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Research report

Research on the safety re-evaluation of existing additives

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June 19, 2015
Committee on Food Additives, Food Sanitation Commission,
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Research on the safety re-evaluation of existing additives

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(June 19, 2015, Committee on Food Additives, Food Sanitation Commission,
Pharmaceutical Affairs and Food Sanitation Council)

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Research report

Research on the safety re-evaluation 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 identified in the Hayashi Group Report as requiring further study, 7 additives remained, excluding those listed below that have already been reviewed for safety or removed from the list of existing additives. Of these 7 additives, two additives, Grape seed extract and Lac colour, for which safety test results could be newly collected, were evaluated. The results of a 90-day or longer repeated dose study and a mutagenicity study were available for 2 investigated additives, and basic safety could be evaluated based on the study results. In conclusion, the two 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." In addition, in the "Research on safety evaluation of existing additives" (Senior Researcher: Inoue Tohru) or the "Research on safety evaluation of existing additives" (Senior Researcher: Nishikawa Akiyoshi) published in FY 2003, 2004, 2006, 2007, 2008, 2009, 2010, 2011 and 2013, there are 16, 14, 7, 8, 7, 6, 5, 1 and 3 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 2 among 7 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 7 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 2 additives for which the required results of a 90-day or longer repeated-dose study and a mutagenicity study were available.

D. Results
The study result for the 2 additives whose safety has been reviewed in this research is summarized in the Annex.
Regarding Grape seed extract and Lac colour, there was no study result that suggested an effect on human health at present.

E. Discussion
In this research, among the 7 existing additives for which safety confirmation was required in Hayashi Group Report and which had not yet been reviewed, two were found to have both repeated-dose test results of at least 90 days or more and mutagenicity test results. Based on the evaluation of the test results, none of the products were considered to have any toxicity that would cause adverse effects on human health to the extent that they are currently used as additives.
The Ministry of Health, Labour and Welfare deleted existing additives that are not in actual use from the list of existing additives 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 2 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 H27.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)

·3 additives reported in FY 2013 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Nishikawa Akiyoshi)

Annex

Grape seed extract

1. Food additive name:

Grape seed extract (containing proanthocyanidins obtained from the skins of fox grapes or common grapes as the main component.)

2. Origin, method of preparation, and definition:

It is obtained from the seeds of fox grapes (*Vitis labrusca* LINNE) of the Vitaceae family or common grapes (*Vitis vinifera* LINNE) of the Vitaceae family by extraction with hot water, warm ethanol, or room temperature acetone, or from the extract obtained by fermentation with yeast or by hydrolysis with tannase. It consists mainly of proanthocyanidins.

3. Major use:

Antioxidant, food manufacturing agent

4. Summary of safety study results:

(1) Single-dose study

A single-dose toxicity test by gavage was conducted in F344 rats. LD_{50} was 4,000 mg/kg or more in both sexes. [1)]

A single-dose toxicity test by gavage was conducted in 5 male and 5 female albino rats at a dose of 5,000 mg/kg. In this test, one female died on the first day of dosing. Necropsy revealed adhesion of brown material to the forestomach. The oral LD_{50} was 5,000 mg/kg or more in both sexes. [2)]

(2) Repeated-dose study

(i) 90-day repeated-dose study in rats

A 13-week repeated-dose test was conducted in F344 rats by dietary administration (0.05%, 0.5%, and 5.0%; test substance intake of 28.4, 286, and 3,020 mg/kg BW/day for males and 33.4, 335, and 3,530 mg/kg BW/day for females, respectively). As a result, no toxicological changes caused by the test substance were observed in general condition, body weight, food consumption, hematological test, blood biochemical test, gross pathological or histopathological test. In organ weight, the liver/brain weight ratio and kidney/brain weight ratio were significantly decreased in males of the 5.0% group. However, since no changes were observed in histopathological test and the absolute weight of these organs was not significantly different between the treated and control groups, these changes were not considered to be treatment-related. [3)]

A 3-month repeated-dose test was conducted in SD rats by dietary administration (0.63%, 1.25%, and 2.5%; test substance intake of 434, 860 and 1,790 mg/kg BW/day for males and 540, 1,050, and 2,170 mg/kg BW/day for females, respectively). A dose

at which tannins had no inhibitory effect on nutrient absorption was selected as the highest dose. In this test, there were no remarkable changes in general condition, body weight, food consumption, hematological test, or blood biochemical test. Histopathological test revealed no toxic changes caused by the test substance. The no observed adverse effect level is considered to be 2.5% in both sexes (male: 1,790 mg/kg BW/day; female: 2,170 mg/kg BW/day). [4)]

A 90-day repeated-dose test was conducted in F344 rats by dietary administration (0.02%, 0.2%, and 2.0%; test substance intake of 13.3, 129, and 1,410 mg/kg BW/day for males and 14.8, 154, and 1,500 mg/kg BW/day for females, respectively). In this test, there were no notable changes in general condition, body weight, food consumption, water consumption, hematological test, or blood biochemical test. Necropsy, organ weights, and histopathological test also showed no treatment-related toxicological changes. The no observed adverse effect level is considered to be 2.0% in both sexes (male: 1,400 mg/kg BW/day; female: 1,500 mg/kg BW/day). [1)]

