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Research report

Research on the safety re-evaluation of existing additives

FY 2010

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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22 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 11, 2011
Committee on Food Additives, Food Sanitation Commission,
Pharmaceutical Affairs and Food Sanitation Council

Research on the safety re-evaluation of existing additives (FY 2010 Survey)

Research on the safety re-evaluation of existing additives

(May 11, 2011, 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 11, 2011.

Research report

Research on the safety re-evaluation of existing additives

March 2011

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Attachment

A. Summary

In the FY 1996 Health Sciences Research Report, “Research on the safety evaluation of existing natural 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.

Among the 139 additives for which further investigation was required in the Hayashi Group Report, among the 22 additives other than those listed below that have already been reviewed for safety or those that have been excluded from the list of existing additives, this research examined 5 additives for which new safety test results could be collected: Mulberry bark extract, Propolis extract, Shikon colour, Hokosshi extract, and Montan wax.

·13 additives reported in FY 1999 “Research on the safety evaluation of existing additives” (Senior Researcher: Kurokawa Yuji) (hereinafter, referred to as the “Kurokawa Group Report”)

·16 additives reported in FY 2003 “Research on the safety re-evaluation of existing additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY 2003 Inoue Group Report”)

·14 additives reported in FY 2004 “Research on the safety re-evaluation of existing

additives" (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2004 Inoue Group Report")
·7 additives reported in FY 2006 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2006 Inoue Group Report")
·8 additives reported in FY 2007 "Research on the safety re-evaluation of existing additives" (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2007 Inoue Group Report")
·7 additives reported in FY 2008 "Research on the safety re-evaluation of existing natural additives" (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2008 Inoue Group Report")
·6 additives reported in FY 2009 "Research on the safety re-evaluation of existing natural additives" (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the "FY 2009 Inoue Group Report")
·The additives that were previously deleted from the list of existing additives (including 46 additives considered to require safety confirmation)

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 5 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. In conclusion, the five 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 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 Report published in FY 2004, 2006, 2007, 2008 and 2009, there are 14, 7, 8, 7 and 6 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 5 among the 22 out 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 22 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 individually evaluated the safety study results of 5 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 5 additives whose safety has been reviewed in this research is summarized in the Annex.

Regarding Mulberry bark extract and Shikon colour, there was no study result that suggested an immediate effect on human health at present.

Regarding Propolis extract, although decreases in serum TG were observed in the 26-week and 52-week repeated dose studies, no finding suggesting toxicity was observed in the carcinogenicity study, and therefore it was judged that there was no carcinogenicity. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health in the range of use as an additive.

With regard to Hokosshi extract, no findings suggestive of toxicity to the reproductive system were observed in the Hershberger assay, the uterine hypertrophy reaction test, or the 1-year repeated-dose toxicity/carcinogenicity combined study, which were conducted based on the

findings including decreased weight of the reproductive organs observed in the 90-day repeated-dose toxicity study. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health in the range of use as an additive.
Regarding Montan wax, no findings suggesting carcinogenicity were observed in the 1-year repeated-dose toxicity/carcinogenicity study conducted based on the observation of liver disorder in a 90-day repeated-dose toxicity study. When these study results are comprehensively evaluated, it is considered that there is no toxicity which would have a harmful effect on human health in the range of use as an additive.

E. Discussion

In this research, out of 22 items which are existing additives requiring safety confirmation in the Hayashi Team Report and have not been reviewed, regarding 5 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 is currently proceeding with a third deletion procedure for existing additives that are not in actual use, following December 2004 and September 2007.
While the operation of reviewing existing additives is steadily proceeding in this manner, it is considered necessary to continue further investigation such as the actual usage status of existing additives, and to proceed with review efficiently from the required additives.

F. Conclusion

This research showed that the basic safety of 5 additional natural additives was confirmed. It was considered that there was no immediate need to perform a further safety study at the present stage for any of these additives.

