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# Research report

# Research on the safety re-evaluation of existing additives

FY 2008

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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20 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 1, 2009
Department of Food Safety, Pharmaceutical and Food Safety Bureau

Research on the safety re-evaluation of existing additives (FY 2008 Survey)

Research on the safety re-evaluation of existing additives
(September 3, 2009, 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 3, 2009.

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## Research report

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

## March 2009


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## A. Summary

"Research on the safety evaluation of existing additives" (Senior Researcher: Hayashi Yuzo) (hereinafter, referred to as the "Hayashi Group Report") in Health Sciences Research Report FY 1996.

In this study, an investigation was performed on food additives for which safety study results were newly available, including 35 additives among the 139 additives that had been considered to require further investigation regarding safety in the Hayashi Group Report, excluding the following additives reported:

- "Research on the safety evaluation of existing additives" reported in FY 1999 (Senior Researcher: Kurokawa Yuji) (hereinafter, referred to as the "Kurokawa Group Report")
- "Research on the safety re-evaluation of existing additives" reported in FY 2003 (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2003 Inoue Group Report")
- "Research on the safety re-evaluation of existing additives" reported in FY 2004 (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2004 Inoue Group Report")
- "Research on the safety re-evaluation of existing additives" reported in FY 2006 (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2006 Inoue Group Report")
- "Research on the safety re-evaluation of existing additives" reported in FY 2007 (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2007 Inoue Group Report")
- The additives that were previously deleted from the list of existing additives (46 additives are considered to require safety confirmation)

In this report, the results of investigation of the seven additives Catechin, Jamaica quassia extract, Soybean saponin, Tocotrienol, Roasted rice bran extract, Ferulic acid and Gallic acid are summarized.

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 7 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. The seven additives evaluated were not considered to have toxicity that would cause adverse effects on human health to the extent that they are currently used 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 included on the list of existing additives have not been checked individually for safety, therefore, confirmation of their safety is requested in the Diet, etc.

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 and 2007, regarding 14, 7 and 8 additives, respectively, it was also stated that "There are no study results suggesting any immediate adverse effect on human health for these additives for which safety was reviewed in this study."

The present research aimed to investigate the basic safety of natural additives by collecting domestic and overseas study results and evaluating those results for 35 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 deleted from the list of existing additives.

## C. Methods

Among 35 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 7 additives for which the required results of a 90-day or longer repeated-dose toxicity study and a mutagenicity study were available.

## D. Results

Of the 7 additives whose safety has been reviewed in this research, 5 additives except Soybean saponin and Roasted rice bran extract, based on the results of 90-day repeated dose and mutagenicity tests, 1-year repeated dose study and other additional test results were obtained. Each study result is summarized in the Annex.

Regarding Roasted rice bran extract and Soybean saponin, there was no study result that suggested an immediate effect on human health at present.

Regarding Catechin, no findings suggestive of toxicity to the liver or thyroid were found in a 1-year repeated dose toxicity/carcinogenicity study conducted based on the results of a mutagenicity test and a 90-day repeated dose study in which the weight of organs such as thyroid gland increased. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health.

Regarding Jamaica quassia extract, in a 1-year repeated dose toxicity study conducted based on the results of a medium-term hepatic carcinogenicity study, chronic hyperplasia of the thyroid follicular epithelium, thought to be caused by liver enzyme induction, was observed, and carcinogenesis promoting action on the liver by large doses was suggested, but safety within the non-toxic range was confirmed. Given the estimated intake (0.0016 mg/person/day) based on the production survey (FY 2007 Health and Labor Sciences Research Report), it was thought that it would not immediately have a harmful effect on human health within the range currently used as an additive.

Regarding Tocotrienol, in a Hershberger test, uterine hypertrophy test and one-year repeated dose toxicity/carcinogenicity study conducted based on the observation of increased testicle weight and decreased ovary/uterine weight in the 90-day repeated dose study, increased hepatocellular nodular hyperplasia and mild increase of adenomas were observed in high dose group of the 1-year repeated dose toxicity/carcinogenicity study, but there was thought to be no immediate harmful effect on human health within the range currently used as an additive, based on the degree and production survey (FY 2007 Health and Labor Sciences Research Report) intake estimate (1.69 mg/person/day).

Regarding Ferulic acid, no findings suggesting toxicity to the testes were observed in the 1-year repeated dose toxicity/carcinogenicity study conducted based on the observation of degenerative seminiferous epithelium in the testes in a 90-day repeat administration 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.

Regarding Gallic acid, no findings suggesting toxicity to the testes were observed in the 90-day repeated dose study or the 1-year repeated dose toxicity study conducted based on the fact that increased testes weight was observed in the 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.

## E. Discussion

In this research, the study results for safety evaluation were collected for the 35 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 7 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 was thought to be no toxicity with immediate harmful effect on human health for any of these additives in their current range of use as additives.

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 that were not actually used in December 2004, and 32 additives in September 2007.

