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Research report

Research on the safety re-evaluation of existing additives

FY 2006

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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18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.

Data relating to the Pharmaceutical Affairs and Food Sanitation Council

July 4, 2007
Department of Food Safety, Pharmaceutical and Food Safety Bureau

Research on the safety re-evaluation of existing additives (FY2006 Survey)

Research on the safety re-evaluation of existing additives
(July 4, 2007, Committee on Food Additives, Food Sanitation Commission, Pharmaceutical Affairs and Food Sanitation Council)

A report on 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 4, 2007.

Research report

Research on the safety re-evaluation of existing additives

March 2007

Senior Researcher	
Inoue Tohru	Head of the Biological Safety Research Center, National Institute of Health Sciences
Study collaborators:	
Ema Makoto	Director, Division of Risk Evaluation, Biological Safety Research Center, National Institute of Health Sciences
Kanno Jun	Director, Division of Cellular and Molecular Toxicology, National Institute of Health Sciences
Kawanishi Tohru	Director, Division of Biological Chemistry Biologicals, National Institute of Health Sciences
Tanamoto Kenichi	Director, Division of Food Additives, National Institute of Health Sciences
Nagao Minako	Visiting Professor, Kyoritsu College of Pharmacy
Nishikawa Akiyoshi	Director, Laboratory I, Division of Pathology, National Institute of Health Sciences
Hayashi Makoto	
Hirose Masao	Director, Division of Genetics and Mutagenesis, National Institute of Health Sciences
Maitani Tamio	Director, Division of Pathology, National Institute of Health Sciences
	Director, Division of Foods, National Institute of Health Sciences

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Annex

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 as “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 70 out of the 139 additives that had been considered to require further investigation regarding safety in the Hayashi Group Report, excluding the following additives:

- 14 additives reported in FY1999 “Research on the safety evaluation of existing additives” (Senior Researcher: Kurokawa Yuji) (hereinafter, referred to as the “Kurokawa Group Report”)
- 17 additives reported in FY2003 “Research on the safety re-evaluation of existing additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY2003 Inoue Group Report”)
- 14 additives reported in FY2004 “Research on the safety re-evaluation of existing additives” (Senior Researcher: Inoue Tohru) (hereinafter, referred to as the “FY2004 Inoue Group Report”)
- The additives that were previously deleted from the list of existing additives (among them, 24 additives are considered to require safety confirmation)

This report collectively lists investigation results for 7 additives, namely: Urushi wax, Enzymatically hydrolyzed coix extract, Enzymatically decomposed rice bran, Shea nut colour, Jojoba wax, Eucalyptus leaf extract, and Mannentake extract.

The results of a 90-day or longer repeated dose study and a mutagenicity study were available for each of the 7 investigated additives, and basic safety could be evaluated for the individual existing additives based on the study results. In conclusion, as there was presently no study result that suggested any immediate adverse effects on human health, it was considered that there was no immediate need to perform a new toxicity 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 response to this request, 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." 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." In addition, the Inoue Group Report published in FY 2004 stated that "regarding the 14 additives for which safety was reviewed, there were no study results that suggested any immediate adverse effect on human health at present."

The present survey aimed to investigate the basic safety of natural additives by collecting domestic and overseas study results and evaluating those results for 70 out of the 139 additives that had been considered 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 70 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, 7 additives for which the data of both results of 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 7 additives for which safety was reviewed in this survey, there was no study result that suggested an immediate effect on human health at present for any of these additives. A summary is provided in the Annex.

E. Discussion

The study results for safety evaluation were collected for the 70 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 when the study results were evaluated for 7 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, there were no study results that suggested an immediate effect on human health at present for any of these additives. Therefore, it was considered that there was no immediate need to perform a new study for safety evaluation for the 7 existing additives that were evaluated.

Prior to this, 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 was deleted in December 2004 as those additives that are not actually used.

While the operation of reviewing existing additives may be currently undergoing steady progress in this manner, it is considered necessary to continuously investigate the actual usage status of existing additives, and to proceed with reorganization efficiently from the additives that require information.

