

# **Risk Assessment Report**

## **Advantame (Food Additives)**

**Food Safety Commission of Japan (FSCJ)**

**July 2013**

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**◀Chronology of discussions▶**

April 2, 2012	Request for the food safety risk assessment related to designation of food additives by the Minister of Health, Labour and Welfare (Notice No. 2 from the Department of Food Safety, MHLW, 30 March 2010), and acceptance of relevant documents
April 5, 2012	The 426th Meeting of the Food Safety Commission (requested items were explained)
June 26, 2012	The 107th Meeting of the Expert Committee on Food Additives
July 17, 2012	Request for submitting the supplementary documents
July 25, 2012	Acceptance of supplementary documents
July 27, 2012	The 108th Meeting of the Expert Committee on Food Additives
August 10, 2012	Request for submitting the supplementary documents
December 20, 2012	Acceptance of supplementary documents
March 27, 2013	The 116th Meeting of the Expert Committee on Food Additives
May 27, 2013	The 475th Meeting of the Food Safety Commission (Report)
May 28, 2013 to June 26, 2013	Call for public opinion and information
July 23, 2013	Report from the Chairperson of the Expert Committee on Food Additives to the Chairperson of the Food Safety Commission
July 29, 2013	The 483th Meeting of the Food Safety Commission (Report)
July 30, 2013	Notification to the Minister of Health, Labour and Welfare

**◀List of Members of the Food Safety Commission of Japan (FSCJ)▶**

(Up to June 30, 2012)	(From July 1, 2012)
Naoko Koizumi (Chairperson)	Susumu Kumagai (Chairperson)
Susumu Kumagai (Deputy chairperson)	Hiroshi Sato (Deputy chairperson)
Taku Nagao	Yasushi Yamazoe (Deputy chairperson)
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Masao Hirose	Kiyoko Kamiyasuhira
Masatsune Murata	Masatsune Murata

**◁List of Members of the Expert Committee on Food Additives, the Food Safety Commission of Japan (FSCJ)▷**

(Up to June 30, 2012)

Katsumi Imaida (Chairperson)

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Kiyomi Ito

Makoto Ema

Kikue Kubota

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Akemi Morita

Masami Yamada

◁Expert witness▷

Reiko Teshima

## **Executive summary**

The FSCJ conducted a risk assessment of advantame (CAS number: 714229-20-6), a food additive used as a sweetener, using results from various studies.

The results used in the assessment include data on toxicokinetics, genotoxicity, acute toxicity, repeated dose toxicity, carcinogenicity, reproductive and developmental toxicity, allergenicity and general pharmacology in experimental animals and human data on advantame as the test substance.

FSCJ reviewed toxicokinetics of advantame in experimental animals, its general pharmacology and also data on humans, and concluded that these data showed no matters of concern for food safety.

FSCJ concluded that advantame and its degraded-products are of no concern in terms of their genotoxicity.

FSCJ reviewed the acute toxicity, repeated dose toxicity, carcinogenicity, reproductive and developmental toxicity and allergenicity of advantame. In a prenatal developmental toxicity study in rabbits, dams administered advantame exhibited digestive disorders accompanied by deterioration of general conditions at doses of 1,000 mg/kg bw/day or higher. FSCJ attributed this effect to the administration of advantame, and regarded 500 mg/kg bw/day (a dose lower than that caused the abovementioned effect) as the minimum NOAEL of advantame. In addition, FSCJ concluded on the basis of its review that advantame showed no carcinogenicity.

Taking the observed toxicological effects and the estimated intake of the additive "advantame" (3.57 mg/person/day (0.0714 mg/kg bw/day)) after its approval in Japan into account, FSCJ considered that it is necessary to specify an ADI for the additive "advantame". FSCJ specified the ADI of 5.0 mg/kg bw/day, applying a safety factor of 100 to the NOAEL in the prenatal developmental toxicity study in rabbits (500 mg/kg bw/day).

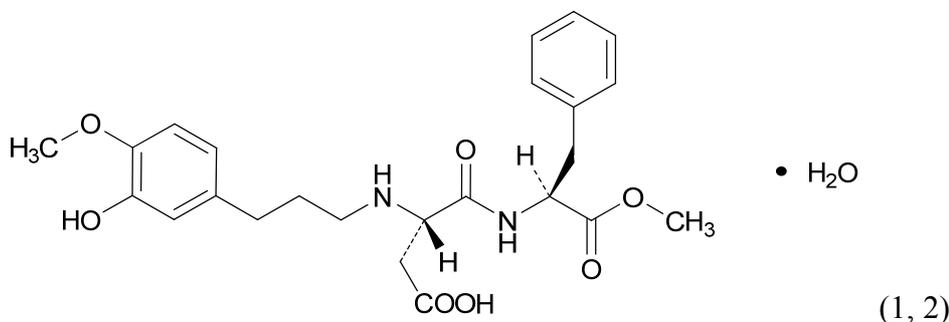
**I. Outline of the items under assessment****1. Use**

Sweetener (1,2)