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

The basic safety of 7 additional natural additives was confirmed in this research. 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**

## Catechin

### 1. Food additive name:

Catechin

### 2. Origin, method of preparation, and definition:

After dry distillation from the stems or leaves of tea (*Camellia sinensis* O.KZE.) of the Theaceae family, the branches of a pegu-catechu (*Acacia catechu* WILLD.) of the Leguminosae family, or the branches or leaves of the gambir (*Uncaria gambir* ROXBURGH) of the Rubiaceae family, Catechin is obtained by extraction with water or ethanol and purification, or by extraction with hot water and then partitioning with methanol or ethyl acetate. It contains catechins.

### 3. Major use:

Antioxidant

### 4. Summary of safety study results:

(1) 90-day repeated-dose study

In a 90-day repeated-dose test by dietary administration (0.3, 1.25, 5.0%) using F344/DuCrj rats, in males of the 5% group, suppressed weight gain, high values for serum ALT and ALP, and increased relative weight of the liver and kidney were observed. The relative weight of the thyroid gland decreased at 1.25% or higher groups, and serum T-Cho decreased at 0.3% or higher groups, but the toxicological significance was considered to be scant. In females of the 5% group, elevated serum AST, ALT and ALP and increased relative weights of the liver, kidney and thyroid were observed, and the weight of the thyroid gland was mildly increased at 1.25% or higher groups.
The no observed effect level was considered to be less than 0.3% (179.9 mg/kg/day) in males and 0.3% (188.5 mg/kg/day) in females, and the nontoxic dose was 1.25% in both sexes (763.9 mg/kg/day in males, 820.1 mg/kg/day in females). [1)]

(2) Genotoxicity study

In the reverse mutation test using bacteria, a 3.2-fold increase in revertant mutant colonies was induced in the TA98 strain in the presence of S9mix, and it was judged to be positive because it showed concentration dependence. [2), 3)]

In the chromosome aberration test using cultured mammalian cells, 72-hour continuous treatment and short-term treatment without addition of S9 mix were positive. [2), 4)]

In the micronucleus test using bone marrow of mice (ICR strain, males), no significant increase in the frequency of polychromatic erythrocytes with micronuclei was found at any dose tested up to the limit dose of 2,000 mg/kgx2, and it was judged to be negative because there was no significant reduction in the proportion of polychromatic

erythrocytes to total erythrocytes. [2), 5)]

From the above results, although genotoxicity was shown *in vitro*, considering the results of *in vivo* bone marrow micronucleus and carcinogenicity tests, it was concluded that there is no genotoxicity which is a particular problem *in vivo*.

(3) 1-year repeated-dose toxicity/carcinogenicity study

In a 1-year repeated-dose toxicity test by dietary administration (0.02, 0.3, 1.0, 3.0%) using Wistar Hannover rats, there were no deaths or effects on general condition related to administration of the test substance. There was a tendency of suppressed body weight gain in females of the 3.0% group. In hematological test, monocytes increased in females of the 3.0% group. In blood chemical test, the A/G ratio increased in females of the 3.0% group. In organ weight, an increase in relative liver weight was observed in males of the 3.0% group. Histopathological test revealed an increase in hypertrophy of centrilobular hepatocytes in males of the 3.0% group, and when induction of cytochrome P450 enzyme (CYP) in the liver was confirmed by immunohistochemical staining, enhancement of CYP3A2 expression that was consistent with hepatocyte hypertrophy in the same group was observed. No increase in hepatocyte proliferation activity or pre-hepatic cancer lesion (GST-P positive foci) was observed.

In a 2-year carcinogenicity test by dietary administration (0.02, 0.3, 1.0, 3.0%) using Wistar Hannover rats, there were no deaths or effects on general condition related to administration of the test substance. There was a tendency of suppressed body weight gain in males and females of the 3.0% group. In the measurement of liver weight, no increase in the actual or relative weight was observed. Histopathological examination revealed an increase in hepatocyte hypertrophy in males of the 3.0% group. Regarding tumorigenesis, no increase related to the administration of the test substance was observed in comparison between the control group and the 3.0% group, and neither the early development of tumors nor the enhancement of malignancy was observed.

Suppressed body weight gain in the 3.0% group in both studies was thought to result from nutritional deficiency due to long-term feeding with the high concentration diet containing 3.0%, since no other apparent toxicological findings related to administration were observed in other test items. Changes in the liver of males in the 3.0% group were not finding suggestive of hepatic injury, and since the expression of CYP3A2 increased, it was thought that they were adaptive changes due to induction of drug metabolizing enzymes, not toxic changes.

