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Research report

Research on the safety re-evaluation of existing additives

FY2004

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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16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.

Administrative Information, Ministry of Health, Labour and Welfare

July 28, 2005-Data relating to the Pharmaceutical Affairs and Food Sanitation Council Research on the safety re-evaluation of existing additives (FY 2004 Survey)

Research on the safety re-evaluation of existing additives

(July 28, 2005, 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 July 28, 2005.

FY 2004 food additive safety confirmation grant research

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

July 2005

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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, an investigation was performed on food additives for which safety study results were newly available, including 108 additives out of the 139 additives that had been considered to require further investigation regarding safety in the Hayashi Group Report, excluding 14 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”) and 17 additives reported in FY 2003 “Research on the review of the safety of existing natural additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “Inoue Group Report”).

This report collectively lists investigation results for 14 additives, namely: Agrobacterium succinoglycan, Linseed gum, Aloe vera extract, Fish scale foil, Sandarac resin, Sphingolipid, Paffia extract, Isodonis extract, Himematsutake extract, Betaine, Carthamus red, Mevalonic acid, Morin and Logwood colour.

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 14 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. In conclusion, as there is presently no study result that suggests any immediate effects on human health, it is considered that there is no immediate need to perform a new safety study.

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 on the list of existing additives have not been checked individually for safety, therefore, confirmation of their safety is requested in the Diet, etc

In FY 1996, the basic safety of 489 existing additives was investigated based on international evaluation results, approval status in Europe and the United States, and safety study results, etc., and was then published as the Hayashi Group Report. In the report, 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." 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."

The present survey aimed to investigate the basic safety of natural additives by collecting domestic and overseas study results and evaluating those results for 108 of the 139 additives that had been considered to require investigation for safety in the FY 1996 Hayashi Group Report, excluding 14 additives that completed the safety review in the FY 1999 Kurokawa Group Report and 17 additives that completed the safety review in the FY 2003 Inoue Group Report.

C. Methods

Among 108 of the 139 existing additives that were considered to require an investigation for safety in the Hayashi Group Report, excluding 14 additives that had completed the safety review in the Kurokawa Group Report and 17 additives that had completed the safety review in the FY 2003 the Inoue Group Report, 14 additives for which the results of both a 90-day or longer repeated-dose toxicity study and a mutagenicity study were available were individually subjected to an evaluation of those safety study results.

D. Results

Regarding the 14 additives for which safety was reviewed in this survey, there is no study result that suggests any immediate effects on human health, at present, for any of these additives (a summary is provided in the Annex).

E. Discussion

Safety study results were collected for the 108 existing additives that had not completed a safety review, among those that had been considered to require an investigation regarding safety in the Hayashi Group Report, and the study results were evaluated for 14 existing additives for which both the results of a repeated-dose toxicity study at least 90 days in length and a mutagenicity study were available. As there were no study results that suggest an immediate effect on human health, at present for any of these additives, it is therefore considered that there is no immediate need to perform a new safety study for the 14 existing additives that were evaluated.

The Ministry of Health, Labour and Welfare deleted Madder colour from the list of existing additives and banned its use in July 2004, based on the evaluation of its carcinogenicity performed by the Food Safety Commission and the Pharmaceutical Affairs and Food Sanitation Council. In addition, 38 existing additives (25 additives that were considered to require safety confirmation) were deleted in December 2004 as existing additives that are not actually used. While the operation of reviewing existing additives is currently undergoing steady progress, it is considered necessary to continuously investigate the actual usage status of existing additives, and to proceed with reorganization efficiently from additives that require information. In addition, see the attached reference materials for details regarding specific safety review status.

F. Conclusion

The basic safety of 14 existing additives was newly confirmed by this survey, based on the collected study results. It is considered that there is no immediate need to perform a safety study, at the present stage for any of these additives.

Annex

Agrobacterium succinoglycan

1. Food additive name:

Agrobacterium succinoglycan (obtained from the culture solution of Agrobacterium; contains succinoglycan as the major component.)

2. Origin, method of preparation, and definition:

Agrobacterium succinoglycan is a polysaccharide obtained from the culture solution of a bacterium (*Agrobacterium tumefaciences*). It consists mainly of succinoglycan.

3. Major use:

Thickening stabilizer

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 0.5, 1.5, and 5%. In the test, no animal deaths or changes in general condition, body weight, or food intake were observed.

There was no significant effect caused by the intake of the additive based on hematological, blood chemical or pathological tests.

