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Research report

Research on the safety re-evaluation of existing additives

FY 2011

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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23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.

May 30, 2012
Committee on Food Additives, Food Sanitation Commission,
Pharmaceutical Affairs and Food Sanitation Council

Research on the safety re-evaluation of existing additives (FY 2011 Survey)

Research on the safety re-evaluation of existing additives (FY 2011)
(May 30, 2012, 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 May 10, 2012.

Research report

Research on the safety re-evaluation of existing additives

May 2012

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A. Summary

In the FY 1996 Health Sciences 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 11 additives excluding those already reviewed for safety and those eliminated from the list of existing additives listed below, Horseradish extract was examined for which safety test results could be newly collected.

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for 1 investigated additive, and basic safety could be evaluated for the individual existing additives based on the study results. As a result, the one investigated additive was concluded to have no toxicity that would be immediately detrimental to human health within the range 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 Report or the "Research on safety evaluation of existing additives" (Senior Researcher: Nishikawa Akiyoshi) published in FY 2004, 2006, 2007, 2008, 2009 and 2010, there are 14, 7, 8, 7, 6 and 5 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 one of the 11 of 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 11 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 been deleted from the list of existing additives, this research individually evaluated the safety study results of 1 additive 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 additive whose safety has been reviewed in this research is summarized in the Annex.

Regarding horseradish extract, there was no study result that suggested an immediate effect on human health at present.

E. Discussion

In this research, out of 11 items which are existing additives requiring safety confirmation in the Hayashi Team Report and have not been reviewed, regarding 1 item 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 1 additional natural additive 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 Evaluation of Existing Additives Review status.pdf

·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)

Annex

Horseradish extract (234)

1. Food additive name:

Horseradish extract (Derived from horseradish roots, consists mainly of isothiocyanate.)

2. Origin, method of preparation, and definition:

It is extracted by steam distillation after pulverizing the roots of horseradish (*Armoracia rusticana* P. GAERTN., B. MEYERet SCHERB.) of Brassicaceae family. It consists mainly of isothiocyanate.

3. Major use:

Antioxidant, food manufacturing agent

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by gavage of Horseradish extract at 20, 40, and 80 mg/kg. Findings included keratosis, hyperkeratosis, and squamous hyperplasia of the mucosal epithelium caused by direct stimulation of the forestomach in males and females in the 40 and 80 mg/kg groups. Other findings such as decreased body weight gain and decreased food consumption are considered to be secondary effects. The no observed adverse effect level was estimated to be 20 mg/kg based on tissue changes in the forestomach. [1)]

(2) Genotoxicity study

In a reverse mutation test in bacteria, since it induced 1.5 times more His+ revertant colonies than the control with or without S9 mix, and concentration dependence was also observed, it was suspected to be positive. [2)]

In addition, negative and positive results have been reported. [3),4)]

A chromosomal aberration test in cultured mammalian cells (CHL) induced chromosomal aberrations and polyploidy by both the direct method and the metabolic activation method. [5),6)]

In a micronucleus test using mice, micronucleus induction was not observed in mouse bone marrow at any dose. [7)]

Based on the above results, it was concluded that there was no genotoxicity which is a particular problem to living organisms.

(3) Chronic toxicity/carcinogenicity study

In a 52-week repeated-dose toxicity test in F344 rats with administration in drinking

water (0.005, 0.01 and 0.04%) via a tube with a nozzle, 1 male in the control group died of malignant lymphoma and 1 female in the 0.04% group died of poor growth with hind limb paralysis, which were considered to be accidental. There were no abnormalities in body weight, food consumption, or organ weight. Hematological test revealed low Seg and high Lympho in males in all administered groups, but no dose response was observed. Moreover, there were no major changes in WBC and no histopathological findings suggestive of a relationship, therefore these findings were considered to have little toxicological significance. Serum biochemical test revealed increased glucose in males in the 0.04% group. Although the possibility of effects of administration cannot be ruled out, no histopathological findings suggesting relationship to the liver or pancreas were observed, and the cause was unknown. Histopathological test revealed PN hyperplasia in the forestomach in males in the 0.04% group, and PN hyperplasia in the bladder in females in the 0.04% group. The no observed adverse effect level was determined to be 0.01% for both sexes (male: 7.0 mg/kg BW/day; female: 8.4 mg/kg BW/day), based on increased glucose and PN hyperplasia in the forestomach or bladder. [8)]