In conclusion, the no observed effect level was considered to be 1.0% (416.44 mg/kg/day) in males and 3.0% (1539.80 mg/kg/day) in females, and the nontoxic dose was 3.0% in both sexes (1265.77 mg/kg/day in males, 1539.80 mg/kg/day in females). [6)]

**(References)**


1. Hirose Masao: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Director, Division of Pathology, National Institute of Health Sciences
2. Hayashi Makoto: The Datasheet of Mutagenicity Assessment of Food Additives by the Welfare Ministry, etc. (FY 1979 - 1998)
3. Miyabe Masaki: FY 1995 Study on the Safety Evaluation, etc. of Food Additives, Nagoya City Public Health Research Institute

4. Sofuni Toshio: FY 1995 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, Biological Safety Research Center, National Institute of Hygienic Sciences
5. Kurita Toshishiro: FY 1995 Study on the Reevaluation, etc. of the Safety of Food Additives, The Institute of Environmental Toxicology
6. Nakae Dai: FY 2005 Health and Labor Sciences Research Grant, Studies on Carcinogenicity of Natural Additives

## Jamaica quassia extract

### 1. Food additive name:

Jamaican quassia extract (obtained from the trunks or barks of Jamaica quassia, with quassin and neoquassin as main components.)

### 2. Origin, method of preparation, and definition:

It is obtained by extraction with water from the trunks and branches or tree barks of Jamaica cassia (*Quassia excelsa* SW.) of the Simaroubaceae family. Active ingredients are quassin and neoquassin.

### 3. Major use:

Bitterant, etc.

### 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 with the test substance at concentrations of 0.005, 0.05, and 0.5%. As a result, no animal deaths were observed, and no changes were observed in general condition, food intake, or body weight.

In the blood chemical test, an increase in γ-GTP was observed in females of the 0.5% group, suggesting an effect on the liver.

In protein and non-protein nitrogen, TP and Alb increased in both sexes of the 0.5% group. In lipids, TG decreased in males of the 0.5% group and T-Cho increased in females of the 0.5% group. In electrolytes, Ca increased in both sexes of the 0.5% group and P increased in females of the 0.5% group. Among these, the changes in TP, Alb, Ca and P are considered to be within the normal range, and although statistically significant differences were observed, they were not considered to be toxicologically meaningful.

In organ weight, increases in absolute and relative liver weight were observed in both sexes of the 0.5% group. Increase in the relative weight of the kidney was observed in males, but no change suggestive of kidney damage was observed in other tests including histopathological test.

Histopathological test revealed hypertrophy of hepatocytes and hyperplasia of thyroid follicular cells in both sexes of the 0.5% group.

In conclusion, the no observed adverse effect level is considered to be 0.05% in both sexes (male: 27.9 mg/kg/day; female: 30.1 mg/kg/day). [1)]

(2) Genotoxicity study

In a reverse mutagenicity test using bacteria (*S. typhimurium* TA98, TA100, TA1535,

TA1537 and *E. coli* WP2*uvr*A/pKM101), TA98, TA100, TA1537 and WP2*uvr*A/pKM101 were positive in the presence of S9 mix. [2)]

In a chromosome aberration test using mammalian cultured cells (CHL/IU), dose-dependent induction of chromosome structural abnormalities was observed by the short-term treatment method (- and +S9 mix). [3)]

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 induction was observed at any dose. [4)]

Using male Sprague-Dawley SPF rats, a single gavage dose of the test substance was administered at 500 and 2,000 mg/kg, and an *in vivo* rat liver unscheduled DNA synthesis test (liver UDS test) was performed. As a result, no significant increase in the uptake of radioactive thymidine was observed in either the long-term treatment and the short-term treatment as compared with the negative control group. Therefore, it was concluded that there was no DNA damage in the rat liver cells (negative). [5)]

From the above results, although genotoxicity was shown *in vitro*, considering the results of *in vivo* bone marrow micronucleus and unscheduled DNA synthesis tests, it was concluded that there is no genotoxicity which is a particular problem *in vivo*.

(3) Medium-term liver carcinogenicity test

Using F344 rats, a single intraperitoneal dose of diethylnitrosamine was administered at 200 mg/kg, dietary administration of the test substance at doses of 0.05, 0.5 and 3.0% was started at week 2, and partial resection of the liver was performed at week 3. Administration was continued, and in the results of sacrifice and necropsy at week 8, in the 3.0% group as with the positive control phenobarbital, the number and area of hepatocellular foci that were positive for glutahione S-transferase placental form enzymatic mutation showed increase, and although there was no significant difference, a dose-dependent trend was seen even at the lower dose of 0.5%.

The above suggested that there is a carcinogenesis promoting effect on the rat liver at high doses. [6)]

(4) One-year repeated-dose toxicity study

A 1-year repeated-dose toxicity test was performed in F344 rats by dietary administration with the test substance at concentrations of 0.0005, 0.005, 0.05, 0.5%. In the results, 2 deaths were observed in females in the 0.5% group, but otherwise, no changes were observed in general condition, body weight or food intake. In the 0.0005 and 0.005% groups, no effect by administration of the test substance was observed.

Hematological test revealed decreases in Hb, Ht, MCV and MCH in both sexes in the 0.5% group.

In blood chemical test, increased TP and Alb in males of the the 0.05% group, and increased TP and Alb and decreased ALP and T-Bil in both sexes, increased Glc, T-Cho, PL and γ-GTP in females, and increased TG and decreased ChE in males of the 0.5% group were observed.