Based on these results shown above, the no observed adverse effect level of Agrobacterium succinoglycan is considered to be 5% (3.64 g/kg) for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate and yielded negative results regardless of the presence or absence of metabolic activation. [2)]

In a chromosomal aberration test performed in cultured mammalian cells (CHL/IU) up to 5.0 mg/mL (physiological limit concentration), no induction of chromosomal aberrations was observed under any treatment condition. [3)]

A micronucleus test in the bone marrow of mice (ICR male) was performed up to 2,000 mg/kg×2, which was the limit dose, and no induction of micronuclei was observed at any dose level. [4)]

(References)

1. Harada Masaoki: Kanagawa Cancer Center Research Institute
2. Honma Masamitsu: Health Sciences Research Grant, Division of Genetics and

Mutagenesis, National Institute of Health Sciences
3 and 4. Tanaka Noriho: Health Sciences Research Grant, Food and Drug Safety Center

Linseed gum

1. Food additive name:

Linseed gum (obtained from linseeds; contains polysaccharides as the major component.)

2. Origin, method of preparation, and definition:

Linseed gum is obtained from the endosperms of linseed (i.e., the seeds of flax (*Linum usitatissimum* LINNE) of the Linaceae family) by extraction with room temperature to a warm water or hydrous alcohol. It consists mainly of polysaccharides.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by gavage with the test substance at doses of 100, 300, and 1,000 mg/kg. It showed no animal deaths, and no change that was considered to be caused by the test substance was observed in any of general condition, food intake, ophthalmological test, urinalysis, hematological test, blood chemical test, organ weight, macroscopic observation, or histopathological test.

Mild suppression of body weight gain was observed in both males and females of the 1,000 mg/kg group.

Based on these above results, the no observed adverse effect level of Linseed gum is considered to be 300 mg/kg for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5 mg/plate and yielded negative results regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5 mg/mL, resulting in no induction of chromosomal aberration by either short-term treatment or continuous treatment. [3)]

A micronucleus test was performed in the peripheral blood of mice (ddy male, 7 weeks of age) with Linseed gum suspended in olive oil at up to 2,000 mg/kg (10 ml/kg) ×2, and no induction of micronuclei was observed at any dose. [4)]

Based on the above results, it was judged that Linseed gum has no genotoxicity.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Food and Drug Safety

Center

2. Kojima Akinori: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Mochizuki Nobuhiko: FY 2001 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center
4. Honma Masamitsu: Health Sciences Research Grant, National Institute of Health Sciences

Aloe vera extract

1. Food additive name:

Aloe vera extract (obtained from the leaves of aloe; contains polysaccharides as the major component).

2. Origin, method of preparation, and definition:

Aloe vera extract is obtained from the leaves of aloe (*Aloe vera* LINNE) of the Liliaceae family by squeezing. It consists mainly of polysaccharides.

3. Major use:

Thickening stabilizer

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats with the test substance at doses of 100, 300, and 1,000 mg/kg by gavage. Then no changes were observed in body weight, food intake, or ophthalmological examination. Significant differences were observed in some test items in hematological test, blood chemical test, urinalysis and organ weight. However, the changes were considered to be accidental or of little toxicological significance, based on background data, dose-dependency, or histopathology. Autopsy showed stunted, softened testicles and atrophy in the seminiferous tubules in one male of the 1,000 mg group, both of which were judged as naturally occurring lesions.

Based on these results, the no observed adverse effect level of Aloe vera extract is considered to be 1,000 mg/kg for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKm101) was performed up to 5 mg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in mammalian cells up to the maximum dose of 5 mg/ml (CHL/IU), resulting in negative results regardless of the presence or absence of metabolic activation. [3)]

A micronucleus test in the bone marrow of mice (ICR male) was performed up to the limit dose of 2,000 mg/kg, and no induction of micronuclei was observed at any dose. [4)]

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Food and Drug Safety Center
2. Matsushima Taijiro: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center
3. and 4. Iwamoto Tsuyoshi: FY 1999 Study on the Preparation of Standards and Criteria, etc.

for Food Additives, The Institute of Environmental Toxicology

Fish scale foil

1. Food additive name:

Fish scale foil (fine fish scales collected and purified from the scales of *Trichiurus lepturus* LINNE, *Clupea pallasi* CUVIER et VALENCIENNES, *Sardinops melanosticta* TEMMINCK et SCHLEGEL, etc. It consists mainly of guanine.)

