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Research report

Research on the safety re-evaluation of existing additives

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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25 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.

April 23, 2014
Committee on Food Additives, Food Sanitation Commission,
Pharmaceutical Affairs and Food Sanitation Council

Research on the safety re-evaluation of existing additives

Research on the safety re-evaluation of existing additives
(April 23, 2014, Committee on Food Additives, Food Sanitation Commission,
Pharmaceutical Affairs and Food Sanitation Council)

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Research report

Research on the re-evaluation safety of existing additives

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A. Summary

In the FY 1996 health and welfare science grant research report, “Research on the safety evaluation of existing additives” (Senior Researcher: Hayashi Yuzo) (hereinafter referred 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, among the 139 additives for which further investigation was required in the Hayashi Group Report, among the 10 additives excluding those already reviewed for safety and those eliminated from the list of existing additives listed below, this research examined 3 additives for which new safety test results could be collected: Rumput roman extract, Grape skin extract, and Mastic gum.

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 3 investigated additives, and basic safety could be evaluated for those 3 existing additives based on the study results. In conclusion, the three 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 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 "Research on safety evaluation of existing additives" (Senior Researcher: Kurokawa Yuji) published in FY 1999, 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 "Research on safety evaluation of existing additives" (Senior Researcher: Inoue Tohru) published in FY 2003 (hereinafter referred to as the “Inoue Group Report”) 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 or the "Research on safety evaluation of existing additives" (Senior Researcher: Nishikawa Akiyoshi) published in FY 2004, 2006, 2007, 2008, 2009, 2010 and 2011, there are 14, 7, 8, 7, 6, 5 and 1 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 3 among the 10 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 10 out 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 evaluated the safety study results of 3 additives for which the required results of a 90-day or longer repeated dose study and a mutagenicity study were available.

D. Results

Each study result for 3 additives whose safety has been reviewed in this research is summarized in the Annex.

Regarding Rumput roman extract, Grape skin extract, and Mastic gum, there was no study result that suggested an immediate effect on human health at present.

E. Discussion

In this research, out of 10 items which are existing additives requiring safety confirmation in the Hayashi Team Report and have not been reviewed, regarding 3 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, Labour and Welfare deleted existing additives that are not in actual use for the third time in May 2011, following December 2004 and 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

This research showed that the basic safety of 3 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.

(Reference) Status of Review on the Safety of Existing Additives Review status H26.pdf

(Reference) Status of Review on the Safety Evaluation of Existing Additives

·13 additives reported in FY 1999 "Research on the safety evaluation of existing additives" (Senior Researcher: Kurokawa Yuji)

·16 additives reported in FY 2003 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru)

·14 additives reported in FY 2004 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru)

·7 additives reported in FY 2006 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru)

·8 additives reported in FY 2007 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru)

·7 additives reported in FY 2008 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru)

·6 additives reported in FY 2009 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru)

·5 additives reported in FY 2010 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Nishikawa Akiyoshi)

·1 additive reported in FY 2011 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Nishikawa Akiyoshi)

Annex

Rumput roman extract (109)

1. Food additive name:

Rumput roman extract (Containing capillin obtained from the entire plant of Capillary wormwood as the principal component.)

2. Origin, method of preparation, and definition:

It is obtained from the entire plant of *Artemisia capillaris* THUNB. of Asteraceae family by extraction with ethanol or hydrous ethanol at room temperature, or by steam distillation. The active ingredients include capillin and etc.

3. Major use:

Preservative

4. Summary of safety study results:

(1) 90-day repeated-dose study

A 90-day repeated-dose toxicity test was conducted in Crl/CD(SD) rats by gavage (0, 0.02, 0.2, and 2 mg/kg as capillin). In this test, no death was observed in any group of animals, and there were no toxicologically significant changes in general condition, detailed clinical observations, functional tests, body weight changes, food consumption, ophthalmological test, urinalysis, necropsy findings, organ weights, or histopathological test that were considered to be caused by the test substance.
Blood biochemical test revealed significantly higher glucose levels in females in the 2 mg/kg group than in the control group, but there was no difference from the ethanol-containing control group, suggesting that the change was related to the ethanol contained in the vehicle. Females in the 2 mg/kg or higher groups had significantly higher β-globulin fraction ratio, but this was not considered to be related to the test substance because there were no changes related to other fractions.
From on the above, the no observed adverse effect level was determined to be 2 mg/kg as capillin in both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA100, TA1535, TA98, TA1537, WP2*uvrA*) was performed at doses up to 5,000 μg/plate and growth inhibition was observed in the absence of S9 mix. In TA98 without S9 mix and TA1537 with S9 mix, the number of revertant colonies increased in a dose-dependent manner to at least twice that in the negative control, indicating that the test substance was positive. [2)]
Using cultured mammalian cells (CHL/IU), the frequency of cells having structural

aberrations was clearly increased with dose-dependency in the presence of S9 mix in the chromosomal aberration test, without S9 mix in the confirmation test, and in 24-hour treatment. The cell growth rates at the highest doses that could be observed microscopically in each culture series (0.13 mg/mL without S9 mix, 2 mg/mL with S9 mix, and 0.11 mg/mL in 24-hour treatment) were all less than 50%. From the above results, clastogenicity was judged to be positive. [3)]