Urinalysis showed an increase in protein in females of the 0.05% group and above and in males of the 0.5% group.

In organ weight, increased liver weight in males of the 0.05% group, and increased weight of the liver, kidneys, adrenal gland and thyroid in both sexes, and increased heart weight and relative lung weight and decreased relative spleen weight in females of the 0.5% group were observed.

Histopathological test revealed hepatocyte hypertrophy in males of the 0.05% group, and hepatocyte hypertrophy, chronic progressive nephropathy and chronic hyperplasia of the thyroid follicular epithelium in both sexes, and increased incidence (number of animals) and size of mutant hepatocellular foci, and mild hyperactivation of erythroblast extramedullary hematopoiesis in the spleen in males of the 0.5% group In the females in the 0.5% group that died, nephroblastoma was found in the kidney of animal necropsied in week 33, and pheochromocytoma was observed in the adrenal glands in the animal died in week 51.

In conclusion, the no observed adverse effect level was estimated to be 0.005% in both sexes (male: 2.1±0.6 mg/kg/day; female: 2.5±0.6 mg/kg/day). In addition, the incidence and size of mutant hepatocellular foci were increased in males of the 0.5% group, which suggested the possibility that carcinogenesis is promoted in the liver by large doses. [7)]

**(References)**


1. Sekita Kiyoshi: Health and Labor Sciences Research Grant, Product Safety Research Center, National Institute of Health Sciences
2. Kojima Akinori: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Mochizuki Nobuhiko: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, 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
5. Ono Hiroshi: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Hatano Research Institute, Food and Drug Safety Center
6. Hirose Masao: FY 2004 Study on the Reevaluation of the Safety of Food Additives, Division of Pathology, National Institute of Health Sciences
7. Sekita Kiyoshi: FY 2005 Health and Labor Sciences Research Grant, Studies on Carcinogenicity of Existing Additives

## Soybean saponin

### 1. Food additive name:

Soybean saponin (obtained from the seeds of soybeans; contains saponin as the major component.)

### 2. Origin, method of preparation, and definition:

It is obtained by pulverizing the seeds of soybean (*Glycine max* MERRILL) of the Leguminosae family, extracting with water or ethanol, and purifying it. It consists mainly of saponins (such as soyasaponin).

### 3. Major use:

Emulsifier

### 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 with the test substance at concentrations of 1.25, 2.5, and 5%. In the results, death of animals was not observed, and although significant suppression of body weight gain was observed compared to the control group in males of the 5% group and females of the 2.5% and 5% groups from week 2 of administration, and in females of the 1.25% group from week 11, no change in intake was observed.

In hematological test, RBC and Ht were significantly low and MCV was significantly high in males of the 5% group, suggesting an anemic tendency.

In serum chemical test, BUN increased in males at 5% group and in females at 2.5% or higher groups, and the relative weight of the kidney increased in males at 5% group and in females at 1.25% or higher groups, and although effects of administration were suggested, histopathological changes were not observed. TP and Alb showed significantly high values in males at 2.5% or higher groups, and TG decreased significantly in females at 1.25% or higher groups.

In the liver weight, the relative weight increased in males at 1.25% or more, and in females at 2.5% or more, and although it was considered to be an effect of administration, there was no histopathological change.

In addition, atrophy of the ventral prostate lobe was seen in all males of the 5% group, and enhanced vaginal mucus production with atrophy of the epithelium, and increased atretic follicles in the ovaries were observed in females at 2.5% or more.

In conclusion, the no observed adverse effect level in both sexes is considered to be less than 1.25% (male: 707.2 mg/kg/day; female: 751.8 mg/kg/day). [1)]

(2) Genotoxicity study

The reversion mutation test using bacteria has been reported as negative, the chromosomal aberration test using mammalian cultured cells (CHL/IU) as false positive, and the *in vivo* micronucleus test (mouse bone marrow) as negative. The result of the chromosomal aberration test is thought to be a nonspecific result dependent on cytotoxicity. [2) 3 )4 )5)] The *in vitro* micronucleus test was also reported to be negative. [2)]

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

**(References)**


1. Hirose Masao: FY 2004 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Division of Pathology, National Institute of Health Sciences
2. Hayashi Makoto: The Datasheet of Mutagenicity Assessment of Food Additives by the Welfare Ministry, etc. (FY 1979 - 1998)
3. Miyabe Masaki: FY 1996 Study on the Safety Re-evaluation, etc. of Food Additives, Nagoya City Public Health Research Institute
4. Sofuni Toshio: FY 1996 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, Safety Biological Research Center, National Institute of Hygienic Sciences
5. Hachiya Noriyuki: FY 1996 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Akita University Faculty of Medicine

## Tocotrienol

### 1. Food additive name:

Tocotrienol

### 2. Origin, method of preparation, and definition:

Tocotrienol is obtained from rice bran oil of rice (*Oryza sativa* LINNE) of the Poaceae family, palm oil, etc. of oil palm (*Elaeis guineensis* JACQ.) of the Arecaceae family by separation. It contains tocotrienol.