In a 104-week carcinogenicity test in F344 rats by administration in drinking water (0.005, 0.01 and 0.04%) via a tube with a nozzle, no abnormalities were observed in general condition or food consumption. Body weight gain was suppressed or tended to be suppressed in males and females in the 0.04% group, and a decrease in the weight of the liver and an increase in the relative weight of the brain were observed in males in the 0.04% group. The relative weight of the brain, lung, heart, and kidney increased in females in the 0.04% group, which was attributed to low body weight because no histopathological changes were observed. In histopathological tests, simple hyperplasia, PN hyperplasia, papilloma, and transitional cell carcinoma were sporadically observed in the urinary bladder in each male and female group. However, there were no dose-relationships and changes related to the administration. Therefore, it was judged to be non-carcinogenic in rats. [8)]

(4) Short-term administration/2-stage carcinogenicity study

In a short-term administration test in male F344 rats by administration with drinking water via a water bottle (tap water for the first 5 weeks of the experiment, and drinking water mixed with 0.005, 0.01 and 0.04% of Horseradish extract from the 5th week), no abnormalities were observed in general condition. In body weight, a tendency of decrease was observed in the 0.04% group. Water consumption decreased in a dose-dependent manner. Histopathological changes in the 0.04% group were simple hyperplasia in 1/5 animals on Day 3; simple hyperplasia in 4/5 animals, PN hyperplasia in 1/5 animals and submucosal edema/cell infiltration in 2/5 animals in

Week 1; and simple hyperplasia in 5/5 animals and PN hyperplasia in 4/5 in Week 2. The BrdU positive rate significantly increased on Day 1 in Weeks 1 and 2 in the 0.01 and 0.04% groups. [8)]
No abnormalities were observed in general condition of male F344 rats administered water in a water bottle (0.05% N-n-butyl -4 hydroxybutylnitrosamine (BNN) in deionized water for 4 weeks after the start of the experiment, followed by deionized water for 1 week, and combined drinking water with horseradish extract at 0.005, 0.01, and 0.04% from 5 weeks after the start of the experiment). Body weight was low in the 0.04% group. A tendency of decreased food consumption was observed in the 0.04% group. Water consumption decreased in a dose-dependent manner. In histopathological changes at Week 13, PN hyperplasia in all groups, papilloma in the 0.01 and 0.04% groups, and transitional cell carcinoma in the 0.04% group were significantly increased. There were significant increases in PN hyperplasia in the 0.04% group and in papilloma and transitional cell carcinoma in the 0.01 and 0.04% groups at Week 32. As proliferative lesions, significant increases in PN hyperplasia, papilloma, and transitional cell carcinoma were observed, and therefore it was judged to have a bladder carcinogenesis-promoting effect in rats. [8)]

(5) Chronic toxicity study
In a 104-week repeated-dose chronic toxicity test of Horseradish extract in drinking water (0.01 and 0.04%) administered via water bottles to male F344 rats, no abnormalities were observed in general condition. Although water intake and food intake decreased in a dose-dependent manner, water intake was considered to decrease due to the strong spiciness of horseradish, and food intake decreased accordingly. Body weight was also affected, and inhibition of increase was observed in males in the 0.04% group. In organ weight, absolute weight of the brain, heart, and liver decreased in 0.04% group, which is considered to be associated with suppression of weight gain, and relative weight of the brain, which is considered to be less affected by changes in body weight, increased in the 0.04% group. As there were no histopathological changes, it was considered to be due to decreased body weight. Although relative weight of the spleen increased, hematological test revealed no changes in erythroid parameters. Therefore, it was considered to be of little toxicological significance. Histopathological test revealed simple hyperplasia of the urinary bladder in all treatment groups. Although PN hyperplasia, papilloma, and transitional cell carcinoma were observed in the 0.04% group, PN hyperplasia and papilloma were also observed in the control group, with no statistically significant difference. Although neoplastic lesions were observed in various organs, based on the incidence of these lesions, it was considered that the changes were not dose-related. Therefore, it was judged to be non-

carcinogenic in rats. [8)]

5. Results

From the above study results, no test results suggesting human health effects that could be a particular problem were found.

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