2. Origin, method of preparation, and definition:

Fish scale foil is obtained by collecting the epithelium of the fish body of *Sardinops melanosticta* TEMMINCK et SCHLEGEL of the Clupeidae family, *Trichiurus lepturus* LINNE of the Trichiuridae family or *Clupea pallasi* CUVIER et VALENCIENNES of the Clupeidae family, washing with water or weakly alkaline aqueous solution at room temperature, and extracting with ethanol at room temperature. The principal coloring component is unknown but contains guanine. Fish scale foil is white to light yellow-gray.

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 gavage with the test substance at doses of 100, 300, and 1,000 mg/kg. It showed no animal deaths, and no change was observed in food intake or body weight.

Hematological test showed increased mean red blood cell hemoglobin content in the female 300 mg/kg group. However, this increase was mild and not observed in the 1,000 mg/kg group and was accordingly judged not to have been caused by the test substance.

Blood chemical test showed no changes caused by the test substance.

Regarding organ weight, higher absolute weights for the brain and spleen were observed in the male 1,000 mg/kg group, lower absolute weight for the thyroid was observed in the female 300 mg/kg group, and lower relative weight for the thyroid was observed in the female 300 mg/kg and 1,000 mg/kg groups. However, as these observations were not associated with histopathological changes, etc., it was judged that they were not caused by the test substance.

Histopathological test showed no changes caused by the test substance.

Based on the above results, the no observed adverse effect level of Fish scale foil is considered to be 1,000 mg/kg for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537,

and TA1538) was performed up to 25 mg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) with the addition of a 1%CMC suspension, up to the maximum dose of 5 mg/mL. In this test, structural aberrations were observed in less than 10% of the cells in continuous treatment for 24 and 48 hours, and the results were reported as a suspected positive. [3)]

In a micronucleus test in the bone marrow of mice (ddy male, 8 weeks of age), a suspension in 1% tragacanth solution was administered orally up to the maximum dose of 2,000 mg/kg/day×2. The results were judged as negative because there was a decreasing trend in the polychromatophilic erythrocyte (PCE) ratio, but no significant increase in the percentage of polychromatophilic erythrocytes with micronuclei. [4)]

Based on the above results, it is judged that Fish scale foil has no genotoxicity.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Food and Drug Safety Center
2. Miyabe Masaki: FY 1997 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Sofuni Toshio: 1997 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences
4. Kishi Michiko: FY 1997 Results of Study on Reevaluation, etc. of the Safety of Food Additives, Kanagawa Prefectural Institute of Public Health

Sandarac resin

1. Food additive name:

Sandarac resin (obtained from the secretion of sandarac; contains sandaracopimaric acid as the major component).

2. Origin, method of preparation, and definition:

Sandarac resin is obtained from oleoresin obtained from the secretion of *Tetraclinis articulata* (VAHL.) MAST. of the Cupressaceae family, by extraction with ethanol at room temperature, followed by distillation of ethanol from the filtrate. It consists mainly of sandaracopimaric acid.

3. Major use:

Gum base

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 test substance concentrations of 0.2, 1.0, and 5.0%. It showed no animal deaths, and no changes caused by the test substance were observed in general condition or food intake.

Hematological test showed decreased Ht in males and females of the 5.0% group and increased MCHC in males. Blood chemical test showed increased BUN and decreased TG in males of the 5.0% group, and decreased AIT in males of the 1.0% group. All these changes were judged to be of little toxicological significance.

Regarding organ weights, no differences between groups were observed in the absolute weights of major organs. Regarding relative weights, a significant increase in that of the liver was observed in males of the 1.0% and 5.0% groups and in females of the 5.0% group. Histopathological test also showed mild centrilobular hepatocellular hypertrophy (with no significant difference) in 2 animals in the male 5.0% group, which may be involved in the observed increased relative liver weight.

Pathological test, including the observation of mild centrilobular hepatocellular hypertrophy, showed no significant differences in frequency or severity.

The no observed adverse effect level is considered to be 0.2% (123.6 mg/kg/day) for males, and 1% (686.6 mg/kg/day) for females. [1)]

(2) Genotoxicity study

A reverse mutation test was performed in *Salmonella typhimurium* (TA98, TA10, TA1535, TA1537, and WP2*uvr*A/pKM101) up to 5,000 μg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU), and the results showed no induction of chromosomal aberrations under any treatment condition. However, polyploids were induced at a high dose, where precipitation was observed. [3)]

A micronucleus test in the bone marrow of mice (ICR male, 8 weeks of age) was performed up to the limit dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. [4)]

Therefore, the chromosomal aberration inducibility observed *in vitro* was not considered to cause any particular problem in the body, as it was not observed in the micronucleus test in rodents, in which properly high doses had been investigated.