### 3. Major use:

Antioxidant

### 4. Summary of safety study results:

(1) Repeated-dose study

In a 90-day repeated dose test by dietary administration (0.19, 0.75, 3%) using F344 rats, in hematological test, decrease in MCH was observed in males of the 3% group, decrease in Hb and MCHC in females of the 3 and 0.75% groups, and decrease in Ht in females of the 3% group. In blood chemical test, increase in ALT was observed in males and females of the 3% group, increase in AST and γ-GT was observed in females of the 3% group, and histopathologically, hepatocyte hypertrophy was observed in males of the 0.75% group or more. In 3% group, increase in testis weight and decrease in ovary and uterus weight were observed.

Since anemic tendency was observed in females at 0.75% or more, and hepatocyte hypertrophy was observed in males at 0.75% or more, the nontoxic dose was considered to be 0.19% (male: 119.0 mg/kg/day, female: 129.8 mg/kg/day). Although decreased MCV hematological examination of males, increased A/G and ALP in serum chemical test, and increased adrenal weight were mild, the no observed effect level could be obtained in this study since they were also observed in the 0.19% administered group. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, 100, 1535, 1537 and 1538) was performed up to 50 mg/plate, and was negative with or without S9 mix. [2)]

In a chromosome aberration test using mammalian cultured cells (CHL) performed up to 5,000 μg/mL, no clear induction of chromosomal abnormality by tocotrienol treatment was observed in either continuous treatment and the short-term treatment, and it was negative. [3)]

In a micronucleus test using mice performed up to 3,000 mg/kg×2, exceeding the limit dose, the frequency of micronucleus-containing polychromatic erythrocytes was not significant compared with the negative control group, and it was negative. [4)]

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

(3) Hershberger test

Using Sprague-Dawley rats with testes removed, the test substance was subcutaneously or orally administered at doses of 100, 300 and 1,000 mg/kg/day in order to investigate the androgenic effect. In order to examine the anti-androgen effect, testosterone propionate was subcutaneously administered at a dose of 0.4 mg/kg/day, and at the same time the test substance was subcutaneously or orally administered at doses of 100, 300 and 1,000 mg/kg/day. In each of the tests, administration was repeated for 10 days, and the animals were sacrificed, and organ weight was measured about 24 hours after the final administration.

In the results of subcutaneous administration, no deaths or abnormalities of the general condition were observed in any administration group, but decreased food intake was observed in the 1,000 mg/kg group (androgenic effect test), and suppression of body weight gain and decreased food consumption were observed in the 1,000 mg/kg group (antiandrogen effect test). In the organ weight, increased liver weight was observed in the 1,000 mg/kg group in both tests, but no significant change suggesting androgenic effect or antiandrogen effect was observed.

In oral administration, no death was observed in any dose group, and no change was observed in general condition, body weight or food consumption. In organ weight, no significant change suggesting an androgenic effect or antiandrogenic effect was observed in any of the organs measured.

From the above results, it was judged that tocotrienol does not show androgenic effect or antiandrogen effect *in vivo* by subcutaneous or oral administration. [5)]

(4) Uterotrophic response test

Using Sprague-Dawley rats with ovaries removed, the test substance was subcutaneously or orally administered at doses of 30, 100, 300 and 1,000 mg/kg/day in order to investigate the estrogenic effect. In order to examine the antiestrogenic effect, ethinyl estradiol was subcutaneously administered at a dose of 0.6 μg/kg/day, and at the same time the test substance was subcutaneously or orally administered at doses of 100, 300 and 1,000 mg/kg/day. In each of the tests, administration was repeated for 7 days, and the animals were sacrificed, and uterus weight were measured about 24 hours after the final administration.

In the results of subcutaneous and oral administration, no death was observed in any dose group, and no change was observed in general condition or body weight. In the uterus weight, no significant change suggesting an estrogenic effect or antiestrogenic effect was observed in any dose group.

From the above results, it was judged that tocotrienol does not show estrogenic effect or antiestrogenic effect *in vivo* by subcutaneous or oral administration. [5)]

(5) 1-year repeated-dose toxicity/carcinogenicity study

In a 1-year repeated dose toxicity test by dietary administration (0.08, 0.4, 2.0%) using Wistar Hannover rats, 6 males died in the 2.0% group, but no death or abnormality in general condition was observed in the other groups. In hematological test, males showed decreased MCV at 0.4% or more, and significant decreases in Hb, Ht and MCH and prolongation of prothrombin time at 2.0% group, and females showed decreases in Hb, Ht, MCV and MCH and increase in MCHC at 2.0% group. In blood chemical test, males showed decreased TG and glucose and increased Na and Cl at 0.4% or more, increased A/G ratio, P, AST, ALT, ALP, direct Bil and prothrombin time and decreased LDH at 2.0% group, and decreased cholesterol ester ratio in all dose groups, and females showed increased TP and ALP and decreased total Bil, direct Bil and indirect Bil at 2.0% group. In organ weight, in the 2.0% group the relative weight of the brain, lung, heart, adrenal gland, kidneys and testes increased in males, the relative weight of the brain, heart, liver, adrenal gland and kidneys increased in females. Histopathological test revealed high frequency of hepatocellular nodular hyperplasia and spongiform degeneration in the liver and focal aggregation of foam cells in the alveoli in males and females in the 2.0% group. At necropsy of the 6 animals that died, hemorrhage in the base of the brain and mesenteric lymph nodes, and liver spongiform degeneration in all cases were observed, and hemorrhagic foci were observed in the thoracic lymph nodes, subendocardium, submucosa of the bladder, etc.