**2. Names of the principal components**

English name: Advantame

CAS number: 714229-20-6

IUPAC: Methyl *N*-[3-(3-Hydroxy-4-methoxyphenyl) propyl]-*L*- $\alpha$ -aspartyl  
-*L*-phenylalaninate monohydrate (1, 2)**3. Molecular and structural formulae** $C_{24}H_{30}N_2O_7 \cdot H_2O$ **4. Molecular weight**

476.52 (2)

**5. Characteristics**

The applicant recently requested for the designation of this item as a food additive and for setting its standards and criteria, and submitted the ingredients standards (draft) of the additive “advantame” to the Ministry of Health, Labour and Welfare. The ingredient standards (draft) define that the content of the item as advantame anhydrate is 97.0-102.0% and the appearance is a white to yellowish white powder. Also, the items required in its purity test are “specific rotation $[\alpha]_D^{20}$ :  $-39^\circ$  to  $-46^\circ$  (0.2 g, ethanol (99.5, 100 mL, anhydrate) <sup>(1)</sup>,” “lead: no more than 1  $\mu\text{g/g}$  as Pb,” “*N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl)propyl]-*L*- $\alpha$ -Aspartyl]-*L*-phenylalanine (ANS9801-acid<sup>(2)</sup>) no more than 1.0%,” and “other analogous substances no more than 1.5 %” (2)

<sup>1</sup> According to the applicant, no stereoisomer of advantame was detected in 12 batches produced in the practical production process (less than 0.02%).

<sup>2</sup> Please refer to Appendix 1 for the formal names of the abbreviations used in this article.

## 6. Stability

According to 2007a, a long term testing on advantame at room temperature (25°C /relative humidity (RH) 60%, 60 months) was conducted. The result showed that the content of advantame is 99.6-100.6% at the beginning of the tests, 98.5-99.7% after 36 months, and 98.7-99.4% after 60 months. The total amount of related compounds contained as trace impurities were 0.44-0.51% at the beginning of the test and 0.55-0.61% after 60 months. Thus, the test performer concluded that no apparent degradation of advantame was observed and advantame was stable throughout the preservation period of 60 months. (3)

According to 2002a and 2004a, an accelerated testing on advantame (40°C/RH 75%, 6 months, dark place) was conducted. As a result, it is reported that no change in the content of advantame was observed; 99.6-100.6% and 99.6-100.3% at the beginning of the test and after 6 months, respectively. A slight increase was observed in the total amount of related compounds of advantame contained as trace impurities; 0.44-0.51% and 0.60-0.67% at the beginning of the test and after 60 months, respectively. (4, 5)

According to 2009a and 2010a, advantame is gradually hydrolyzed in acidic solution to form ANS9801-acid. (6, 7)

According to the abovementioned 2009a, a 26-week stability test on the additive “advantame” (5, 20, 30, 35°C) was conducted in acidic solutions (pH2.8, 3.2, 3.8, 4.5) assuming acidic beverages. As a result, after 8 weeks under the storage condition of pH3.2, 20°C, more than 1% of the initial content of advantame was found as ANS9801-acid. The amounts of other following trace degradants observed were less than 1% of the initial content of advantame; *N*-[*N*-[3-(3-Hydroxy-4-methoxyphenyl)propyl]-*L*-β-aspartyl]-*L*-phenylalaninate methyl ester (β-ANS9801), *N*-[*N*[3-(3-Hydroxy-4-methoxyphenyl)propyl]-*L*-β-aspartyl]-*L*-phenylalanine (β-ANS9801-acid), *N*-(3-(3-Hydroxy-4-methoxyphenyl)propyl)-*L*-aspartimide-*L*-phenylalaninate methyl ester (ANS9801-imide), *N*-[3-(3-Hydroxy-4-methoxyphenyl)propyl]-*L*-aspartic acid (HF-1), and *L*-phenylalaninate methyl ester. (7)

According to 2006a, a 26-week storage test was conducted, in which a Coke-type carbonated beverage supplemented with the additive “advantame” was stored under the condition of pH3.2, 25±2°C, and RH of 60±5%. As a result, 47.8% of advantame remained and degradants formed during the storage period were ANS9801-acid, β-ANS9801, and β-ANS9801-acid. (8)

According to 2007b, a 36-month storage test ( $25\pm 2^{\circ}\text{C}$ , RH:  $60\pm 5\%$ ) of tabletop sweeteners was conducted. As a result, 97.3% and 84.6% of advantame remained after 12 months and 36 months, respectively. The main degradant was ANS9801-acid. (9)

According to the applicant, since the additive “advantame” has secondary amino groups, Maillard reaction may occur between advantame and dextrose or maltodextrin contained in tabletop sweeteners. However, from the results of the tests including the abovementioned storage test of tabletop sweeteners, no possible product by Maillard reaction was detected and mass balance could be explained by the remaining amounts of advantame and its degradants mentioned above. Thus, it was predicted that interactions with other food ingredients such as Maillard reaction would not produce interaction products in the detectable concentration range under the condition where advantame is assumed to be used. (2, 7, 9,10,11,12,13)