(References)

1. Sekita Seiji: Health and Labor Sciences Research Grant, Division of Toxicology, National Institute of Health Sciences
4. Nakajima Madoka: Health Sciences Research Grant, Biosafety Research Center
3. Matsumoto Kyomu: Health and Labor Sciences Research Grant, The Institute of Environmental Toxicology
4. Miyakawa Makoto: Mitsubishi Chemical Safety Institute Ltd.

Sphingolipid

1. Food additive name:

Sphingolipid (obtained from the bovine brain or rice bran; contains sphingosine derivatives as major components).

2. Origin, method of preparation, and definition:

Sphingolipid is obtained from the extract of the brain of cattle (*Bos taurus* LINNE) of the Bovidae family, or rice bran obtained from the seeds of rice (*Oryza sativa* LINNE) of the Poaceae family or the wheat germs (*Triticum aestivum* LINNE) by extraction at from room temperature to a warm temperature with ethanol, hydrous ethanol, isopropyl alcohol, acetone, hexane, or ethyl acetate. It consists mainly of sphingosine derivatives.

3. Major use:

Emulsifier

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in Wistar Hannover (GALAS) rats by gavage with test substance doses of 60, 250, and 1,000 mg/kg. It showed no animal deaths, and no changes caused by the test substance were observed in general condition, body weight gain, hematological and serum biochemical test, organ weight or histopathological test.

The no observed adverse effect level is considered to be 1,000 mg/kg for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in S*almonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5,000 μg/mL, resulting in no induction of chromosomal aberrations under any treatment conditions. [3)]

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

(References)

1. Mitsumori Kunitoshi: Health and Labor Sciences Research Grant, Professor, Department of Veterinary Medicine, Tokyo University of Agriculture and Technology
2. Matsushima Taijiro: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Japan Bioassay Research Center

3. Mochizuki Nobuhiko: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center
4. Iwamoto Tsuyoshi: The Institute of Environmental Toxicology

Paffia extract

1. Food additive name:

Paffia extract (obtained from the roots of paffia; contains ecdysteroid and saponin as the major components).

2. Origin, method of preparation, and definition:

Paffia extract is obtained from the roots of paffia (*Paffia iresinoides* SPRENGEL) of the Amaranthaceae family by extraction with aqueous ethanol at lukewarm temperature. It consists mainly of ecdysteroid, saponin, etc.

3. Major use:

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 with the test substance at doses of 100, 300, and 1,000 mg/kg. It showed no animal deaths, and no changes that were considered to be caused by the test substance were observed in any of general condition, food intake, ophthalmological examination, urinalysis, hematological test, blood chemical test, organ weight, visual examination, or histopathological test.

In addition, sporadic changes were observed in several test items; however, these were judged to be of no toxicological significance, and to not be caused by the test substance, as there was no dose relationship, etc.

Based on the above results, the no observed adverse effect level of Paffia extract is considered to be 1,000 mg/kg for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5 mg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5 mg/mL, resulting in no induction of chromosomal aberrations at any dose, regardless of the presence or absence of metabolic activation. [3)]

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

(References)

1. Ogawa Hiroshi: FY 2003 Study on the Safety of Existing Additives, Food and Drug Safety Center

2. Matsushima Taijiro: FY 2002 Study on the Safety Evaluation, etc. of Food Additives, Japan Bioassay Research Center
3. Iwamoto Tsuyoshi: FY 2002 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology
4. Sato Shuji: FY 2002 Results of Study on Reevaluation, etc. of the Safety of Food Additives, Kanagawa Prefectural Institute of Public Health

Isodonis extract

1. Food additive name:

Isodonis extract (obtained from the stems or the leaves of *Isodon japonicus* HARA; contains enmein as the major component).

2. Origin, method of preparation, and definition:

Isodonis extract is obtained from the stems or leaves of *Isodon japonicus* HARA of the Lamiaceae family by extraction with ethanol. It consists mainly of diterpenoids (e.g., enmein).

3. Major use:

Bitterant, etc.