F. Conclusion

The basic safety of 7 existing additives was considered to be newly confirmed by this survey, based on the collected study results. It was considered that there was no immediate need to perform a further safety study at the present stage for any of these additives.

Annex

Urushi wax

1. Food additive name:

Urushi wax (obtained from the fruit of poison oak; contains glycerol palmitate as the major component.)

2. Origin, method of preparation, and definition:

Urushi wax is obtained from the fruits of poison oak (*Rhus verniciflua* LINNE) of the Anacardiaceae family by melting and exposure. It consists mainly of glycerol palmitate.

3. Major use:

Gum base, polish

4. Summary of safety study results:

(1) Repeated-dose study

A 13-week repeated-dose test was performed in F344/DuCrj SPF rats by gavage (100, 300, and 1,000 mg/kg). It showed no animal deaths, and no changes related to the test substance were observed in general condition, trajectory of body weight, food intake, or ophthalmological test in any groups.

While significant changes such as urine volume and osmolality, reticulocyte ratio, total protein, albumin content, A/G ratio, albumin ratio, β-globulin ratio, ALT, total cholesterol, and phospholipid were observed in laboratory tests, all of these were mild changes within or almost within the physiological variation; in addition, since no changes associated with the administration of test substance were found in organ weight and histopathological test at autopsy, those changes were not considered as effects of the substance on renal function, the hematopoietic system, or liver function.

In conclusion, the no observed adverse effect level is considered to be 1,000 mg/kg in both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative with and without metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the maximum treatment concentration of 5.0 mg/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 maximum tolerated dose of 2,000 mg/kg×2, and no micronucleus induction was observed

at any dose. [4)]

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center
2. Kojima Akinori: FY 2000 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Nagoya City Public Health Research Institute
3. Ono Hiroshi: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Hatano Research Institute, Food and Drug Safety Center
4. Sato Shuji: FY 2000 Results of Study on Reevaluation, etc. of the Safety of Food Additives, Kanagawa Prefectural Institute of Public Health

Enzymatically hydrolyzed coix extract

1. Food additive name:

Enzymatically hydrolyzed coix extract (obtained from the coix seeds by enzymatic degradation.)

2. Origin, method of preparation, and definition:

Enzymatically hydrolyzed coix extract is obtained from the coix (*Coix lachryma-jobi var. ma-yuen* STAPF) seeds by extraction with hot water, followed by enzyme (α-amylase) degradation and treatment with ethanol.

3. Major use:

Preservative

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344/DuCrj (Fischer) SPF rats by gavage (100, 300, and 1,000 mg/kg/day). It showed no deaths, and no changes due to the administration of test substance were observed in any items of general condition, body weight, food intake, ophthalmological examination, urinalysis, water consumption, blood chemical test, organ weight, autopsy, or histopathological test.

Hematological test showed lower hemoglobin levels in males in the 1,000 mg/kg administered group compared with the vehicle administered control group. However, this decrease was rather mild, no changes were observed in other red blood cell parameters, and there was no difference to the ethanol added control group; taken together, these changes were considered to be of little toxicological significance.

In conclusion, the no observed adverse effect level was considered to be greater than 1,000 mg/kg/day in both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative with and without metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the maximum treatment concentration of 5.0 mg/mL, resulting in no induction of chromosomal aberrations under any treatment condition. [3)]

A micronucleus test in the bone marrow of mice (BDF1 male) was performed up to the maximum tolerated dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. [4)]

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, 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. Mochizuki Nobuhiko: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center
4. Mochizuki Nobuhiko: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center

Enzymatically decomposed rice bran

1. Food additive name:

Enzymatically decomposed rice bran (obtained from defatted rice bran; contains phytic acid and peptides as the major components.)

2. Origin, method of preparation, and definition:

Enzymatically decomposed rice bran is obtained from the defatted rice bran from the seeds of rice (*Oryza sativa* LINNE) of the Poaceae family by enzymatic decomposition and extraction with water. It consists mainly of peptides and phytic acid.