In conclusion, the no observed adverse effect level was estimated to be 0.4% in both sexes (male: 297 mg/kg/day; female: 467 mg/kg/day).

In the 2-year carcinogenicity test using Wistar Hannover rats by dietary administration (0.4, 2.0%), deaths increased in males in the 2.0% group, and therefore the dose was reduced to 1.0% from week 50 and the study was continued. In males, there were no differences between groups in the final body weight or organ weight. In females, the final body weight showed a dose-related low value, and dose-related decrease in the absolute weight of the kidneys, dose-related increases in the relative weight of the lungs and heart, and in the high dose (2.0 → 1.0%) group, decreases in the absolute weight of the lungs, heart and spleen and increase in the relative weight of the liver and kidneys were observed. At necropsy, multiple nodular lesions were observed in the liver of males and females in the high dose group, and histopathologically, they were confirmed to be hepatocellular nodular hyperplasia, hepatocellular adenoma or hepatocellular carcinoma. In the high dose group, increased occurrence of hepatocellular nodular hyperplasia in both sexes and slight increase in hepatocellular adenoma in females were observed. [6)]

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etc.

## Roasted rice bran extract (325)

### 1. Food additive name:

Roasted rice bran extract

### 2. Origin, method of preparation, and definition:

Rice bran of rice (*Oryza sativa* LINNE) of the Poaceae family is defatted, roasted and extracted with hot water, then proteins are removed with ethanol while warm. It contains maltol.

### 3. Major use:

Food manufacturing agent

### 4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose test was performed in F344 male and female rats by dietary administration (0.5, 1.5 and 5.0%). It showed no animal deaths, and no changes related to the test substance were observed in general condition, body weight, food intake, hematological test or organ weight in any groups.

In blood chemical test, A/G increased in females of the 5.0% group, but since the degree of change was small and no change was observed in the other items, the change was judged not to be related to the administration of the test substance.

Histopathological test revealed slight or mild necrosis of hepatocytes in males at 5.0% group, and slight necrosis of the salivary glands, mineral deposits in renal tubules in the cortico-medullary junction of the kidney, and mild dilatation of the uterine lumen in females at 5.0% group, but since the frequency of occurrence of these findings was low and no statistical difference from the control group was observed, it was judged that it was not a change related to the administration of the test substance.

In conclusion, the no observed adverse effect level was judged to be 5.0% in both sexes (male: 2,893 mg/kg/day; female: 3,096 mg/kg/day). [1)]

(2) Genotoxicity study

Reverse mutation tests using bacteria (TA98, TA100, TA1535, TA1537, TA1538) were performed up to 200 μl/plate, and in the presence of metabolic activation, His+ revertant colonies were induced in TA98 and TA1538 strains at 1.5 to 1.7 times the solvent control with concentrations of 100 μl/plate or more, and in the T100 strain at 1.6 times the solvent control with a concentration of 200 μl/plate, and showed concentration dependence. Since reproducibility was also observed, it was judged to be false positive. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL) up to the maximum treatment concentration of 5,000 μg/mL in both short-term and continuous treatment, resulting in no induction of chromosomal aberration. [3)]

A micronucleus test in the bone marrow of mice (ddY, male) was performed up to 3,000 mg/kg×2, and no significant increase in micronucleus frequency was observed at any dose. [4)]

From the above, although false positive results were obtained in the reverse mutation test using bacteria, from comprehensively evaluation as negative in *in vivo* micronucleus tests at sufficiently high doses, Roasted rice bran extract is not thought to have genotoxicity which becomes a particular problem especially *in vivo*.

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## Ferulic acid

### 1. Food additive name:

Ferulic acid

### 2. Origin, method of preparation, and definition:

Rice bran oil obtained from rice bran of rice (*Oryza sativa* LINNE) of the Poaceae family is partitioned with hydrous ethanol and hexane under weak alkalinity at room temperature, then γ-oryzanol obtained in the water-containing ethanol fraction is hydrolyzed with sulfuric acid by heating under pressure and purified, or bacteria (*Pseudomonas*) are cultured in a media containing clove oil obtained by steam distillation from buds and leaves of clove (*Syzygium aromaticum* MERRILL et PERRY) of the Myrtaceae family, or eugenol obtained by purification from clove oil is obtained by separating and purifying the culture solution. It contains ferulic acid.