A micronucleus test in male Crlj:CD1(ICR)SPF mice was performed by oral administration up to the limit dose of 2,000 mg/kg/day × 2, and the incidence of micronuclei was not significantly increased at any dose as compared to the negative control group. Therefore, it was judged to be negative. [4)]

A 28-day repeated-dose mutation study was conducted by gavage in C57BL/6JJmS1c-Tg strain (*gpt* delta) mice. The liver and stomach (glandular) were collected 3 days after the last dose to measure the gene mutation frequency (MF). As a result, no significant increase in MF was observed in any of the organs treated with the test substance. Therefore, it was judged that the test substance had no gene mutation inducing effect (negative) in the liver or stomach (glandular). [5)]

From the above results, *in vitro* mutagenicity and clastogenicity were not confirmed in the *in vivo* test system, and therefore are not considered to be particularly problematic for a living body.

5. Results

From the results of these studies and the estimation of intake, there was no concern about human health effects.

(References)

1. Matsuura Masao: FY 2010 Study on the Safety of Existing Additives, Safety Research Institute for Chemical Compounds Co., Ltd.
2. Yamauchi Hisami: FY 2009 Safety Study of Existing Additives, BoZo Research Center Inc.
3. Kikuchi Masanori: Chromosomal Aberration Test of Capillary Wormwood Extract in Cultured Mammalian Cells, SRD Biology Center Co., Ltd.
4. Ishii Takahiro: Micronucleus Test of Capillary Wormwood Extract in Mice, BoZo Research Center Inc.
5. Ono Hiroshi: FY 2012 Safety Tests of Designated Additives, *Gpt* delta Transgenic Mouse Mutation Test of Capillary Wormwood Extract, Food and Drug Safety Center

Grape skin extract (366)

1. Food additive name:

Grape skin extract (Containing polyphenol obtained from the skins of fox grapes or common grapes as the main component.)

2. Origin, method of preparation, and definition:

It is extracted with room temperature to slightly warmed ethanol from the peels of fox grapes (*Vitis labrusca* LINNE) of Vitaceae family or common grapes (*Vitis vinifera* LINNE) of Vitaceae family for eating or for brewing Koshu, Chardonnay or Riesling. It consists mainly of polyphenol.

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 rats by dietary administration (1.25, 2.5, and 5.0%). In this study, no death was observed in any group of animals, and there were no toxic changes in general condition, body weight, water intake, ophthalmological test, urinalysis, hematological test, blood biochemical test, or organ weights that were considered to be caused by the test substance. Gross pathological test revealed hypertrophy of the parotid gland in all males and females in the 5.0% and 2.5% groups, and histopathological findings included hypertrophy of acinar cells in the parotid gland in both males and females in all groups treated with the test substance. In addition, in females in the 5.0% and 2.5% groups, the incidence and severity of mineral deposition at the cortico-medullary junction in the kidney increased. The no observed adverse effect level was considered to be less than 1.25% in both sexes (770 mg/kg BW for males and 830 mg/kg BW for females) and could not be determined in this study. [1)]

To confirm the reproducibility of the effects on parotid gland/acinar cells and kidneys, and to identify the NOAEL, a 90-day repeated-dose toxicity study was conducted in F344/DuCrj rats by dietary administration (0.2, 1.0, 5.0%). 2)As a result, no death was observed in any group of animals, and there were no toxicologically significant changes in general condition, body weight, food consumption, organ weights, hematological test, or blood biochemical test that were considered to be caused by the test substance. Histopathological test revealed diffuse basophilic change and severe hypertrophy of the acinar epithelial cells of the parotid gland in all males and females in the 5.0% group. In addition, the number of animals with mineral deposition in the cortex and medulla of the kidney that was mild but more severe than in other groups

was significantly increased in females of the 5.0% group. Mineral deposition in the kidneys of females in the 5.0% group was considered to be toxicity of the test substance. In addition, basophilic change and diffuse hypertrophy of acinar epithelial cells of the parotid gland were observed in males and females of the 5.0% group, although the toxicological significance to the body was unclear. Therefore, the NOAEL was determined to be 1.0% in both sexes (600 mg/kg BW/day for males and 700 mg/kg BW/day for females). [2)]