3. Major use:

Antioxidant

4. Summary of safety study results:

(1) Repeated-dose study

A 90-day repeated-dose test was performed in F344/DuCrj (Fischer) SPF rats by gavage (100, 300, and 1,000 mg/kg/day). It showed no deaths, and no changes due to the administration of test substance were observed in any items of general condition, ophthalmological examination, urinalysis, water consumption, hematological test, blood chemical test, organ weight, autopsy, or histopathological test.

Body weights in males in the 1,000 mg/kg administered group were slightly lower than those in the control group during the first half of the administration period. However, the change was temporal and rather mild, and no differences were observed in mean body weights at the end of dosing period; therefore, these changes were considered to be of little toxicological significance.

Food intake in males in the 300 and 1,000 mg/kg administered groups were slightly lower than that in the control group sporadically at some measurement points. However, since the change was rather mild, with no effects on body weights at the end of dosing period, the change was considered to be of little toxicological significance.

Taken together, the no observed adverse effect level was considered to be greater than 1,000 mg/kg/day in both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 5,000 μg/plate, and the results were negative with and without metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the maximum treatment concentration of 5.0 mg/mL, resulting in no induction of chromosomal aberrations under any treatment condition. [3)]

A micronucleus test in the bone marrow of mice (BDF1 male) was performed up to the maximum tolerated dose of 2,000 mg/kg×2, and no micronucleus induction was observed at any dose. [4)]

(References)

1. Ono Hiroshi: FY 2003 Study on the Safety of Existing Additives, Hatano Research Institute, Food and Drug Safety Center
2. Matsumoto Kyomu: FY 2001 Health Sciences Research Grant (The Health Science Special Research Project), Study on the Safety with a Focus on Mutagenicity of Existing Natural Additives, etc., The Institute of Environmental Toxicology
3. Nakajima Madoka: FY 2001 Health Sciences Research Grant (The Health Science Special Research Project), Study on the Safety with a Focus on Mutagenicity of Existing Natural Additives, etc., Biosafety Research Center
4. Nakajima Madoka: FY 2001 Health Sciences Research Grant (The Health Science Special Research Project), Study on the Safety with a Focus on Mutagenicity of Existing Natural Additives, etc., Biosafety Research Center

Shea nut colour

1. Food additive name:

Shea nut colour (obtained by extraction from the fruits or seeds coat of shea tree.)

2. Origin, method of preparation, and definition:

Shea nut colour is obtained from the fruits or seed coats of shea tree (*Butyrospermum parkii* KOTSCHY.) of the Sapotaceae family by extraction with weak alkaline aqueous solution at room temperature and neutralization. Shea nut colour is brown.

3. Major use:

Color

4. Summary of safety study results:

(1) Repeated-dose study

A 13-week repeated-dose test was performed in Wistar Hannover rats by dietary administration (0.007, 0.31, 1.25, and 5%). It showed no test animal deaths, and no changes were observed in general condition, body weight or food intake.

There were no significant differences between the groups in hematological test. Additionally, no significant differences were also observed between the groups in the classification of leukocyte differentiation types.

Serum biochemical test showed decreased Ca in the male 0.07% group, as well as increased Ca and decreased Na in the male 1.25 and 5% groups. However, the changes occurred sporadically and were considered to be of little toxicological significance.
Regarding organ weight changes, the decreased relative lung weight was seen only in the male 5% group. However, the decrease means weight saving and was determined to be of little toxicological significance.