### 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 F344 rats by dietary administration with the test substance at concentrations of 0.32, 0.8, 2.0, and 5.0%. Hair loss was observed in males and females of the 5.0% group after 3-7 weeks in the test. The final body weight was low value in males and females of the 5.0% group. Both food intake and water intake showed low values in the 5.0% group. In hematological test, decrease in platelets was observed in males and females of the 5.0% group. In the test items of blood chemistry, ALB, ALP, AMY increased in both sexes of the 5.0% group, and cholesterol increased in females of the 2.0% group. In organ weight, increased relative weight of the liver and kidney, and decreased relative weight of the thymus, prostate, ovary, uterus in both sexes of the 5.0% group, and increased relative weight of the liver and kidney in males of 2.0% group were observed. In histological tests, hepatocyte hypertrophy accompanied by eosinophilic change was observed with dose correlation in both sexes, and seminal epithelial degeneration of the testes, parotid acinar epithelium atrophy, and decreased thickness of the femoral trabecular bone and cortical bone were observed in males of the 5.0% group. [1)]

In conclusion, the non-toxic level was considered to be 0.8% in both males and females.

(2) Genotoxicity study

A reverse mutation test in bacteria was performed up to 20 mg/plate, and did not induce His+ revertant colonies at 1.5 times or more the solvent control with or without S9 mix. [3)]

A chromosomal aberration test using mammalian cultured cells (CHL) showed reproducibility in the frequency of structural abnormalities and polyploidy cells, both of

which were judged to be false positives. [2), 4)]

A micronucleus test in mice was performed up to the limit dose of 2,000 mg/kg×2, and there was judged to be no micronucleus inducibility at any dose. [2), 5)]

From the above results, although false positive results were obtained in the chromosomal aberration test, considering the results of *in vivo* bone marrow micronucleus and carcinogenicity tests, it was concluded that there is no genotoxicity which is a particular problem *in vivo*.

(3) 1-year repeated-dose toxicity/carcinogenicity study

In a 1-year repeated dose toxicity test in F344 rats by dietary administration (0.5, 1.0, 2.0%), 1 death was confirmed in males of the 0.5% group, and hair loss was observed at 3-8 weeks in the test substance groups. There were no significant changes in body weight and food consumption. In hematological test, no significant change was observed in males, while in females, although significant changes were observed in low white blood cell and platelet counts, high red blood cell count, and low ratio of neutrophils and high ratios of lymphocytes and monocytes in differential leukocyte percentages, there was no concentration dependence in any of them, and they were considered to be toxicologically meaningless changes. In blood chemical test, a low value of CRE was observed in males at 1.0% or more, but it was considered a change of little toxicological significance. In organ weight, males showed high values for the actual weight of the brain at 0.5% or more, and high values for the relative weight of the pancreas and kidneys in the 2.0% group, while in females, low values for actual weight of the adrenal gland, high values for relative weight of the liver and low values for relative weight of the adrenal glands were observed at 2.0% group, but all were slight and considered to be incidental changes. In histopathological test, there were no lesions that could be attributed to the test substance in any of the organs.

In conclusion, the no observed adverse effect level was estimated to be 2.0% in both sexes (male: 557.6±117.6 mg/kg/day; female: 717.4±221.6 mg/kg/day). [6)]

In a 2-year carcinogenicity test in F344 rats by dietary administration (0.5, 1.0, 2.0%), no death considered to be caused by administration of the test substance was observed, and although transient hair loss was observed in the test substance administered groups, there were no other clinical signs. No changes were observed in hematological test. In blood chemical test, low values for TP in males at 1.0% group or more, and high AG ratio females at 2.0% group were observed, but both were considered incidental changes unrelated to administration. In males, there was no significant difference in final body weight, and in females, the final weight increased only in the 0.5% group, the actual weights of the pancreas and the adrenal gland were low in the 1.0% group, and the actual weights of the pancreas, adrenal gland and heart and relative weight of the adrenal gland were low in the 2.0% group. Histopathological test revealed a high incidence of intrahepatic bile duct proliferative lesions in females in the 2.0% group, thought to be reactive changes to intrahepatic pericholangitis.

In conclusion, no increase in the incidence of neoplastic lesions considered to be attributable to ferulic acid administration was observed, and carcinogenicity was not observed. [7)]

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## Gallic acid

### 1. Food additive name:

Gallic acid

### 2. Origin, method of preparation, and definition:

Gallic acid is obtained from tannin extracted with water, ethanol or organic solvent from gallnut produced by sumac (*Rhus javanica* LINNE) of the Anacardiaceae family or gallnut produced by beech (*Quercus infectoria* OLIV.) of the Fagaceae family, or tannin extracted with hot water from the fruit of tara (*Caesalpinia spinosa* (MOLINA) KUNTZE) of the Leguminosae family, by hydrolysis with alkali or enzyme (tannase). It contains gallic acid.

