90-day repeated dietary dose toxicity study of Olibanum in rats), BoZo Research Center Inc.
2. Oguma Yoshihiro: FY 2007 Safety study of existing additives (Bacterial reverse mutation test of Olibanum), BoZo Research Center Inc.
3. Sono Akira: FY 2007 Safety study of existing additives (Chromosomal aberration test of Olibanum in cultured mammalian cells), BoZo Research Center Inc.
4. Teramoto Shoji: FY 2007 Safety Study of Existing Additives (Micronucleus test (Olibanum)), Institute of Environmental Toxicology

Roasted soybean extract

1. Food additive name:

Roasted soybean extract (obtained from the seeds of soybeans; contains maltol as the major component.)

2. Origin, method of preparation, and definition:

It is obtained by degreasing and roasting soybean seeds, extraction with hot water and then removing proteins with hot ethanol. It contains maltol as a component.

3. Major use:

Food manufacturing agent

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 13-week repeated-dose test was performed in Wistar Hannover GALAS rats by administration in drinking water (1.25, 2.5, and 5%). It showed no animal deaths, and no changes caused by the test substance were observed in body weight, food intake, or water intake.

Hematological test revealed decreased WBC count in males in the 5% group, but since histopathological test revealed no abnormality in the hematopoietic system, it was not considered to be related to the test substance.

Serum biochemical test revealed decreased ALB in males in the 5% group, but the range of variation was small, and it was not considered to be toxicologically significant.

In organ weight measurement, changes in pituitary gland, thyroid gland, kidney, and liver weight were observed, but there were no histological changes, and therefore these were considered to be incidental changes.

In conclusion, the no observed adverse effect level was considered to be 5% in both sexes (male: 3.41 g/kg/day; female: 5.40 g/kg/day). [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 the results were negative with and without metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) at the maximum treatment concentration of 5 mg/mL, resulting in negative results by short-term treatment regardless of the presence or absence of metabolic activation. However, structural chromosomal aberrations were observed at the highest dose of 5 mg/mL in continuous treatment. In the *in vitro* micronucleus test in cultured mammalian cells (CHL/IU) in the absence of metabolic activation, micronuclei were observed in all treatment groups. Although all positive responses were observed only at the highest dose of 5 mg/mL, they were concluded to be mutagenic due to their high frequency of induction and their reproducibility. [3)]

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

From the above results, although positive results were obtained in the chromosomal

aberration test, considering the negative results of the *in vivo* micronucleus test, which was conducted at sufficiently high doses, it was concluded that there is no genotoxicity which is a particular problem *in vivo*.

(3) One-year repeated-dose toxicity study

In a 1-year repeated dose toxicity test in F334 rats by administration in drinking water (0.63, 1.25, 2.5, 5%), no animal deaths or changes in general condition were observed, and urinalysis showed no changes attributable to the test substance.

In body weight, food consumption, and water consumption, suppressed body weight gain was observed in females in the 2.5% or higher groups and males in the 5% or higher groups, a tendency of decreased food consumption in males and females in the 2.5% or higher groups, and a tendency of decreased water consumption in females.

Hematological test revealed increased Ht and MCV and decreased MCHC in males in the 5% group. Blood biochemical test showed increased Na in males in the 2.5% or higher groups, and decreased ALP, BUN, and K in males, and decreased TP, Alb, and T-Cho, and increased γ-GTP and Ca in females in the 5% group. However, these changes were considered to be of little toxicological significance because they were very slight and there were no other related changes such as pathological findings.

In organ weight, decrease in absolute weight of uterus and increase in relative weight of brain were observed in females of the 2.5% or higher groups, and in the 5% group, increase in absolute/relative weight of brain, decrease in absolute weight of liver, and increase in relative weight of kidney in males, and decrease in absolute weight of liver, and increases in relative weight of brain, kidney and ovary in females were observed. However, these changes were considered to be of no toxicological significance because they were very slight and not associated with histopathological changes.

Histopathological test revealed no findings that suggested effects of the test substance.

In conclusion, the no observed adverse effect level was considered to be 2.5% (2.17 g/kg/day) in males and 1.25% (1.32 g/kg/day) in females. [5)]

A One-year repeated-dose toxicity test in transgenic mice (*Revl* homo mice) has been reported, but this test was not included in the determination of NOAEL as it was a test using special genetically modified mice.

(References)

1. Mitsumori Kunitoshi: FY 2001 Study on the Preparation of Standards and Criteria for Food Additives, Faculty of Agriculture, Tokyo University of Agriculture and Technology
2. Ikka Tsuguo: FY 2005 Study on Standards and Criteria for Food and Food Additives, Safety Research Institute for Chemical Compounds
3. Makoto Hayashi: FY 1991 Study on the Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Health Sciences
4. Hachiya Noriyuki: FY 1999 Food Additive Safety Reevaluation Study, Akita University School of Medicine
5. Kenji Kamiya: Health and Labour Sciences Research Grant "Safety evaluation study on carcinogenicity, of existing additives - Development of model animals and search

for carcinogenicity of roasted soybean extract -", Research Institute for Radiation Biology and Medicine, Hiroshima University

Peach gum

1. Food additive name:
Peach gum (obtained from peach secretions; contains polysaccharides as the major component)

2. Origin, method of preparation, and definition:
It is obtained by separating the resin components of the main branch of the peach (P*runus persica* BATSCH) of Rosaceae family. 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%, 5.0%). It showed no death in any group of animals, and there were no toxicologically significant changes in general condition, body weight, food intake, water intake, ophthalmic examination, urinalysis, or hematological test that were considered to be caused by the test substance.