## **7. Chronology of assessment request**

The structure-activity relationship study on the sweetener “aspartame” conducted in the applicant’s laboratory showed that advantame which shares a common structure with phyllodulcin, a natural sweetening compound, had sweetness 100 times or higher than that of aspartame and was also superior in stability. (2,14,15,16,17)

According to the applicant, the sweetness of advantame was approximately 14,000 to 48,000 times higher than that of sugar, depending on the type or formulation of the food in which advantame was used. (1)

In 2011, Food Standards Australia New Zealand (FSANZ) concluded that there was no problem in using the additive “advantame.” (18, 19)

Recently, the applicant requested for the designation of advantame as a food additive and for setting its standards and criteria to the Ministry of Health, Labour and Welfare, and the relevant documents were submitted. The Food Safety Commission was then requested to conduct a risk assessment, according to Article 24, Paragraph 1, Item 1 of the Food Safety Basic Law. (1)

## **8. Outline of the designation of food additives**

The Ministry of Health, Labour and Welfare is to investigate whether to designate “advantame” as a new additive and to set its standards and criteria after receiving the

notification of the risk assessment results from FSCJ. Standards for use are not to be defined. (1)

## **II. Outline of toxicological findings**

### **1. Disposition**

#### **(1) Absorption**

According to 2002d, stability tests on  $^{14}\text{C}$ -advantame and  $^{14}\text{C}$ -ANS9801-acid in artificial gastric juice and in intestinal fluids (37°C, 120 minutes) were conducted. As a result, advantame was stable in the artificial gastric juice, but was rapidly converted to ANS9801-acid in the intestinal fluids containing pancreatin. ANS9801-acid was stable in the artificial gastric juice and in the intestinal fluids. The applicant considered that advantame is mainly absorbed in the form of ANS9801-acid, but some part is absorbed as the undegraded form and then converted to ANS9801-acid in serum. (2,20)

According to 2004b and 2005c, a single forced oral administration test (5, 150 mg/kg bw) and a single intravenous administration test (5 mg/kg bw) on  $^{14}\text{C}$ -advantame were conducted in Wistar rats (3 rats each in male and female in each group in 2004b, and 4 rats each in male and female in each group in 2005c). As a result, in the oral administration test, the total radioactive concentration in serum reached its maximum 0.25 to 0.75 hours after administration, and its  $C_{\text{max}}$  and AUC increased almost dose-dependently, and its  $T_{1/2}$  was 6.0 to 8.1 hours. The serum concentration of advantame was below the quantitation limit in the 5 mg/kg bw group and was 1/24 to 1/53 of the total concentration of ANS9801-acid after sulfatase treatment in the 150 mg/kg bw group. The total serum concentration of ANS9801-acid after sulfatase treatment reached its maximum 0.25 to 1.0 hours after administration, its  $C_{\text{max}}$  and AUC increased almost dose-dependently, and its  $T_{1/2}$  was 1.9 to 3.6 hours. The absorption rate was estimated, from the ratio among urinary excretion rates of radioactivity from  $^{14}\text{C}$ -labeled forms, as 4 to 8%. In the intravenous administration test, the total radioactive concentration in serum reached its maximum within 0.1 hours after administration. The total serum concentration of ANS9801-acid after sulfatase treatment reached its maximum within 0.1 hours after the administration, and its  $T_{1/2}$  was 0.6 hours. (21, 22, 23)

According to 2005d, a single forced oral administration test (5, 150 mg/kg bw) and a single intravenous administration test (5 mg/kg bw) on  $^{14}\text{C}$ -advantame in beagle dogs (3 dogs each in male and female in each group) were conducted. As a result, in the single forced oral administration test, the total radioactive concentration in

serum reached its maximum 6 to 8 hours after administration, and its C<sub>max</sub> and AUC increased dose-dependently with a slightly lower increase rate than the doses, and its T<sub>1/2</sub> was 80.6 to 85.6 hours. The serum concentration of advantame was below the quantitation limit in the 5 mg/kg bw group, and was only 1/32 to 1/578 of that of ANS9801-acid in the 150 mg/kg bw group. The serum concentration of ANS9801-acid reached its maximum 0.5 to 1.0 hours after administration, and its C<sub>max</sub> and AUC increased dose-dependently with a slightly lower increase rate than the dose, and its T<sub>1/2</sub> was 4.2 to 7.1 hours. The absorption rate was estimated, from the ratio among urinary excretion rates of radioactivity from <sup>14</sup>C-labeled forms, as 10 to 20%. In the single intravenous administration test, the total radioactive concentration in serum reached its maximum right after administration. The serum concentration of ANS9801-acid reached its maximum right after administration, and its T<sub>1/2</sub> was 0.5 to 0.6 hours. (24)