### 3. Major use:

Antioxidant

### 4. Summary of safety study results:

(1) Acute toxicity study

The 50% lethal dose (LD50) for oral treatment in mice was not less than 5 g/kg for both males and females. [1)]

(2) 90-day repeated-dose toxicity study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with the test substance at concentrations of 0.2, 0.6, 1.7, and 5.0%. It showed no animal deaths, and no changes were observed in general condition or food intake. In body weight, significant weight gain suppression was observed in the 5% group in both sexes starting the study at week 1.

In hematological test, in males, dose-dependent decreases in Hb content and Ht value were observed at 0.6% or more, RBC count decreased in the 0.6 and 5.0% groups, and decreases in MCV and MCH were observed only in the 5% group. In females, dose-dependent decrease in MCV at 1.7% or higher groups, significant decreases in RBC count, Hb content and Ht value in the 5% group and decrease in MCH in the 5% group were observed. Nucleated red blood cells increased in both sexes of the 5% group.

In organ weight, the relative weights of the liver, kidneys and testes increased in males at 1.7% or higher groups, but except for the liver they were mild. In the 5% group, the absolute weight of the spleen and liver was increased. In females, increased relative weight of the liver at 1.7% or higher groups and increased relative weight of the kidneys and spleen at 5% group were observed.

Histopathological test revealed hemosiderin deposition, enhanced extramedullary hematopoiesis and congestion in the spleen in both sexes of the 5% group. In the liver, hypertrophy of centrilobular hepatocytes was observed at 1.7% or higher groups in both sexes. Increases in Alb and ALP were observed in males, but there was no change in A/G

ratio, AST, or ALT. In females, no changes were observed except tendencies for A/G ratio to decrease and GPT to increase. In the kidneys, BUN showed mildly high values in both sexes of the the 5% group, and mild increases or decreases in CRN and some electrolytes were observed. The relative weight of the kidney increased by 16% at maximum compared to the control group, brown pigmentation was observed pathologically in the proximal tubule epithelium of the highest dose group, and it was suggested that these changes were effects of the test substance.

Based on the above results shown, the no observed adverse effect level of gallic acid is considered to be 0.2% (119 mg/kg). [2)]

(3) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) was performed up to 10 mg/plate, and the results were negative with and without metabolic activation. [3)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5 mg/mL, and induction of chromatid exchange abnormalities were seen in the system with metabolic activation. However, the nonphysiological condition of the pH of the treatment fluid must be considered. [4)]

A micronucleus test in the bone marrow of mice (8-week-old ddy male mice, aqueous solution, 2,000 mg/kg/day×2) was performed up to the limit dose of 2,000 mg/kg, and no micronucleus induction was observed at any dose. [5)]

In addition, DNA damage was not observed in Rec-assay. [6)]

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

(4) 90-day repeated-dose toxicity study

In a 90-day repeat-dose test by dietary administration (0.2, 1, 5%) using F344 rats, no deaths or abnormalities in the general condition were observed. Suppressed body weight gain was observed in both sexes in the 5% group, and decreased food consumption was observed in females of all test substance administration groups. In hematological, mild anemia was observed in both sexes of the 5% group, and in blood chemical test, increase in bilirubin and decrease in creatinine were observed in both sexes of the 5% group. In organ weight, the absolute and relative weights of the liver and spleen were increased in both sexes of the 5% groups, and the absolute weight of the pituitary, ovary and uterus was increased in females of the 5% group. In males of the 5% group, the absolute and relative weights of the ventral prostate were decreased, and the relative weight of the pituitary, testis and epididymis was increased, but no abnormality was observed in the sperm and hormone examinations, and no differences in the prostate were observed in histopathological test. Pathological test revealed splenic congestion, brown pigmentation and extramedullary hematopoiesis, brown pigmentation of proximal renal tubular epithelial cells and mild diffuse thyroid follicular epithelial hypertrophy in the thyroid in both sexes in the 5% group, hypertrophy of centrilobular hepatocytes in males and perilobular hepatocyte hypertrophy in females in the 5% group were observed. [7)]

(5) One-year repeated-dose toxicity study

In a 1-year repeat-dose test by dietary administration (0.2, 0.6, 1.8%) using F344 rats, no deaths or abnormalities in the general condition were observed. Suppressed body weight gain was observed in females of the 1.8% group, but no difference in food consumption was observed. In hematological test, mild anemia was observed in both sexes of the 1.8% group. In blood biochemical test, there were increases in direct bilirubin, total cholesterol, AST, ALT, γ-GTP and low values for creatinine in males of the 1.8% group, and low values for creatine in females of the same group. In organ weight, increased absolute and relative weight of the liver was observed in males of the 1.8% group. Histopathological examination revealed mild hypertrophy of centrilobular hepatocytes in males in the 1.8% group and mild perilobular hepatocyte hypertrophyt in females in the same group. There was no occurrence of a tumor showing significant increase.

In conclusion, the no observed adverse effect level is considered to be 0.6% (male: 107.4 mg/kg/day; female: 117.8 mg/kg/day). [7)]

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