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by gavage with the test substance at doses of 100, 300, and 1,000 mg/kg. It showed no animal deaths, no effect on their general condition and body weight, and no effect on the results of urinalysis and autopsy caused by the administration with the test substance. Significant differences were observed in some test items in food intake, ophthalmological test, hematological test, blood chemical test, organ weight, and histopathological test; however, these were accidental and without a dose relationship, and were judged to be of little toxicological significance. [1)]

Based on the above results, the no observed adverse effect level of Isodonis extract is considered to be 1,000 mg/kg for both males and females.

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5 mg/plate. A slight increase in revertant colonies was observed at the maximum dose in WP2*uvr*A/pKM101 without metabolic activation. However, neither dose-dependency nor reproducibility were observed, and it was comprehensively judged as negative. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 1.25 mg/ml, resulting in increased cells with structural aberrations only at higher doses for which 50% or higher cell growth inhibition was observed in a cell growth inhibition assay, regardless of the presence or absence of metabolic activation. [3)]

A micronucleus test in the bone marrow of mice (ICR male, 7 weeks of age) was performed up to the limit dose of 2,000 mg/kg, and no micronucleus induction was observed at any dose. [4)]

Isodonis extract was not considered to express genotoxicity in the body, considering that the abnormal induction observed in the chromosomal aberration test in cultured mammalian cells was a response obtained only at doses at which cytotoxicity was expressed, that there was no clear dose relationship, and that the micronucleus test in rodents performed up to the limit dose showed negative results.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Food and Drug Safety Center
2. -4. Matsumoto Kyomu: Health and Labor Sciences Research Grant, The Institute of Environmental Toxicology

Himematsutake extract

1. Food additive name:

Himematsutake extract (obtained by extraction from the mycelia or fruiting bodies of himematsutake or its culture solution).

2. Origin, method of preparation, and definition:

Himematsutake extract is obtained by extraction with water from the mycelia or fruiting bodies of the basidiomycete himematsutake (*Agaricus blazei* MURR.) or its culture solution.

3. Major use:

Bitterant, etc.

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 0.63, 1.25, 2.5, and 5%. It showed no animal deaths, and little effect was observed on general condition, body weight gain and food intake.

Hematological test showed a significant increase in MCV in the female 5% group; however, a dose relationship was not observed, and it was not considered to be the effect of the test substance.

Serum biochemical test showed a significant increase in BUN in the male 2.5% group and higher. However, CRN showed a significant decrease in the 0.63% group and higher to the contrary, and there was no relationship with kidney weight or changes in kidney tissues. Accordingly, this was considered to be of little toxicological significance. In addition, significant differences were observed in ALB, TC, etc., although a dose relationship was not observed. In females, only BUN showed a significant increase in the 1.25% group, and was considered to be an accidental change.

Regarding organ weight, relative liver weight increased in the male 1.25% and 2.5% groups, and relative brain weight increased significantly in the female 2.5% group. However, there was no dose response, and these changes were considered to be accidental.

Histopathological test showed microgranuloma and extramedullary hematopoiesis in the liver and basophilic renal tubules and hyaline deposits in the kidney in males and females, and mineralization and mild inflammatory cell infiltration in the myocardium in females. These are naturally occurring lesions and were considered to be accidental and of little toxicological significance.

The no observed adverse effect level is considered to be 5% (male: 2,645 mg/kg/day; female: 2,965 mg/kg/day) for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in Salmonella typhimurium (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5,000 μg/mL, resulting in no induction of chromosomal aberrations under any treatment condition. [3)]

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

(References)

1. Nishikawa Akiyoshi: Health and Labor Sciences Research Grant, Division of Pathology, National Institute of Health Sciences
2. Ajimi Shozo: Health Sciences Research Grant, Chemicals Evaluation and Research Institute
3. Nakajima Madoka: Health and Labor Sciences Research Grant, Biosafety Research Center
4. Kishi Michiko: Kanagawa Prefectural Institute of Public Health

Betaine

1. Food additive name:

Betaine

2. Origin, method of preparation, and definition:

Betaine is obtained from the molasses of sugar beet (*Beta vulgaris* LINNE var. *rapa* DUMORTIER) of the Chenopodiaceae family by separation. It consists mainly of Betaine.

3. Major use:

Flavoring agent

4. Summary of safety study results:

(1) Repeated-dose study

A 52-week chronic toxicity test (1.0, 2.3, and 5.0%) and a 104-week carcinogenicity test (1.0% and 5.0%) were performed in F344 rats by dietary treatment.

No marked differences were observed in general condition in either the chronic toxicity test or carcinogenicity test. Body weight increased gradually throughout the test period in all groups. Food intake was significantly lower, sporadically at some weeks in the administered group compared to the control group in both the chronic toxicity and carcinogenicity tests.