According to 2004c and 2005e, a single oral administration test (0.25 mg/kg bw) on <sup>14</sup>C-advantame in healthy human (6 men) and a single oral administration test (0.10, 0.25, 0.50 mg/kg bw) on non-labeled advantame in healthy human (8 men in each group) were conducted. As a result of the test with <sup>14</sup>C-advantame, the total radioactive concentration in serum reached its maximum 1.25 hours after administration and its C<sub>max</sub> was 30.1±3.2 ng equiv./g. The serum concentration of ANS9801-acid reached its maximum 1.75 hours after administration and its C<sub>max</sub> was 22.7±5.1 ng/mL. T<sub>1/2</sub>'s of the total concentration in serum of radioactivity and that of ANS9801-acid were 3.9 hours and 5.7 hours, respectively. Advantame in serum was detected only at 2 time points each in 3 subjects, and the most of the radioactivity in serum was derived from ANS9801-acid. The ratio of the AUC of ANS9801-acid to the AUC of total serum radioactivity was 82.1-89.2%. In the test with non-labelled advantame, advantame was detected only 1 to 4 time points for each subject when administered at 0.25 mg/kg bw and 0.50 mg/kg bw, and the level of advantame was below the quantitation limit in all time points when administered at 0.1 mg/kg bw. The C<sub>max</sub> and AUC of ANS9801-acid increased almost in proportion to the doses. (23,25,26)

## **(2) Distribution**

According to 2002e and 2004d, a single forced oral administration test on <sup>14</sup>C-advantame (5 mg/kg bw) in colored Lister Hooded rats (3 males) was conducted to measure the concentrations in tissues up to 48 hours after administration, and a single oral administration test on <sup>14</sup>C-labeled advantame (5 mg/kg bw) in Wistar rats (1 male and 1 female at each time point, female rats include pregnant and

non-pregnant rats) was conducted to investigate the distribution by whole-body autoradiography. The result of the test in colored Lister Hooded rats showed that the radioactive concentrations reached its maximum 15 minutes after administration in most tissues. The radioactive concentration in serum reached its maximum 15 minutes after administration and decreased to approximately 10% of the maximum value within 6 hours after administration. As for the radioactive concentrations in tissues other than gastrointestinal tract, the radioactive concentrations higher than that in serum were observed only in bladder, liver, and kidney 15 minutes after administration. The radioactive concentrations in tissues rapidly decreased and no accumulation or congestion was observed in any tissue. The radioactive concentration in gastrointestinal tract accounted for the most of the recovered radioactivity, and the most of which was excreted within 24 hours. The highest radioactive concentration 15 minutes after administration was observed in stomach contents, 1 and 2 hours after administration in small intestine contents, and 6 and 12 hours after administration in cecum and in large intestine contents. The test in Wistar rats showed that no difference in distribution in tissues or in disappearing patterns was found between the sexes or between pregnant and non-pregnant rats, and no migration of radioactivity into placentas or fetuses was detected. Radioactive level reached its maximum shortly after administration and decreased rapidly thereafter. The radioactive levels were high in stomach, gastrointestinal tract, liver, kidney, and bladder 0.25 to 2 hours after administration, and low in other tissues. The radioactivity was detected only in excretory organs 6 and 12 hours after the administration. No accumulation of radioactivity in particular tissues was observed. (27, 28)

According to 2002f, a single forced oral administration test on  $^{14}\text{C}$ -advantame (5 mg/kg bw) in beagle dogs (1 male for each time point) was conducted to measure the concentrations in tissues 6, 72, 144, and 288 hours after administration. As a result, the radioactive concentration in each tissue reached its maximum approximately within 6 hours after administration and the highest concentration was observed in large intestine and in bile. The radioactive concentration in large intestine and in bile 6 hours after administration was higher than that in serum. However, the radioactive concentrations in the other tissues at the same time point and in all tissues 72 hours after administration or later were lower than those in serum. The applicant concluded that the radioactive concentrations in tissues were derived from circulating serum perfusing in tissues, since the ratios of radioactive concentrations in the most of the tissues to that in serum were almost stable throughout the test period. The radioactive concentration was significantly lower in spinal cord, brain and eyes than

that in serum. (29)

According to 2004e, a test was conducted in which <sup>14</sup>C-advantame was added to the serum of dogs and humans (dog: 20-20,000 ng/mL, human: 10-1,000 ng/mL), <sup>14</sup>C-ANS9801-acid was added to the serum of rats, dogs, and humans (rat: 10-10,000 ng/mL, dog: 100-25,000 ng/mL, human: 10-5,000 ng/mL) and then serum protein binding ratios were determined by ultrafiltration. As a result, serum protein binding ratio of ANS9801-acid was 91-92% in rats in the concentration range of 10-10,000 ng/mL. In dogs, serum protein binding ratio of advantame was 63-65% in the concentration range of 20-20,000 ng/mL, and that of ANS9801-acid was 62-71% in the concentration range of 100-25,000 ng/mL. In human, serum protein binding ratio of advantame was 81-92% in the concentration range of 10-1,000 ng/mL, and that of ANS9801-acid was 96-97% in the concentration range of 10-5,000 ng/mL, and saturation was not observed in the concentration ranges investigated. (23,30)