Review on the Safety of Existing Additives (as of May 2011): Review status.pdf

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Annex

Mulberry bark extract (159)

1. **Food additive name:**
   Mulberry bark extract (obtained from the skins of mulberry rhizomes, containing stilbene derivatives and flavonoids as principal components)

2. **Origin, method of preparation, and definition:**
   It is the extract obtained from the skins of the rhizome of mulberry (*Morus bombycis* KOIDZ.) of Moraceae family by extraction with water, heated ethanol, or room temperature to warm acetone. It consists mainly of flavonoids and stilbene derivatives.

3. **Major use:**
   Food manufacturing agent

4. **Summary of safety study results:**

(1) 90-day repeated-dose study

A 90-day repeated-dose toxicity test by dietary administration (0, 6,400, 16,000, 40,000 ppm) was conducted in Crl/CD(SD) rats. As a result, no death was observed in any group of animals, and there were no toxicologically significant changes in general condition, detailed clinical observations, functional tests, food consumption, ophthalmological test, urinalysis, necropsy findings, organ weights, or histopathological test that were considered to be caused by the test substance. The body weight of females in the 40,000 ppm group showed a continuous trend toward lower values after 50 days of administration.

Hematological test revealed prolonged prothrombin time and activated partial thromboplastin time in males in the 40,000 ppm group. In addition, decrease in reticulocyte count was observed in females in the test article groups, but no tendency such as anemia was observed. In males in the 40,000 ppm group, a significant decrease in segmented leukocyte ratio was observed, but no change was observed in leukocyte count, and therefore all of these changes were judged to be of no toxicological significance.

Blood biochemical test revealed increased AST and ALT in females in the 6,400 ppm or higher groups, increased γ-GTP in the 16,000 ppm or higher groups, and decreased TG in the 40,000 ppm group. However, they were judged to have no toxicological significance because the changes in AST and ALT were not dose-dependent, there were no changes in liver weight or histopathological findings, and similar findings were not observed in males.

In conclusion, the no observed adverse effect level was considered to be 16,000

ppm in both sexes (male: 1,105.9 mg/kg/day; female: 1,248.9 mg/kg/day). [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvrA*) was performed up to 5,000 μg/plate and the results were negative with and without S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum treatment concentration of 250 μg/mL, resulting in no induction of chromosomal aberrations under any treatment condition. [3)]

A micronucleus test in mice (ICR, male) was performed up to the limit dose of 2,000 mg/kg × 2, and was judged to be negative because the frequency of micronuclei showed no significant increase at any dose compared with the negative control group. [4)]

Therefore, it was concluded that genotoxicity is not observed.

(References)

1. Ikka Tsuguo: FY 2007 Study on the Safety of Existing Additives, Safety Research Institute for Chemical Compounds
2. Hisami Yamauchi: FY 2007 Safety Study of Existing Additives, BoZo Research Center Inc.
3. Sono Akira: Chromosomal Aberration Test of Mulberry bark Extract in Cultured Mammalian Cells, BoZo Research Center Inc.
4. Teramoto Shoji: FY 2007 Safety Study of Existing Additives, Institute of Environmental Toxicology

Propolis extract (376)

1. Food additive name:
Propolis extract (A flavonoid obtained from a bees' nest as principal components.)

2. Origin, method of preparation, and definition:
It is obtained by extraction with ethanol from the nest of honeybees (*Apis* spp.). It consists mainly of flavonoids.

3. Major use:
Antioxidant

4. Summary of safety study results:

(1) Repeated-dose study

Repeated-dose studies were conducted for 26 and 52 weeks (Week 26 and Week 52, respectively) by dietary administration at 0.1, 0.5 and 2.5% to F344 rats. (The test substance was manufactured using Brazilian propolis as raw material, with 50% soluble starch added as a powdering agent.) The control group received a diet containing only 2.5% soluble starch. An untreated control group (without soluble starch) was also included. As a result, there were no test article-related deaths in any of the animals in any group, and no test article-related changes in general condition, hematological test, or histopathological test. Body weight gain in the 2.5% group tended to be suppressed and was significantly suppressed in males at Week 26.