(2) Genotoxicity study

In a reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, WP2*uvr*A), a dose-dependent increase in revertant colonies was observed in the TA98 strain with and without S9 mix. Therefore, the test was considered positive. The maximum specific activity was 22 (Rev/mg), indicating very weak mutagenicity. [3)]It has also been reported that a reverse mutation test (TA97, TA98, TA100, TA102) was negative regardless of the presence or absence of S9 mix. [4)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) at the maximum treatment concentrations of 313 μg/mL (short-term treatment, + S9 mix), 156 μg/mL (short-term treatment, -S9 mix), and 40.0 μg/mL (continuous treatment, 24-hour and 48-hour treatment). As a result, no induction of chromosomal aberrations was observed under any treatment conditions. [5)]

In the micronucleus test in mice (BDF1, male), the test was performed up to the limit dose of 2,000 mg/kg × 2. Since no significant increase in the incidence of micronuclei was observed at any dose as compared to the negative control group, the test article was considered negative. [6)]

From the above results, although a positive result was obtained in the bacterial reverse mutation test, the mutagenicity was very weak, and the results were negative in the *in vivo* micronucleus test, which was conducted at sufficiently high doses. Based on the comprehensive evaluation, this additive is considered to have no genotoxicity that is of particular concern to living organisms.

5. Results

Based on the results of these studies, there was no study result that could be of concern for human health.

(References)

1. Ono Hiroshi: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., DIMS Institute of Medical Science, Inc.
2. Ogawa Kumiko: FY 2011 Study on the Reevaluation, etc. of the Safety of Food Additives, Division of Pathology, Biological Safety Research Center, National Institute of Hygienic Sciences

3. Yamauchi Hisami: FY 2007 Safety Study of Existing Additives (Bacterial Reverse Mutation Test of Grape Skin Extract), BoZo Research Center Inc.
4. Aiub C, Stankevicins L, da Costa V, Ferreira F, Mazzei J, Ribeiro da Silva A, Soares de Moura R, Felzenszwalb I. Genotoxic evaluation of a vinifera skin extract that present pharmacological activities. Food Chem Toxicol.42(6):969-73,2004.
5. Sono Akira: FY 2007 Safety Study of Existing Additives (Chromosomal Aberration Test of Grape Skin Extract in Cultured Mammalian Cells), BoZo Research Center Inc.
6. Mochizuki Nobuhiko: FY 2007 Study on Standards and Criteria for Food and Food Additives (Micronucleus Test of Grape Skin Extract in Mice), Biosafety Research Center

Mastic gum (421)

1. Food additive name:

Mastic gum (A substance obtained from secretion of the mastic tree, containing masticadienonic acid as the main component.)

2. Origin, method of preparation, and definition:

It is obtained from the secretion of the mastic tree (*Pistacia lentiscus* LINNE) of Anacardiaceae family by distilling off the portion with low-boiling point, extraction with hot ethanol, and distilling away the ethanol. It consists mainly of masticadienonic 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 F344 rats by dietary administration with the test substance at concentrations of 0.22, 0.67, and 2%. As a result, no animal deaths were observed, and no changes were observed in general condition or food intake. Suppressed body weight gain was observed in males in the 2% group and females in the 0.67% or higher groups. As changes related to administration, hematological findings included increased platelets in males in the 0.67% or more groups and increased white blood cells in the 2% group. Blood biochemical test revealed increased TP and ALB and decreased TG in males of the 0.67% or higher groups, and increased ALP, TC, and Ca in males of the 2% group. In females, P decreased and -GT and TC increased in the 0.67% or more groups, and TP and BUN increased in the 2% group. In organ weight, increases in absolute and relative liver weight were observed in both sexes of the 0.67% or above groups. Histopathological test revealed no treatment-related findings.

In conclusion, the no observed adverse effect level is considered to be 0.22% in both sexes (male: 120 mg/kg BW/day; female: 132 mg/kg BW/day). [1)]