### (3) Metabolism

According to 2005c, the major metabolite in serum in Wistar rats was ANS9801-acid and almost no undegraded form of advantame was detected in oral administration groups. In urine, no undegraded form of advantame was detected in oral administration groups and the major metabolite was ANS9801-acid. The proportions of the detected metabolite to doses was 0.3-0.6% for ANS9801-acid in oral administration groups and 19.8-22.6% for ANS9801-acid in intravenous administration groups. Seven types of metabolites including HF-1 and 3-(3-hydroxy-4-methoxyphenyl)-1-propylamine (HU-1) were detected in urine in both groups. However, the detected amount of each metabolite was not more than 0.3% of the dose in oral administration groups. No undegraded form of advantame was detected in feces in both groups. The major metabolites in feces were ANS9801-acid and its desmethyl, RF-1. The proportions of detected metabolites to doses for ANS9801-acid, RF-1 (demethylated ANS9801-acid) and RF-1 (demethylated ANS9801-acid) were 28.8-29.1%, 40.9-41.1% and 11.5-11.9% in the 5 mg/kg bw oral administration group, and 20.7-26.2%, 26.9-27.6% and 8.1-8.3% in the 5 mg/kg bw intravenous group in the 150 mg/kg bw oral administration group. In the 150 mg/kg bw oral administration group, the proportion of detected amount of ANS9801-acid to doses was 86.2-88.1% and no RF-1 was detected. (22, 23)

According to 2005d, the major metabolite in serum in beagle dogs was ANS9801-acid. No undegraded form of advantame was detected in the 5 mg/kg bw

oral administration group and a little amount of undegraded form of advantame was detected at some time points after administration in the 150 mg/kg bw oral administration group. Also, sulfate conjugate of ANS9801-acid was detected in serum in all groups. In urine, undegraded form of advantame was less than 0.1% of the doses in the oral administration group and the major metabolite was ANS9801-acid. The proportions of metabolites detected in urine to doses in the oral administration group were 1.6-3.1%, 0.3-1.5%, 0.2-0.3%, and 0.5-1.0% for ANS9801-acid, HF-1, HU-1, and D3 (unidentified metabolite), respectively, while in the intravenous administration group were 22.9-25.1%, 0.9-1.4%, 0.2-0.3%, and 4.5-5.1% for ANS9801-acid, HF-1, HU-1, and D3, respectively. No sulfate conjugate was detected in urine. In feces, no undegraded form of advantame was detected in both groups, and the major metabolite was ANS9801-acid. The proportions of metabolites detected in feces to doses were 70.3-78.2% for ANS9801-acid, 1.0-4.1% for HF-1 in the oral administration group, and 23.3-23.7% for ANS9801-acid and 2.7-3.6% for HF-1 in the intravenous administration group. No sulfate conjugate was detected in feces. (23, 24)

According to above mentioned 2005e, the major metabolite in serum in healthy men was ANS9801-acid and only a little amount of advantame was detected. In urine, no undegraded form of advantame was detected and the major metabolite was ANS9801-acid. The proportions of metabolites detected in urine to doses were  $2.3 \pm 0.6\%$  for ANS9801-acid,  $1.0 \pm 0.6\%$  for HF-1, and  $1.9 \pm 1.9\%$  for HU-1. In feces, no undegraded form of advantame was detected and the major metabolites were ANS9801-acid and HF-1. The proportions of metabolites detected in feces to doses were  $52.0 \pm 13.0\%$  for ANS9801-acid and  $30.0 \pm 12.0\%$  for HF-1. (23, 26)