Blood biochemical test revealed significant decreases in TG in males in the 2.5% group at Week 26, and in males in the 0.1% or higher groups and females in the 2.5% group at Week 52. In males at Week 52, PL decreased at 0.5% or higher, and TCho significantly decreased at 2.5%. All the decreases in lipid parameters were dose-dependent. In addition, the decreased TG in males in the 2.5% group at Week 26 and in males and females in the 2.5% group at Week 52 was significantly lower than TG in the untreated control group, with marked decreases to about 50-60% of that in the untreated control group. Although these effects suggest a relation to suppressed body weight gain, no related findings were observed in histopathological examination, and the toxicological significance was unclear. However, the possibility that decreased TG in the 2.5% group was an adverse effect could not be ruled out.

In organ weight, at Week 26 in the 2.5% group, increased relative weight of brain, liver, testis, and adrenal gland was observed in males, and increased relative weight of liver and decreased relative weight of spleen were observed in females. At Week 52 in the 2.5% group, increased relative weight of the brain, kidney, and testis were observed in males, and increased relative weight of the brain and kidney were observed in females, and in females of the 0.5% or higher groups increased relative weight of the liver were observed. However, histopathological examination revealed no effects of the test article on any

organs.
Based on the above, the NOAEL was 0.5% for both males and females (male: 236.9 mg/kg/day, female: 287.9 mg/kg/day), based on the significant and severe decreases in serum TG in males and females in the 2.5% group [4)]

(2) Genotoxicity study

Reverse mutation assay using bacteria (TA98, TA100, TA1535, TA1537, WP2*uvr*A/pKM 101) induced His+ revertant colonies that were at least twice those of the vehicle control in strains TA98 (+S9 mix) and TA1537 (+S9 mix), and also showed dose-dependency (0.0125, 0.125 mg/plate). Since reproducibility was also observed, it was judged to be positive. [1)]
In a chromosomal aberration test using cultured mammalian cells (CHL/IU), chromosomal aberration was induced in a dose-dependent manner (144, 294 μg/mL) regardless of the presence or absence of S9 mix. The potency was moderate (D_{20} 0.104 mg/mL; TR 218). [2)]
Mice (ICR, SPF, male, 3 at each dose) were administered aqueous solutions twice by gavage at 500, 1,000 and 2,000 mg/kg to perform a bone marrow micronucleus test. Since there was no significant increase in the frequency of micronucleated polychromatic erythrocytes, and no dose-dependency was observed, the test substance was considered negative. [3)]
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) Carcinogenicity study

In a 2-year carcinogenicity test in Wistar Hannover rats fed diet containing 0.5% or 2.5% test substance (manufactured from Brazilian propolis), there were no test article-related effects on general condition, but the survival rate was significantly higher in males in the 0.5% group and females in the 2.5% group than in the control group. Suppression of body weight gain was observed in males and females in the 2.5% group, which could result from malnutrition caused by long-term feeding with feed containing a high concentration of the test substance. There was no significant difference in organ weight between each treated group and the control group. Histopathological test did not reveal any test substance-related increase in either non-neoplastic or neoplastic changes, but there was a significant decrease in the incidence of lymphoma/leukemia in males and females in the 2.5% group, and a significant decrease in the incidence of pituitary tumor in males in the 0.5% group and females in the 2.5% group. Although the incidence of thyroid C-cell adenoma was significantly increased in females administered groups, the incidence of C-cell adenoma was reported to be about 10% in the existing background data, and the significant difference was thought to be because no occurrence was observed in females in the control group in this study.
Based on the above, it was concluded that the Propolis extract was not carcinogenic. [5)]

(References)

1. Kojima Akinori: Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
2. Mochizuki Nobuhiko: Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center
3. Iwamoto Tsuyoshi: Test Results on the Reevaluation, etc. of the Safety of Food Additives, The Institute of Environmental Toxicology
4. Kanno Jun: Effects of Long-term Administration of Propolis on F344 rats by 1-year Repeated-dose Toxicity Study, Toxicology Division, Biological Safety Research Center, National Institute of Health Sciences
5. Wanibuchi Hideki: FY 2005 Health and Labor Sciences Research Grant, Studies on Carcinogenicity of Natural Additives

Shikon colour (211)

1. Food additive name:

Shikon colour (the main component of shikonin obtained from the purple gromwell root.)