(2) Chronic toxicity/carcinogenicity study

In a 52-week repeated-dose toxicity test by dietary administration (0.07, 0.2, 0.6%) in F344/DuCrlCrlj rats, no deaths occurred in any group, and no treatment-related changes were observed in clinical signs, food consumption, ophthalmological test , urinalysis, or gross pathological test. Hematological test revealed increased platelets in males in the 0.2% or higher groups and females in the 0.6% group, and decreased red blood cells, HGB, and HTC in males in the 0.6% group. Blood biochemical test

revealed significantly increased γ-GT in females in the 0.2% or higher groups and increased TC in females in the 0.6% group. In organ weight, higher relative weight of liver in males and lower relative weight of spleen in females were observed in the 0.2% or higher groups, and significantly higher absolute weight of liver in males and females and lower absolute weight of spleen in females were observed in the 0.6% group. Histopathological test revealed that the incidence of small proliferative foci in the liver was significantly increased in males in the 0.6% group as compared with the control group.
In a 104-week carcinogenicity study in F344/DuCrlCrlj rats by dietary administration (0.07, 0.2, 0.6%), 1 male in the 0.6% group died in Week 41, and some animals died or were sacrificed moribund thereafter. However, there was no difference between groups in the survival rate at the end of administration in either males or females. General condition, food consumption, and gross pathological test showed no treatment-related changes. Body weight showed significantly low values or tendency of low values continuously in males of the 0.2% group and above, which was considered to be the effect of administration of the test substance. In organ weights, the absolute and relative liver weights were significantly higher in males in the 0.6% group and females in the 0.2% or higher groups. Increased absolute and relative kidney weights were observed in females of the 0.6% group. Histopathological test revealed significant increase or tendency of increase in the incidence of altered hepatocellular foci in the liver in males in the 0.2% and 0.6% groups, and significant increase in the incidence of bile duct hyperplasia in females in all administered groups. However, no increase in the incidence of neoplastic lesions was observed.

Based on the above, considering that increased platelets in males, significantly increased γ-GT in females, increased relative liver weight in males and decreased relative spleen weight in females were observed in the 0.2% or higher groups in the chronic toxicity study, and that the absolute and relative liver weights were also increased in females in the 0.2% or higher groups in the carcinogenicity study, the NOAEL was considered to be 0.07% for both sexes (31.6 mg/kg BW/day for males and 39.5 mg/kg BW/day for females).
In the carcinogenicity test, no obvious carcinogenicity was observed in any organs or tissues. In the chronic toxicity study, an increase in the number of altered hepatocellular foci was observed in males at 0.6%. In the carcinogenicity study, continuous decrease in body weight and tendency of increased number of altered hepatocellular foci were observed in males at 0.2% or more, and increased bile duct hyperplasia was observed in males in all test substance groups. Therefore, although toxicological effects on the liver were observed under the conditions of this study,

there was no obvious carcinogenicity. [2)]

(Reference Information)
A medium-term rat liver carcinogenicity test (Ito method) was conducted in 6-week-old male F344 rats. 200 mg/kg of diethylnitrosamine (DEN) was intraperitoneally administered, and 2 weeks later, Mastic gum was administered in the diet at concentrations of 0, 0.001, 0.1, and 1%. At Week 3, two-thirds partial hepatectomy was performed, and administration was continued until Week 8. As a result, liver weight increased dose-dependently. The number and extent of GST-P-positive cell foci per observation area increased in the 1% treatment group.
Immunodouble staining of BrdU and GST-P showed that BrdU-labeled cells were the highest among GST-positive cell foci in the 1% treatment group. There was no difference in 8-OHdG level.
These results suggest that Mastic gum has a carcinogenesis-promoting effect in the 1% (521 mg/kg BW/day) group under the conditions of this study. [3)] However, since carcinogenicity was not observed in the combined chronic toxicity/carcinogenicity study, and it is not considered to be particularly problematic for the living body in genotoxicity studies, the results of this study are also not considered to be particularly problematic for the living body.

(4) 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 metabolic activation. [4)]
In a chromosomal aberration test in cultured mammalian cells (CHL/IU) up to the concentration at which cytotoxicity was observed, statistically significant induction of chromosomal aberrations was observed only in the presence of metabolic activation, but the incidence was low at 6%. [5)]
A micronucleus test in the bone marrow of mice was performed up to the limit dose of 2,000 mg/kg, and no micronucleus induction was observed at any dose. [5)]
Therefore, the clastogenicity observed *in vitro* was not confirmed in the *in vivo* test system, and is not considered to be a particular problem for living organisms.

5. Results
Based on the results of these studies, there was no study result that could be of concern for human health.

(References)
1. Wanibuchi Hideki: Health and Labour Sciences Research Grant, Osaka City

University Graduate School of Medicine
2. Seiko Tamano: FY 2011 Study on Standards and Criteria for Food and Food Additives, etc., DIMS Institute of Medical Science, Inc.
3. Doi K: Enhancement of preneoplastic lesion yield by Chios Mastic Gum in a rat liver medium-term carcinogenesis bioassay. Toxicol Appl Pharmacol. 2009 Jan 1;234(1):135-42
4. Ajimi Shozo: Health Sciences Research Grant, Chemicals Evaluation and Research Institute
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