There was no difference from the control group in the survival rate in the carcinogenicity test for either females or males.

Hematological test showed decreased mean corpuscular volume in male groups at 2.3% and higher and female groups at 1.0% and higher and decreased mean red blood cell hemoglobin content in the female and male 5.0% groups in the chronic toxicity test, as well as decreased mean corpuscular volume and decreased mean red blood cell hemoglobin content in the female 5.0% group in the carcinogenicity test. Moreover, an increasing trend in platelet count was observed in the male and female administered groups with Betaine in both the chronic toxicity and carcinogenicity tests.

Blood biochemical test performed in the chronic toxicity test showed decreased total protein concentration, albumin concentration, A/G ratio and GOT in the female and male administered groups with Betaine, and decreased ALP and GPT in the male administered groups with Betaine.

Regarding organ weight, increased liver and kidney weights were observed in the female and male 5.0% groups in both the chronic toxicity study and the carcinogenicity test.

Histopathological test showed no differences from the control group in frequency and severity in either females or males in both the chronic toxicity test and the carcinogenicity

test. There were no tumor or nonneoplastic changes for which the frequency increased in a dose-dependent manner.

Based on the above results, the no observed adverse effect level was 5.0% (male: 4,821 mg/kg/day; female: 4,150 mg/kg/day), and no carcinogenicity was shown at concentrations up to 5.0%. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed at up to 5,000 μg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5,000 μg/mL, resulting in no induction of chromosomal aberrations under any treatment condition. [3)]

A micronucleus test in the bone marrow of mice (ICR male) was performed up to the limit dose of 2,000 mg/kg, and no micronucleus induction was observed at any dose. 4)

(References)

1. Ono Hiroshi: Food and Drug Safety Center
2. Kojima Akinori: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. and 4. Iwamoto Tsuyoshi: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

Carthamus red

1. Food additive name:

Carthamus red (obtained from the flowers of safflower; contains carthamin as the major component).

2. Origin, method of preparation, and definition:

Carthamus red is obtained from the flowers, or the fermented or enzymatically treated flowers of safflower (*Carthamus tinctorius* LINNE) of the Asteraceae family, by removing yellow pigments, followed by extraction at room temperature with a weakly alkaline aqueous solution, and neutralization. It consists mainly of carthamin as a principal coloring component. Carthamus red is red.

3. Major use:

Color

4. Summary of safety study results:

(1) Acute toxicity study

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

(2) Repeated-dose study

A 90-day repeated-dose test was performed in F344 rats by dietary administration with the test substance at concentrations of 0.5, 1.5, and 5.0% (color valency 1,792). As a result, no animal deaths were observed, and no changes were observed in general condition. Regarding body weight and food intake, there were no differences between groups in body weight, while food intake showed an increasing trend in the male 5.0% group.

Hematological and blood chemical test showed a significant increase in WBC count in the 5.0% male group; however, this was not considered to be a change related to the test substance, as neither a dose response nor a change in WBC differentials was observed.

Regarding organ weight, increases were observed in male relative kidney weight and female absolute and relative liver weight in the 5.0% group; however, these were not considered to be toxicological changes, because no changes suggestive of damage in these organs were observed in hematological, blood chemical or histopathological tests results.

Based on the above results, the no observed adverse effect level of Carthamus red is considered to be 5.0% (male: 3,056 mg/kg/day; female: 3,224 mg/kg/day). [2)]

(3) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) was performed up to 20 mg/plate, and the results were negative regardless

of the presence or absence of metabolic activation by S9 mix. [3), 6)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5 mg/mL, resulting in weak chromosomal aberration inducibility in the system with metabolic activation. [4), 6)]

There has been a report that a micronucleus test in the bone marrow of mice was negative. [6)]

Moreover, a Rec-assay was performed, and it was considered that no DNA injury potential was observed without the addition of S9 mix. [5), 6)]

Based on the above results, for the weak chromosomal aberration inducibility observed in cultured mammalian cells, the micronucleus test in the bone marrow of mice showed negative results. Accordingly, it is considered that Carthamus red has no genotoxicity and will cause no particular problems in the body.