2. Origin, method of preparation, and definition:

It is obtained by extraction from the roots of purple gromwell (*Lithospermum erythrorhizon* SIEBOLD et ZUCCARINI) of Boraginaceae family with ethanol at room temperature. It consists mainly of shikonin as a principal coloring component. It is red to red-purple.

3. Major use:

Color

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration (0.02, 0.06, 0.17, 0.5%). As a result, no animal deaths were observed, and no changes related to the test substance were observed in general condition, food intake or hematological test. Suppression of body weight gain was observed in females in the 0.17% or higher groups and males in the 0.5% group.

Blood biochemical test revealed increased P in males and females in the 0.5% group and increased K in females in the 0.06% or higher groups. However, both of these changes were mild and clear dose relation was not observed, suggesting that they were of little toxicological significance.

In organ weight, decrease in absolute weight of lung, heart, and spleen and increase in relative weight of brain, liver, kidney, and testis in males in the 5.0% group, increase in relative weight of brain, heart, spleen, and kidney in females in the 0.17% or higher groups, and increase in relative weight of thymus, lung, and liver in females in the 0.5% group were observed, but all changes other than the kidney were mild, and there was no clear dose relation.

Macroscopic observation at necropsy showed black discoloration of the kidneys in males and females in the 0.17% or higher groups, and histopathological examination of the kidneys revealed deposition of yellowish brown granules in the cytoplasm of proximal tubular epithelial cells in the cortex in females in the 0.17% or higher groups and males in the 0.5% group, showing positive Schmorl reaction. Eosinophilic bodies were observed in males in the 0.17% or higher groups, and regenerative tubules were observed in males in the 0.5% group. Eosinophilic bodies are highly specific lesions in males, and were considered to have little toxicological significance.

In conclusion, the no observed adverse effect level was considered to be 0.17%

(96.0 mg/kg/day) in males and 0.06% (34.4 mg/kg/day) in females. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2 *uvrA*) was performed up to 5,000 μg/plate and the results were negative with and without S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the maximum treatment concentrations of 8.00 μg/mL (short-term treatment: +S9 mix) and 2.00 μg/mL (short-term treatment: -S9 mix). The results showed that the short-term treatment method was negative in the presence of S9 mix, but structural chromosomal aberrations were observed at the highest dose of 2.00 μg/mL in the absence of S9 mix (D20 value: 0.0037 mg/mL, TR value: 5,000). No induction of numerical chromosomal aberrations was observed. [3)]

A micronucleus test in mice (ICR, male) was performed up to 1,000 mg/kg × 2, and was judged to be negative because the frequency of micronuclei showed no significant increase at any dose compared with the negative control group. [4)]

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

(References)

1. Nishikawa Akiyoshi: FY 2007 Study on the Preparation of Standards and Criteria, etc. for Food Additives (90-day Repeated-dose Toxicity Study of Shikon Colour in F344 Rats), National Institute of Health Sciences
2. Yamauchi Hisami: FY 2007 Safety Study of Existing Additives (Bacterial Reverse Mutation Test of Shikon Colour), BoZo Research Center Inc.
3. Sono Akira: FY 2007 Safety Study of Existing Additives (Chromosomal Aberration Test of Shikon Colour in Cultured Mammalian Cells), BoZo Research Center Inc.
4. Teramoto Shoji: FY 2007 Safety Study of Existing Additives (Micronucleus test (shikon colour)), Institute of Environmental Toxicology

Hokosshi extract (411)

1. Food additive name:

Hokosshi extract (obtained from the seeds of hokosshi; contains bakuchiol as the major component.)

2. Origin, method of preparation, and definition:

It is obtained by extraction with ethanol from the seeds of hokosshi *(Psoralea corylifolia* O.KZE.) of Fabaceae family. It consists mainly of bakuchiol.