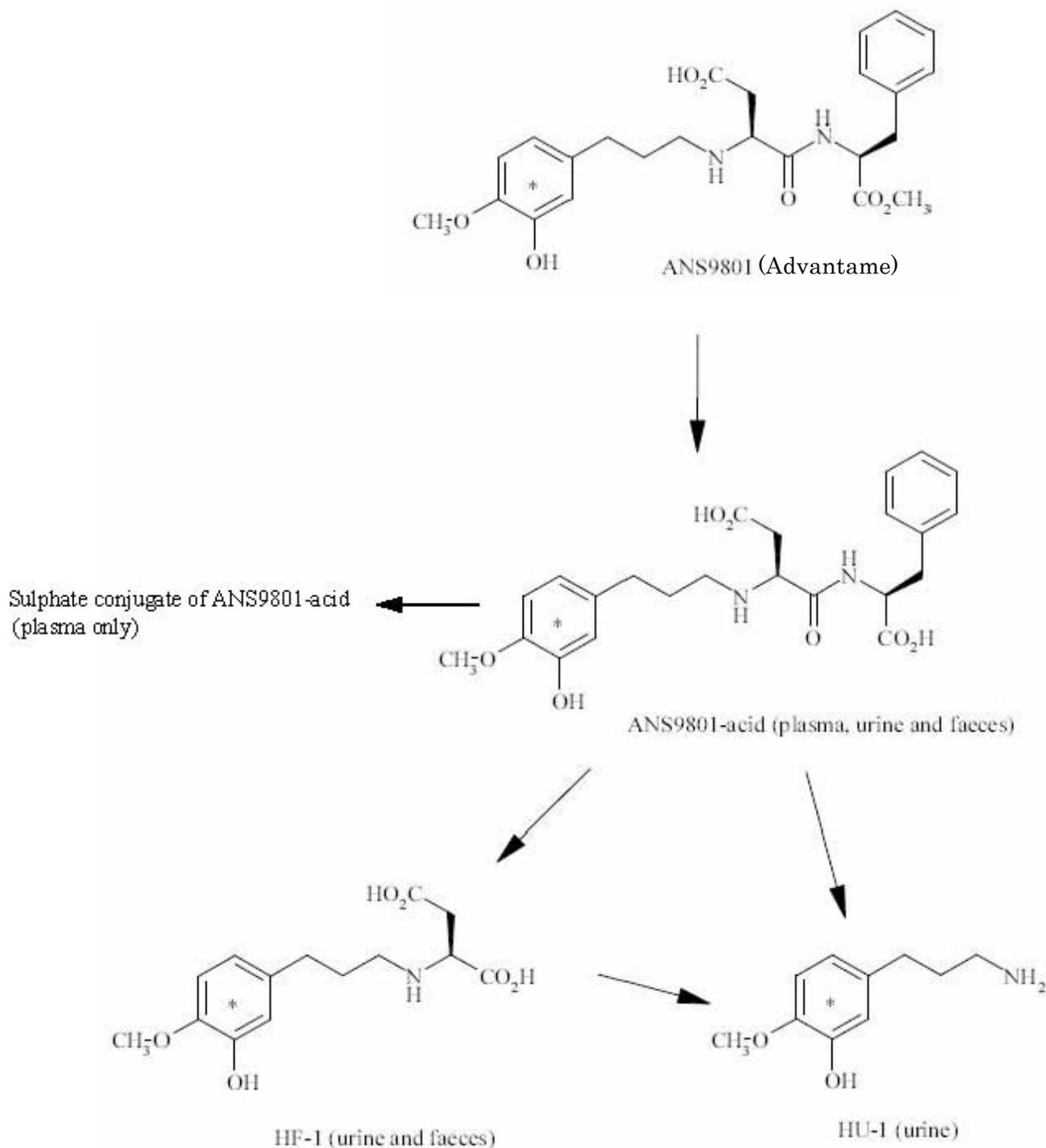
Hence, the applicant estimated that advantame was mainly metabolized to methanol and ANS9801-acid by deesterification, and a part of ANS9801-acid was metabolized to HF-1 or HU-1 by hydrolysis of peptide or by C-N bond break. The applicant also confirmed that sulfate conjugate of ANS9801-acid existed as a metabolite in serum in dogs. The major metabolic pathway of advantame inferred from test results in animals (rats and dogs) and in human is shown in Figure 1, and the major and minor metabolites of each animal and human when orally administered are shown in Table 1. Thus, it was confirmed that the safety in human could be reasonably presumed from the results of safety tests in animals, since any of the metabolites detected in human was also detected in rats and dogs in toxicology tests. (2)

**Table 1. Major and minor metabolites in animals and in human in oral**

**administration**

Species	Serum	Urine	Feces
Rat	ANS9801-acid	ANS9801-acid HF-1 HU-1	ANS9801-acid RF-1 RF-2
Dog	ANS9801-acid Sulfate conjugate of ANS9801-acid	ANS9801-acid HF-1 HU-1 D3	ANS9801-acid HF-1
Human	ANS9801-acid	ANS9801-acid HF-1 HU-1	ANS9801-acid HF-1

**Figure 1. Major metabolic pathway of advantame based on the test results in animals (rats and dogs) and in human (2)**



**(4) Excretion**

According to the abovementioned 2005c, urinary excretion rates up to 96 hours after administration in Wistar rats were 0.97-1.94% in the oral administration group and 23.90-25.84% in the intravenous administration group, and fecal excretion rates up to 96 hours after administration in Wistar rats were 95.92-99.52% in the oral

administration group and 72.27-72.70% in the intravenous administration group. Ninety percent or more of doses was excreted within 48 hours after administration and no difference was found between sexes. (22, 23)

According to the abovementioned 2005d, urinary excretion rates up to 120 hours after administration in beagle dogs were 3.74-7.37% in the oral administration group and 37.11-39.10% in the intravenous administration group, and fecal excretion rates were 82.35-88.94% in the oral administration group and 41.90-47.43% in the intravenous administration group. Approximately 90% or more of the dose was excreted within 48 hours after administration and no difference was found between sexes. (23, 24)

According to abovementioned 2005e, urinary excretion rate in healthy men up to 168 hours after administration was  $6.22 \pm 3.11\%$  as in total radioactivity, and fecal excretion rate was  $89.48 \pm 2.03\%$  as in total radioactivity. Almost all of the amount administered was excreted within 120 hours after administration in all subjects. (23, 26)

## **(5) Others**

According to the applicant, the risk caused by an increase in phenylalanine intake by ingesting advantame is negligible, since, when advantame is ingested, ANS9801-acid is excreted in urine or feces as a major metabolite and, although advantame is an L-phenylalanine compound, the rate of elimination of phenylalanine *in vivo* is very low. (2)