A 90-day repeated-dose test was conducted in SD rats by dietary administration (0.5%, 1.0%, and 2.0%; test substance intake of 348, 642, and 1,586 mg/kg BW/day for males and 469, 883, and 1,928 mg/kg BW/day for females, respectively). As a result, there were no test substance-related effects on general condition, hematological test, gross pathological test, or histopathological test. There was an increase in food consumption in both males and females in the 2.0% group, but no associated body weight gain. Serum iron concentration was significantly decreased in males of the 2.0% group. Given that these changes were within the range of background data, the no observed adverse effect level was estimated to be 2.0% for both sexes (male: 1,586 mg/kg BW/day, female: 1,928 mg/kg BW/day). [5)]

(ii) Six-month and 12-month repeated-dose studies in mice

A 12-month repeated-dose study was conducted in male B6C3F1 mice by dietary administration (100 mg/kg BW/day). Mice were subjected to necropsy at 3, 6, 9, and 12 months after the start of administration. In this study, there were no treatment-related changes in blood biochemical test, organ weights, necropsy, or histopathological test. Subsequently, a 6-month repeated-dose test was conducted in female B6C3F1 mice by dietary administration (100, 250, or 500 mg/kg body weight/day). As a result, no noteworthy changes were observed in any test item, and histopathological test revealed no toxic changes caused by the test substance. Although the NOAEL was not described in the report [2)], the no observed adverse effect level was considered to be 500 mg/kg BW/day.

(3) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, and TA1537) was performed at doses up to 1,250 μg/plate (TA98, TA100) or 5,000 μg/plate (TA1535,

TA1537), and all results were negative with and without S9 mix. [1)]

In a chromosomal aberration test in Chinese hamster lung cells (CHL/IU), the test was performed by short-term treatment (6 hours) in the presence and absence of S9 mix and long-term treatment (24, 48 hours; -S9 mix), up to a maximum dose of 5.0 mg/mL. Clastogenicity was negative up to the concentration at which 50% cell growth inhibition was observed. [1)]

In a micronucleus test using mouse bone marrow, the test substance was orally administered twice at doses of 500, 1,000, or 2,000 mg/kg with an interval of 24 hours, and observation was performed 24 hours after the final administration. No induction of micronuclei or inhibition of bone marrow cell proliferation was observed at any dose. [1)]

Another micronucleus test using mouse bone marrow has also been reported. The test substance was orally administered once at a dose of 500, 1,000, or 2,000 mg/kg, and observation was performed at 24 or 48 hours. Suppression of bone marrow cell proliferation was observed at the highest dose, demonstrating that bone marrow tissue was appropriately exposed to the test substance, but induction of micronuclei was not observed in any treatment group. [6)]

Based on the above results, Grape seed extract was considered to have no genotoxicity.

5. Results

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

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Lac colour

1. Food additive name:
Lac colour (Containing laccaic acid as the main component, obtained from the lac insect.)

2. Origin, method of preparation, and definition:
It is obtained by extracting the resin-like substance secreted from the lac insect (*Laccifer lacca* KERR) with room temperature to hot water. It consists mainly of laccaic acid as a principal pigment.

3. Major use:
Color

4. Summary of safety study results:

(1) Repeated-dose study

(i) 13-week repeated-dose study

A 13-week repeated-dose test was performed in F344 rats by dietary administration (0.313, 0.625, 1.25, 2.5, and 5.0%). The results showed histological findings of hypertrophy of the parotid gland and calcification in the kidney, but there were no marked changes in body weight, food and water consumption, hematological test, blood biochemical test, or organ weight. Hypertrophy of the parotid gland was observed in the 2.5% or more groups in both males and females. Calcification in the kidney was observed in males in the 1.25% or higher administered groups, and in females in the control group (1/10 animals) and all administered groups. [1)]

(ii) 78-week repeated-dose study

A 78-week repeated-dose test was conducted in F344 rats by dietary administration (0.313%, 1.25%, and 5%; test substance intake of 136, 562, and 2,270 mg/kg BW/day for males and 169, 690, and 2,820 mg/kg BW/day for females, respectively). In this study, parotid gland hypertrophy and the severity of renal calcification, which is a spontaneous lesion, were associated with administration, but no necrotic, inflammatory or proliferative lesions were observed. No marked changes were observed in body weight, food and water consumption, hematological test, or blood biochemical test. Parotid gland hypertrophy was observed in all males and females in the 5% group. When the severity of calcification in the kidney was classified as no finding, slight, mild, moderate, or severe, in males it was slight in 1/20 in the control group, slight in 1/17 and mild in 1/17 in the 0.313% group, slight in 6/20 and mild in 1/20 in the 1.25% group, and slight in 12/19 and mild in 5/19 in the 5% group. In females it was slight in 11/15 and mild in 4/15 in the control group, slight in 11/18 and mild in 7/18 in the 0.313% group, slight in 7/18, mild in 9/18 and moderate in 2/18 in the 1.25% group, and mild in 3/19, moderate in 14/19 and severe in 2/19 in the 5% group. In both males and females, the number of affected animals and the severity of calcification increased dose-

dependently. [2)]