(References)

1. Takizawa Yukio: FY 1991 Study on the Safety of Food Additives, Akita University School of Medicine
2. Kanno Jun: Grant for Study on Food Additives, Division of Toxicology, National Institute of Health Sciences
3. Yamamoto Katsuhiko: FY 1991 Study on the Safety of Food Additives, Nagoya City Public Health Research Institute
4. Sofuni Toshio: FY 1991 Study on Reevaluation, etc. of the Safety of Food Additives, Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences
5. Ishizaki Mutsuo: FY 1991 Study on Reevaluation, etc. of the Safety of Food Additives, Ibaraki Prefectural Institute of Public Health
6. Hayashi Makoto: Maita Keizo: FY 1994 Study on the Establishment of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

Mevalonic acid

1. Food additive name:

Mevalonic acid

2. Origin, method of preparation, and definition:

Mevalonic acid is derived from the fermentation culture solution of yeast (*Saccharomycopsis fibuligera*) containing corn steep liquor or peptone, from casein as the major ingredient by extraction with organic solvent. It consists mainly of Mevalonic acid.

3. Major use:

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 dietary administration with the test substance at concentrations of 400, 2,000, and 10,000 ppm. It showed no animal deaths, and no changes were observed in general condition or food intake. Body weight showed significant gain suppression in both females and males of the 10,000 ppm group.

Hematological test showed significant decreases in red blood cell count in males, and in hemoglobin and hematocrit in females of the 10,000 ppm group, and a mild trend to anemia respectively, while no histopathological changes were observed in the hematopoietic organs.

Blood chemical test showed increased ALT in both females and males, increased AST in males, and decreased A/G ratio and significantly increased total cholesterol and triglyceride in females of the 10,000 ppm group.

Organ weight showed increased absolute and relative liver weights in both females and males of the 10,000 ppm group.

Histopathological test showed fatty metamorphosis in centrilobular hepatocytes in the liver in all females and males of the 10,000 ppm group, and minute fatty metamorphosis of hepatocytes was observed not only in the centrilobular part, but in the whole organ. Moreover, mild accumulation foci of inflammatory cells were observed sporadically in the liver parenchyma in all females and males of the same group. No changes caused by the test substance were observed at 2,000 ppm or less.

Based on the above results, the no observed adverse effect level of Mevalonic acid is considered to be 2,000 ppm (149.5 mg/kg/day) for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537,

and WP2*uvr*A/pKM101) was performed up to 5 mg/plate, and the results were negative regardless of the presence or absence of metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) up to the maximum dose of 5 mg/ml, resulting in no clear dose-dependency, while an increase in cells with structural aberrations was observed under all treatment conditions at the maximum dose, for which cytotoxicity was also observed. [3)]

A micronucleus test in the bone marrow of mice (ICR male, 7 weeks of age) was performed up to the limit dose of 2,000 mg/kg × 2, and no micronucleus induction was observed at any dose. [4)]

Mevalonic acid was not considered to express genotoxicity in the body, considering that the induction of aberrations observed in the chromosomal aberration test in cultured mammalian cells was a response obtained only at the dose where cytotoxicity was expressed, that there was no clear dose relationship, that decreased pH was observed, albeit mild, and that the micronucleus test in rodents, which was performed up to the limit dose, was negative.

(References)

1. Nakae Dai: Health and Labor Sciences Research Grant, Sasaki Foundation
2. and 3. Nakajima Madoka: Health and Labor Sciences Research Grant, Biosafety Research Center
4. Matsumoto Kyomu: Health Sciences Research Grant, The Institute of Environmental Toxicology

Morin

1. Food additive name:

Morin

2. Origin, method of preparation, and definition:

Morin is obtained from the trunks and branches or roots of *Broussonetia xanthoxylum* MARTIUS of the Moraceae family by extraction with ethanol and purification. It consists mainly of Morin.

3. Major use:

Antioxidant

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 0.625, 1.25, 2.5, and 5.0%. It showed no animal deaths or abnormalities in general condition throughout the treatment period. Food intake increased in both males and females with a dose relationship. Hematological test showed mild anemia in males at 0.625% or higher, and increased Seg. and decreased Lymph were also observed in the 5.0% group. However, the toxicological significance of these changes was not clear.

In females, an increasing trend in WBC was observed in groups at 0.625% or higher, and increased Seg. and decreased Lymph were also observed in the 5.0% group. However, the toxicological significance of these changes was not clear. Blood biochemical test showed increased AST, ALT, ALP, and γ-GT, and increased relative weight and absolute weight in both the liver and kidney were observed in the female groups at 0.625% ad higher and male groups at 2.5% or higher, suggesting damage in the liver, kidney etc. However, as histopathological test showed no damage in those organs, the toxicological significance is not clear. The no observed adverse effect level was estimated to be 0.625% (299 mg/kg/day) for males, and less than 0.625% (less than 366 mg/kg/day) for females. [1)]

In addition, while the no observed adverse effect level for females was less than 0.625% (less than 366 mg/kg/day), based on the level of toxicity, it can be evaluated to be not much less than the no observed adverse effect level estimated for males.