## **2. Toxicity**

### **(1) Genotoxicity**

#### **i. Advantame**

##### **a. Tests using gene mutation as an index**

###### **(a) Reverse mutation test using microorganisms**

According to 2001a, reverse mutation tests on advantame (maximum dose of 5,000  $\mu\text{g}/\text{plate}$  for both the preliminary and the main test) by preincubation method using bacteria (*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and *Escherichia coli* WP2 *uvrA/pKM101*) were conducted and the results were negative, regardless of the presence or absence of the metabolic activation system. (31, 32)

###### **(b) Mouse lymphoma TK assay**

According to 2002g, a mouse lymphoma TK assay on advantame (maximum concentration: 5,000 µg/mL for short-term method and 1,500 µg/mL for 24-hour continuous method) using L5178Y mouse lymphoma cells was conducted and the result was negative, regardless of the presence or absence of the metabolic activation system. (32,33)

**b. Tests using chromosome aberration as an index**

**(a) Micronucleus assay using rodents**

According to 2001c, a single forced oral administration test on advantame (500, 1,000, 2,000 mg/kg bw) in ICR mice (5 mice in negative control group, 7 male mice in each advantame-administered group) was conducted. As a result, in advantame-administered groups, no increase in the development rate of immature erythrocyte in femur marrow was observed 24 and 48 hours after administration and the result was negative. (34)

**ii. Degraded-products of advantame**

Among degraded-products of advantame, ANS9801-acid and HU-1, a major and a trace metabolites in rats, dogs, and human, are detected in serum. Since almost none of undegraded form of advantame is detected in excrement, genotoxicities of ANS9801-acid and HU-1 are considered to be also assessed in the micronucleus assays on advantame using rodents. Therefore, genotoxicities of other degraded-products ( $\beta$ -ANS9801,  $\beta$ -ANS9801-acid, HF-1 and ANS9801-imide) were assessed based on the following tests.

**a. Tests using gene mutation as an index**

**(a) Reverse mutation tests using microorganisms**

According to 2009d, 2009e, 2009f, 2009g, reverse mutation tests on  $\beta$ -ANS9801,  $\beta$ -ANS9801-acid, HF-1, and ANS9801-imide (maximum dose of 5,000 µg/plate for both the preliminary and the main tests) by preincubation method using bacteria (*S. typhimurium* TA98, TA100, TA1535, TA1537, and *E.coli* WP2 *uvrA*) were conducted and the results were negative, regardless of the presence or absence of the metabolic activation system. (35, 36, 37, 38)

**(b) Mouse lymphoma TK assay**

According to 2009h, a mouse lymphoma TK assay on  $\beta$ -ANS9801 (maximum concentration: 4,600 µg/mL for both short-term method and 24-hour continuous method) using L5178Y mouse lymphoma cells was

conducted and the result was negative, regardless of the presence or absence of the metabolic activation system. (39)

According to 2009i, a mouse lymphoma TK assay (maximum concentration: 4,500 µg/mL for both short-term method and 24-hour continuous method) on β-ANS9801-acid using L5178Y mouse lymphoma cells was conducted and the result was negative, regardless of the presence or absence of the metabolic activation system. (40)

According to 2009j, a mouse lymphoma TK assay on HF-1 (maximum concentration: 1,000 µg/mL for both short-term method and 24-hour continuous method) using L5178Y mouse lymphoma cells was conducted and the result was negative, regardless of the presence or absence of the metabolic activation system. (41)

According to 2009k, a mouse lymphoma TK assay on ANS9801-imide (maximum concentration: 400 µg/mL for short-term method in the absence of metabolic activation system, 178 µg/mL for short-term method in the presence of metabolic activation system, and 500 µg/mL for 24-hour continuous method) using L5178Y mouse lymphoma cells was conducted and the result was positive. (42)

## **b. Tests using chromosome aberration as an index**

### **(a) Micronucleus assay using rodents**

According to 2009l, a single forced oral administration test on ANS9801-imide (500, 1,000, 2,000 mg/kg bw) in ICR mice (5 males each in a negative control group, a positive control group, and advantame-administration groups) was conducted. As a result, no increase in the development rate of immature erythrocyte in femur marrow was observed in ANS9801-imide group 24 hours after administration and the result was negative. (43)

Hence, FSCJ concluded that advantame, and its metabolites such as β-ANS9801, β-ANS9801-acid, HF-1 and HU-1, have no genotoxicity relevant to human health. Also, although the result of the mouse lymphoma TK assay of ANS9801-imide, a degraded-product of advantame, was positive, the result from the micronucleus assay in rodents was negative, and hence FSCJ judged that ANS9801-imide has no genotoxicity relevant to human health.