In conclusion, the no observed adverse effect level was considered to be 5% (male: 1.00g/rat/day, female: 0.78g/rat/day) because of no toxicological changes probably due to the test substance. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria and a chromosomal aberration test in cultured mammalian cells (CHL/IU) were performed, and both results were negative. [2)] Also, a DNA repair test by using *Bacillus subtilis* (Rec-assay) showed negative. [2)]

A micronucleus test in the bone marrow of mice (BDF1 male) was performed up to the maximum tolerated dose of 2,000 mg/kg×2, and no micronucleus was judged to be induced at any dose. [3)]

(References)

1. Hirose Masao: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Division of Pathology, National Institute of Health Sciences
2. Hayashi Makoto: The Datasheet of Mutagenicity Assessment of Food Additives by the Welfare Ministry, etc. (FY 1979 - 1998)
3. Mochizuki Nobuhiko: FY2004 Study on Standards and Criteria for Food and Food Additives, etc., Biosafety Research Center

Jojoba wax

1. Food additive name:

Jojoba wax (obtained from the fruit of jojoba; contains icosenoic acid icosenil as the major component.)

2. Origin, method of preparation, and definition:

Jojoba wax is a high melting point wax separated from jojoba oil extracted from the fruits of jojoba (*Simmondsia californica* NUTT.) of the Buxaceae family. It consists mainly of icosenoic acid icosenil.

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/DuCrj rats by dietary administration (0.625, 1.25, 2.5, and 5%). It showed no animal deaths, and no changes were observed in general condition in any groups.

Among males there was a tendency for body weight gain in the 0.625 and 1.25% groups, and a tendency for decrease in body weight gain in the 2.5 and 5% groups; however, among females no differences were observed between the test substance administered groups and the control group. Food intake tended to decrease in the male 2.5% or higher and female 0.625% or higher groups.

Hematological test showed decreased WBC, increased RBC, increased Ht, decreased MCH, and increased PLT in the male 0.625% or higher groups. While increase or decrease of RBC, Hb, Ht, MCV, MCH, and MCHC was seen in females in the 1.25% or higher groups, these values had no dose relationship and were considered to be of poor toxicological significance.

Among males blood biochemical test showed decreased TP, TC, and A/G ratio in the 0.625% group, decreased BUN in the 1.25% group, increased TC and Na and decreased BUN in the 2.5% group, as well as increased ALP, TC, and Cr in the 5% group. Among females, decreased ALT in the 0.625% group, decreased A/G ratio and IP and increased Cr in the 1.25% group, increased BUN and Cr in the 2.5% group, as well as increased AST, ALT, ALP, and BUN in the 5% group were observed.

There was no change in the organ weight of the liver for both sexes. Though the absolute kidney weights were decreased in some administered groups, no dose relationship was observed. No histopathological changes were observed in the livers and kidneys.
As for other organ weights, among males, decreased absolute/relative weights of the brain, and increased absolute weights of the spleen in the 0.625% group, increased absolute weights of the spleen in the 1.25% group, as well as increased relative weights of the

brain, lungs, spleen, kidneys, and testes in the 5% group were observed; among females, increased relative weights of the brain were seen in the 5% group. However, all these changes were minimal. In addition, no associated histopathological abnormalities were identified in these organs.

In conclusion, the no observed effect level (NOEL) was considered to be 0.625% (294 mg/kg/day) for males and 0.625% (361 mg/kg/day) for females; as for the no observed adverse effect level (NOAEL), 5% (2,351 mg/kg/day) for males and 2.5% (1,401 mg/kg/day) for females. [1)]

(2) Genotoxicity study

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

A chromosomal aberration test was performed in cultured mammalian cells (CHL/IU) to the maximum treatment concentration of 5.0 mg/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×2, and no induction of micronuclei was observed at any dose level.[4)]

(References)

1. Tanaka Takuji: FY 2002 Health and Labor Sciences Research Grant, Pathology I, Kanazawa Medical University
2. Ajimi Shozo: FY 2001 Health Sciences Research Grant (The Health Science Special Research Project), Study on the Safety with a Focus on Mutagenicity of Existing Natural Additives, etc., The Chemicals Evaluation and Research Institute, Japan
3. Ono Hiroshi: FY 2004 Study on Standards and Criteria for Food and Food Additives, etc., Hatano Research Institute, Food and Drug Safety Center
4. Miyagawa Makoto: FY 2001 Health Sciences Research Grant (The Health Science Special Research Project), Study on the Safety with a Focus on Mutagenicity of Existing Natural Additives, etc., Mitsubishi Chemical Safety Institute Ltd.