To confirm the toxicological significance of the parotid gland hypertrophy observed in the 78-week repeated-dose study, a 2-week and 4-week repeated-dose study by dietary administration (5%) using male F344 rats was conducted, and the fine structure of the parotid gland was observed with a transmission electron microscope. In this study, after 2 weeks of administration, the nuclear membrane tended to be deformed and concentrated, the secretory granules were irregularly shaped and swollen, with loss of the limiting membrane, and mitochondrial vacuolation was observed. After 4 weeks of treatment, marked pyknosis with aggregation of nucleoli, loss of the limiting membrane of secretory granules, dilatation of the lumen of rough endoplasmic reticulum, and mitochondrial decay were observed. Lac colour was considered to affect the function of acinar cells. [3)]

Regarding dose correlation of the renal calcification in the 78-week repeated-dose study, the research group on safety review of existing additives performed a trend test (Cochran-Armitage test) to test whether there is a linear tendency between the number of animals with calcification and the dose, and a Mann-Whitney test to test whether there is a difference in the degree of calcification between the control group and the administered groups. The significance level was 5% for both tests. The results of the trend test suggested that the number of animals with renal calcification tended to be significantly linear with the dose in both males and females ($P < 0.001$). The results of the Mann-Whitney U test showed that the degree of calcification was significantly higher in the 1.25% and 5% groups, although no significant difference was observed in either sex in the 0.313% group.

Based on the above results, it was concluded that the number of animals with renal calcification increased dose-dependently with increased severity. The no observed adverse effect level of Lac colour in this study was considered to be 136 mg/kg BW/day (0.313%) in males and 169 mg/kg BW/day (0.313%) in females.

(3) Genotoxicity study

A reverse mutation test of Lac colour in bacteria (TA97, TA98, TA100, and TA102) was performed up to 5,000 μg/plate, and the results were all negative with and without S9 mix. [4)] In chromosomal aberration test of Lac colour and laccaic acid in Chinese hamster lung cells (CHL/IU), chromosomal structural aberrations were induced at doses up to 2.0 mg/mL in long-term treatment (24, 48 hours) without S9 mix. [5)] In a mouse bone marrow micronucleus test of laccaic acid, a single dose of 10 or 20 mg/kg was intraperitoneally administered, and observation was performed 24 hours after administration. No induction of micronuclei was observed. [6)] In a chromosomal aberration test of Lac colour in bone marrow of Chinese hamsters, a single oral dose of 500, 1,000, or 2,000 mg/kg was administered, and observation was performed at 24 and

48 hours after administration, but no induction of chromosomal aberration was observed. [7)]

Based on the above results, Lac colour induced chromosomal aberration *in vitro*, but was negative in both *in vivo* micronucleus test and *in vivo* chromosomal aberration test, and therefore it was considered that there was no genotoxicity of special concern for the living body.

(4) Other Studies

The estrogen effect of Lac colour was investigated using the proliferation activity of human breast cancer-derived cells (MCF-7) as an index, and it was reported that proliferation was enhanced at the concentrations of 1% (10 μg/mL) to 10% (100 μg/mL). [8)] At 100 μg/mL, the cell proliferation rate was 1.8 times (180%) higher than that in the control group, and the molar concentration was estimated to be 2×10^{-4} M, assuming the mean molecular weight of laccaic acid to be 500.

According to the report by Han et al. [9)] which reported the estrogenic effect of chemical substances using a similar test system, it was 220% for 17 β-estradiol (10^{-10} M), 260% for Ethynylestradiol (10^{-8} M), and 210% for Bisphenol A (10^{-8} M). Compared to these known estrogen agonist values, the estrogen effect of Lac colour is 1/2,000,000 of 17 β-estradiol and 1/20,000 of Ethynylestradiol and Bisphenol A. The intake of the test substance in the 5% diet group, which was the highest concentration in the 78-week repeated-dose toxicity test of Lac colour in F344 rats, was 2,265.4 mg/kg body weight/day for males and 2,818.1 mg/kg body weight/day for females [2)]. The exposure concentration of Lac colour when uniformly distributed in the whole body was calculated to be 0.23% to 0.28%, so the 1% *in vitro* concentration is 3.6 to 4.3 times the exposure concentration *in vivo*. In the 13-week and 78-week repeated-dose toxicity tests in rats, no changes in the uterus or mammary gland were observed. [1,2)]

Based on the above, it is concluded that there is no safety concern for humans because the estrogen effect of Lac colour is extremely weak, and exposure at the concentration at which the estrogen effect is observed is not expected in humans.

5. Results

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

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