3. Major use:

Food manufacturing agent

4. Summary of safety study results:

(1) 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 0.38, 0.75, 1.5, and 3.0%. As a result, suppressed body weight gain in administered groups at 0.75% or more in males and 0.38% or more in females, increased γ-GPT in serological tests at 1.5% or more in females and 3.0% in males, decreased absolute and relative weights of the testis and ovary, and histopathological abnormalities in the testis and ovary in the 1.5% or higher groups were observed.

The NOAEL was estimated to be 0.38% (53 mg/kg/day) for males, but could not be determined for females. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and TA1538) was judged to be negative, because His+ revertant colonies were not induced regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test in cultured mammalian cells (CHL) induced structural chromosomal aberrations with and without metabolic activation. [3)]

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

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

(3) Hershberger assay

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 antiandrogen 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 were observed in any administered group, but crust at the administration site was observed in the 1,000 mg/kg group, suppressed body weight gain was observed in the 1,000 mg/kg group (antiandrogen effect test), and decreased food consumption was observed in the 300 mg/kg group (antiandrogen effect test). In the organ weight, increased relative liver weight was observed in the 300 mg/kg or higher groups (androgen effect test), but no significant change suggesting androgenic effect or antiandrogen effect was observed.
After oral administration, no death was observed in any administered group, and no change was observed in body weight or food consumption. In general condition, salivation was observed immediately after administration in the 300 mg/kg or higher groups. 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 Hokosshi extract 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 30, 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 weights were measured about 24 hours after the final administration.
In the results of subcutaneous and oral administration, no death was observed in any administered group, and no change was observed in general condition, but inhibition of weight gain was observed in the 1,000 mg/kg/day group (estrogen effect test). An increase in uterine weight was observed in the 300 mg/kg/day and

higher groups, which confirmed estrogen effect, but no significant change suggesting an antiestrogenic effect was observed in any administered group. From the above results, it was concluded that the Hokosshi extract may have weak estrogenic effect *in vivo* by subcutaneous and oral administration. [5)]

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

In a 1-year repeated-dose toxicity test by dietary administration (0.04, 0.2, 1.0, 1.5%) using Wistar Hannover rats, there were no animal deaths or abnormalities of general condition. In body weight and food consumption, suppressed body weight gain was observed in males in the 1% or higher groups and females in the 0.2% or higher groups, and decreased mean food consumption in males and females in the 1% or higher groups. Hematological test revealed increased white blood cell count in males and females in the 0.04% group and increased lymphocytes (%) in females in the 1% group. Blood biochemical test revealed decreased TG in males and females in the 1% or higher groups and decreased GOT in males and females in the 1.5% group. However, these changes were considered to be of little toxicological significance because the range of variation in these values was very slight and there were no changes in other related parameters. Changes in organ weight were observed in several organs, but they were considered attributable to low body weight caused by administration of the test substance. There were no changes in the weight of the testes or ovaries, and no atrophy of the testes was observed. Histopathological test revealed no specific changes that were attributable to the test article, nor were there any organic changes in the testes or ovaries. The changes in the testes and ovaries observed in the 90-day repeated-dose study in F344 rats could not be confirmed in Wistar Hannover rats, suggesting that the damage to testes and ovaries in F344 rats was due to strain differences. The no observed adverse effect level was estimated to be 0.2% (76.27 mg/kg/day) in males and 0.04% (19.20 mg/kg/day) in females, based on body weight inhibition.

In a 2-year carcinogenicity test by dietary administration (0.04, 0.2, 1%) using Wistar Hannover rats, there were no deaths and no effects on general condition or urinalysis related to administration of the test substance. Decreased food consumption and associated suppression of body weight gain were observed in males in the 1% group and females in the 0.2% or higher groups. In hematological and blood biochemical tests, significant changes were observed in some test items, but these changes were slight and not dose-related, and therefore the effects of the test substance could not be estimated. Blood hormone measurements showed no abnormality in thyroid-stimulating hormone, thyroid hormones (T3, T4), estradiol, or progesterone. A mild increase in testosterone levels was

observed in males in the 1% group. However, it could not be determined to be an effect of the test substance because of the high variability in the levels. Absolute organ weight decreased in several organs, but these changes were estimated to be associated with decreased body weight based on the changes in relative weight. Histopathological test revealed an increase in pituitary tumor in males in the 1% group, but no significant increase in incidence or dose dependence was observed in the other groups. [6)]