Blood biochemical test showed increased BUN in males in the 5% group but was considered to be of little toxicological significance because no abnormality was observed in histopathological examination.

In organ weight, increased relative testis weight was observed in males of the 5.0% group, but since no abnormality was observed in histopathological examination, it was considered to be of little toxicological significance.

Histopathological test revealed no obvious effects of the test substance on other organs or tissues.

In conclusion, the no observed adverse effect level was considered to be 5.0% in both sexes (male: 2,920 mg/kg/day; female: 3,465 mg/kg/day). [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvrA*) 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) up 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 using bone marrow of mice (BDF_1, male) was performed up to the limit dose of 2,000 mg/kg × 2, and was judged to be negative because the frequency of micronuclei showed no significant increase at any dose compared with the negative control group. [4)]

Therefore, it was concluded that genotoxicity is not observed *in vivo*.

(References)

1. Tamano Seiko: FY 2007 Safety Study of Existing Additives (90-day repeated-dose toxicity study of Peach gum), DIMS Institute of Medical Science, Inc.
2. Yamauchi Hisami: FY 2007 Safety Study of Existing Additives (Bacterial Reverse Mutation Test of Peach Gum), BoZo Research Center Inc.
3. Sono Akira: FY 2007 Safety Study of Existing Additives (Chromosomal Aberration Test of Peach Gum in Cultured Mammalian Cells), BoZo Research Center Inc.
4. Mochizuki Nobuhiko: FY 2007 Study on Standards and Criteria for Food and Food Additives (Micronucleus Test of Peach Gum in Mice), Biosafety Research Center

Enzymatically decomposed rutin

1. Food additive name:

Enzymatically decomposed rutin (It is obtained from "rutin (extracts)" and contains isoquercitrin as the main component.)

2. Origin, method of preparation, and definition:

"Rutin (extracts)" is obtained by purification after treatment with enzymes (naringinase, hesperidinase, or rhamnosidase). It consists mainly of isoquercitrin.

3. Major use:

Antioxidant

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose oral test was performed in Wistar rats by dietary treatment with the test substance at concentrations of 0.2, 1.0, and 5.0%. The result showed suppressed body weight gain and decreases in hemoglobin, hematocrit, and triglyceride, which were considered to be caused by enzymatically decomposed rutin, were observed in males of the 5.0% group, but there was no obvious toxic effect attributable to administration of the test substance in female rats. In conclusion, the no observed adverse effect level was estimated to be 1.0% (539 mg/kg/day) in males and 5.0% (3,227 mg/kg/day) in females. [1)]

(2) Genotoxicity study

In the bacterial reverse mutation test (TA98, TA100, TA1535, TA1537, TA1538), induction of His+ revertant colonies in TA98 and TA1537 was at least twice that in the vehicle control group with or without metabolic activation, and they were judged to be positive because concentration dependence and reproducibility were observed. [2)]

In the chromosomal aberration test in cultured mammalian cells (CHL), administration of enzymatically decomposed rutin did not clearly induce chromosomal aberrations in either short-term or continuous treatment methods. [3)]

In a micronucleus assay using mouse bone marrow, it was concluded that there was no micronucleus induction at any dose. [4)]

From the above, although positive results were obtained in the reverse mutation test using bacteria, from the comprehensive evaluation as negative in *in vivo* micronucleus tests at sufficiently high doses, this substance is not thought to have genotoxicity which becomes a particular problem *in vivo*.

(3) One-year repeated-dose toxicity study

In a one-year repeated-dose toxicity test by dietary administration (0.04, 0.2, 1, 5%) using Wistar Hannover rats, there were no animal deaths or effects on hematological test, blood biochemical test, or organ weight related to administration of the test substance. No change in body weight was observed, but food consumption was high in males in the 5% group.

In general condition, chromaturia was observed in males and females in the 5% group. Urinalysis showed dark urine in males and females in the 1% and 5% groups, increased daily calcium excretion in males and females and increased urinary calcium concentration

in males in the 5% group, and increased urinary potassium and chloride concentrations in males in the 1% group, with increased daily excretion of these in males in the 1% or higher groups.

Necropsy revealed yellow discoloration of the femoral surface, suggesting deposition of the test substance, in males in the 1% group and males and females in the 5% group.

Histopathological test in males in the 5% group revealed mineralization of the renal pelvis, and inflammatory cell debris, inflammatory cell infiltration, and transitional cell hyperplasia in the renal pelvis, which were considered to be changes related to mineralization, tended to increase.

Based on the above, the NOAEL was estimated to be 1% in males and females (male: 634 mg/kg/day; female: 788 mg/kg/day for females), considering increased daily calcium excretion in males and females in the 5% group, and increased urinary calcium concentration and mineral deposition in the renal pelvis in males in the 5% group as toxicological findings. [5)]

(References)

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