Eucalyptus leaf extract

1. Food additive name:

Eucalyptus leaf extract (obtained from the leaves of eucalyptus; contains β-diketones as the major components.)

2. Origin, method of preparation, and definition:

Eucalyptus leaf extract is obtained from the leaves of eucalyptus (*Eucalyptus globulus* LABILL) of the Myrtaceae family by steam distillation or extraction with ethanol. It contains β-diketones as active ingredients.

3. Major use:

Antioxidant

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. As a result, no animal deaths were observed, and no changes related to the test substance were observed in general condition, trajectory of body weight, food intake, ophthalmological examination, urinalysis, or blood biochemical test.

Hematological test showed high platelet counts in females in the 300 and 1,000 mg/kg groups, and increased eosinophil ratio in males in each group. However, because the ranges were within the physiological variation and there were no changes in PT and APTT of other coagulation parameters, these results were not considered due to the effect of the test substance with toxicological significance.

At autopsy, black-brown duodenal mucosa was observed in both sexes in the 1,000 mg/kg group, the histopathological test of which indicated deposition of grey-brown pigments in the lamina propria.

As for organ weights, the increased relative liver weights were observed in both sexes in the 300 mg/kg or higher groups.

In conclusion, the no observed effect level is considered to be 100 mg/kg in both sexes. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria (TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101) was performed up to 1,250 μg/plate, and the results were negative with and without metabolic activation. [2)]

A chromosomal aberration test was performed in cultured mammalian cells (CHL) up to

the maximum dose of 625 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 1,000 mg/kg×2, and no micronucleus induction 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

Mannentake extract

1. Food additive name:

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

2. Origin, method of preparation, and definition:

Mannentake extract is obtained from the mycelia or fruiting bodies of mannentake (*Ganoderma lucidum* KARST.) of the Polyporales order or its culture solution by extraction with water, ethanol, or carbon dioxide.

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. As a result, no animal deaths were observed, and no changes related to the test substance were observed in general condition, body weight, food intake, ophthalmological examination, urinalysis, hematological test, autopsy, or histopathological test.

Blood biochemical test showed high albumin (levels) in males in the 1,000 mg/kg group, low β-globulin ratio in males in the 300 and 1,000 mg/kg groups, and high γ-GTP in females in the 1,000 mg/kg group.

As for organ weights, the increased relative liver weights in males in the 300 mg/kg group, as well as the increased absolute and relative liver weights in both sexes in the 1,000 mg/kg group were observed. Also, the increased relative kidney weights in males in the 300 mg/kg group, as well as the increased absolute and relative kidney weights in males in the 1,000 mg/kg group were observed. Additionally, the increased absolute and relative spleen weights in males in the 1,000 mg/kg group, as well as the increased absolute and relative adrenal gland weights in females in the 1,000 mg/kg group were observed.

In conclusion, the no observed effect level is considered to be 100 mg/kg for males and 300 mg/kg for females. [1)]

(2) Genotoxicity study

A reverse mutation test in bacteria, a chromosomal aberration test in cultured mammalian cells, and a rodent micronucleus test have been performed. While the results of the reverse mutation test in bacteria and the rodent micronucleus test were negative, the result of the chromosomal aberration test in cultured mammalian cells has been reported positive with and without metabolic activation. In vitro clastogenicity is observed only at high doses,

and the frequency of abnormal cells is low; in addition, the positive response is shown only in the dose just before the cells are unobservable due to cytotoxicity, and therefore, the clastogenicity is not strong. Also, the result of an in vivo micronucleus test up to sufficiently high doses is negative. Taken together, the additive is not considered to have in vivo genotoxicity causing any adverse effects. [2), 3), 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. Mochizuki Nobuhiko: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, Biosafety Research Center
4. Iwamoto Tsuyoshi: FY 1999 Study on the Preparation of Standards and Criteria, etc. for Food Additives, The Institute of Environmental Toxicology

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Review on the Safety of Existing Additives (as of July 2007):