(References)

1. Hirose Masao: FY 1998 Study on the Preparation of Standards and Criteria, etc. for Food Additives, National Institute of Health Sciences
2. Miyabe Masaki: FY 1997 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Kurita Toshishiro: FY 1997 Study on the Reevaluation, etc. of the Safety of Food Additives, The Institute of Environmental Toxicology
4. Kurita Toshishiro: FY 1997 Study on the Reevaluation, etc. of the Safety of Food Additives, The Institute of Environmental Toxicology
5. Ota Ryo: FY 2003 Study on Standards and Criteria for Food and Food Additives, etc., Hatano Research Institute, Food and Drug Safety Center
6. Kenji Kamiya: Health and Labour Sciences Research Grant (Research on carcinogenicity of existing additives - A 1-year repeated-dose toxicity/carcinogenicity study of Hokosshi extract in rats -)

Montan wax (453)

1. Food additive name:

Montan wax (Composed mainly of fatty acids and tetracosyltriacontanyl alcohol or esters of fatty acids and hexacosyltriacontanyl alcohol obtained from brown charcoal or lignite.)

2. Origin, method of preparation, and definition:

It is obtained from brown charcoal or lignite, extracted with organic solvent. It consists mainly of esters of fatty acids from C_{20} to C_{30} and tetracosyltriacontanyl alcohol or hexacosyltriacontanyl alcohol.

3. Major use:

Gum base, polish

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with the test substance at concentrations of 0.56, 1.67, and 5%. As a result, no animal deaths were observed, and no changes were observed in general condition or food intake.

Hematological test revealed decreases and increases in erythroid parameters and increased white blood cell count in both sexes. In blood biochemical test, effects of the test substance were observed in many parameters in both males and females. In particular, AST and ALT were about 5 times higher than in the control group, indicating severe liver disorder. In many organs, especially the liver, spleen, and lung, increased organ weight was observed in both males and females in all administered groups, both the absolute and relative weight. Histopathological test revealed severe multiple and diffuse granulomatous inflammation in the liver with necrosis of hepatocytes in both males and females. In the lung, small nodular lesions with diffuse lymphocyte infiltration were observed, which were considered to be the cause of increased lung weight. Small granulomas were observed in the mesenteric lymph nodes in a dose-dependent manner.

The NOAEL could not be established. [3)]

(2) Genotoxicity study

A reverse mutation test using bacteria (TA98, TA100, TA1535, TA1537, WP2*uvr*A/pKM101) was negative with and without metabolic activation. [1)]

A chromosomal aberration test was performed using cultured mammalian cells

(CHL/IU). No induction of chromosomal aberrations was observed. [2)]
In a micronucleus test using mouse bone marrow, micronucleus induction was not significantly increased in any dose group. [2)]
Based on the above results, this substance is not considered to be genotoxic.

(3) 1-year repeated-dose toxicity/carcinogenicity study
In a 1-year repeated-dose toxicity test by dietary administration (0.005, 0.05, 0.5%) using F344 rats, there were no deaths or effects on general condition related to administration of the test substance. There were no changes caused by the administration in ophthalmological test, urinalysis, hematological test, organ weights, necropsy, or pathological test. In blood biochemical test, decreased AIP activity and decreased urea nitrogen were observed in females, but they were decreases rather than increases, and decreased glucose was observed, but it was a very slight change, and no change was observed in pathological test of liver. Therefore, all of these changes were considered to be of little toxicological significance.
In conclusion, the no observed adverse effect level was estimated to be 0.5% (male: 301.8 mg/kg/day; female: 349.1 mg/kg/day). [4)]
In a 2-year carcinogenicity test by dietary administration (0.005, 0.05, 0.5%) using F344 rats, there were no deaths or effects on general condition related to administration of the test substance. Body weight and food consumption were high in males and females in each test substance administered groups. Hematological test at the end of the administration period showed no change. Pathological test revealed that there were no neoplastic or non-neoplastic lesions caused by the administration.

(References)

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