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) was judged as positive, because His+ revertant colonies were induced regardless of the presence or absence of metabolic activation by S9 mix. [2)]

A chromosomal aberration test in cultured mammalian cells (CHL) induced chromosomal

aberrations at a frequency of roughly 12% by both the direct method and the metabolic activation method. [3)]

An *in vivo* rat liver unscheduled DNA synthesis test was performed in male SD rats by single gavage at 500 mg/kg and 2,000 mg/kg. As a result, a statistically significant increase in net grain count was observed in the 2,000 mg/kg group by long-term treatment, but was within the range of the background data. In addition, no significant changes were observed by short-term treatment. Accordingly, it was concluded that Morin has no DNA injury potential in the hepatocytes of SD rats under the conditions of this test. [5)]

A micronucleus test in mice (ddY male) was performed at doses up to half (500 mg/kg) of the estimated 50% lethal dose. Exposure to the test substance was proven by the decreased polychromatophilic erythrocyte (PCE) ratio, and it was concluded that Morin had no micronucleus inducibility, at any dose. [4)]

(References)

1. Hirose Masao: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, National Institute of Health Sciences
2. Miyabe Masaki: FY 1995 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Takahide Hidenobu: FY 1995 Study on Reevaluation, etc. of the Safety of Food Additives, Yokohama City
4. Kishi Michiko: FY 1995 Study on Reevaluation, etc. of the Safety of Food Additives, Kanagawa Prefecture
5. Ono Hiroshi: 2003 Study on Standards and Criteria for Food Additives, etc., Food and Drug Safety Center

Logwood colour

1. Food additive name:

Logwood colour (obtained from the heartwood of logwood; contains hematoxylin as the major component).

2. Origin, method of preparation, and definition:

Logwood colour is obtained from the heartwood of logwood (*Haematoxylon campechianum*) of the Fabaceae family by extraction with water at a high temperature. It consists mainly of hematoxylin.

Logwood colour is dark brown.

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 gavage with the test substance at doses of 20, 100, and 500 mg/kg. As a result, no animal deaths were observed, and no changes caused by the test substance were observed in general condition, food intake, ophthalmological test, blood chemical test or autopsy.

In addition, the observations shown below were observed in the 500 mg/kg group. Mild suppression of body weight gain was observed in males. Urinalysis showed a significant increase in urine volume and a decrease in urine specific gravity in females. Hematological test showed a significant decrease in WBC count, associated with significantly decreased lymphocytes and monocytes in females. Organ weight measurements showed a significant increase in relative kidney weight in males. Histopathological test showed a deposition of brown pigment containing lipofuscin in the epithelium of the proximal tubule in the kidney of all females and males. These were all judged as changes of no toxicological significance.

Based on the above results, the no observed adverse effect level of Logwood colour is considered to be 100 mg/kg for both males and females. [1)]

(2) Genotoxicity study

A reverse mutation test in *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5 mg/plate, and the results were negative regardless of the presence or absence of metabolic activation by S9 mix. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU), and an increased frequency of induction of chromosomal structural aberrations of over 10%, with dose-dependency was observed by short-term treatment (+S9), while an

increasing trend was observed by short-term treatment (-S9), although it was not over 10%. [3)]

A micronucleus test in the bone marrow of mice (BDF1 male, 9 weeks of age) was performed up to the limit dose of 2,000 mg/kg. As a result, although dose-dependency was not observed, there was a statistically significant increase in the micronucleus induction ratio at 2,000 mg/kg, compared to the negative control group. In addition, the percentage of polychromatophilic erythrocytes decreased significantly at 2,000 mg/kg, indicating exposure to the test substance. [4)]

The change observed in the chromosomal aberration test was considered to be of no toxicological significance, because the change was only observed at the dose at which cytotoxicity was observed. Moreover, although the changes observed in the micronucleus test were statistically significant, they were affected by the low value of the negative control in the test, and were not considered to be toxicologically significant. Accordingly, it was concluded that these changes were not considered to cause any particular problem in the body.

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Food and Drug Safety Center
2. -4. Nakajima Madoka: Health and Labor Sciences Research Grant, Biosafety Research Center