**(2) Acute toxicity**

According to 2001c, a single forced oral administration test on advantame (5,000 mg/kg bw) in rats was conducted. As a result, no animal deaths or serious changes in general conditions were observed throughout the test period. The test performer concluded that the approximate value of the lethal dose of advantame is above 5,000 mg/kg bw. (44)

**(3) Repeated dose toxicity**

**i. A 13-week administration test in rats**

According to 2004b, a 13-week dietary administration test on advantame (0, 1,500, 5,000, 15,000, 50,000 ppm: 0, 118, 415, 1,231, 4,227 mg/kg bw/day for males; 0, 146, 481, 1,487, 5,109 mg/kg bw/day for females) in 6-week-old Wistar rats (20 or 25 rats each in male and female in each group) was conducted, and consecutively 5 rats each in the 0-, 15,000-, and 50,000-ppm groups were followed by a 4-week recovery period. As a result, no deaths or changes in general conditions, in body weight, or in food intake related to the administration of the test substance were observed in any administration group. Although an increase in water intake was observed in the 15,000- and 50,000-ppm groups, the test performer concluded that the increase was attributable to the quality of taste of the test substance. Although observations including low values in hematocrit, hemoglobin and red blood cell count were obtained in a hematologic test in the 15,000- and 50,000-ppm groups, the test performer concluded that it does not suggest toxicity, since the degree of change was minor and within the background data in the study facilities, and no related change was observed in the other examination items. No effect related to the administration of the test substance was found in ophthalmological examination, neurotoxicity evaluation, blood chemistry examination, urine test, immunotoxicity evaluation, organ weights, autopsy, and histopathologic examination. Hence, the test performer specified the NOAEL in this study as 50,000 ppm (4,227 mg/kg bw/day for males, 5,109 mg/kg bw/day for females), which was the maximum dose applied in this test. (45, 46) FSCJ considered that the assessment by the test performer was appropriate.

**ii. A 13-week administration test in dogs**

According to 2005f, a 13-week dietary administration test on advantame (0, 5,000, 15,000, 50,000 ppm, corresponding mean doses of 0, 205, 667, 2,230 mg/kg bw/day for males and 0, 229, 703, 2,416 mg/kg bw/day for females) in 23- to 26-week-old beagle dogs (4 or 6 dogs each in male and female in each group) was conducted, and

2 dogs each in the 0- and 50,000-ppm groups were followed by a 4-week recovery period. As a result, no death related to the administration of the test substance occurred in any group. As for general conditions, loose stools were observed in all groups including the control group and its occurrence rate was higher in the administration groups. However, the test performer concluded that it did not suggest toxicity. Suppression of weight gain was observed in males in the 50,000-ppm group. In a hematologic test, although observations including low values in hematocrit, hemoglobin and red blood cell counts were obtained in males in the 15,000-50,000-ppm groups, the test performer concluded that it did not suggest toxicity, since the changes were within the background data in the study facilities. In autopsy, a decrease in thymus weight and an increase in the development rate of moderate thymic atrophy were observed in males in the 50,000-ppm group. However, the test performer concluded that it did not suggest toxicity, since the degree and frequency of changes are within the spontaneous degree and frequency. No effect related to the administration of the test substance was observed in food intake, ophthalmological examination, electrocardiogram, and blood chemistry examination. The test performer specified the NOAEL in this study as 15,000 ppm based on the suppression of weight gain observed in the 50,000-ppm group. Meanwhile, since no toxicity observation was obtained in the 50,000-ppm group other than the suppression of weight gain, the applicant judged that the suppression of weight gain was due to the addition of antinutritive compound in a relatively high concentration, and specified the NOAEL in this study as 50,000 ppm (2,230 mg/kg bw/day for males and 2,416 mg/kg bw/day for females), which was the maximum dose applied in this study. (2, 46,47) FSCJ did not consider the assessment by the applicant appropriate, but supported the assessment by the test performer and specified the NOAEL as 15,000 ppm (667 mg/kg bw/day for males and 703 mg/kg bw/day for females).

### **iii. A 52-week administration test in dogs**

According to 2005g, a 52-week dietary administration test on advantame (0, 2,000, 10,000, 50,000 ppm corresponding to 0, 83, 421, 2,058 mg/kg bw/day for males and 0, 82, 406, 2,139 mg/kg bw/day for females) in 22- to 26-week-old beagle dogs (4 or 6 dogs each in male and female in each group) was conducted and 2 dogs each in the 0- and 50,000-ppm groups were followed by a 6-week recovery period. As a result, no deaths or changes in general conditions, body weight and food intake related to the administration of the test substance were observed in any administration group. Although the tendency of increased heart rate was observed in males in the 50,000-ppm group, the test performer concluded that it did not suggest

toxicity, since the degree of the change was not significant but minor, and also no observation related to the administration of the test substance was obtained in other ECG evaluations. No effect related to the administration of the test substance was found in ophthalmological examination, hematologic test, blood chemistry examination, urine test, organ weight, autopsy, and histopathologic examination. Hence, the test performer specified the NOAEL in this study as 50,000 ppm, which was the maximum dose applied in this study (2,058 mg/kg bw/day for males and 2,139 mg/kg bw/day for females). (48, 49) FSCJ considered that the assessment by the test performer was appropriate.

#### **(4) Carcinogenicity**

##### **i. A 104-week administration test in mice**

According to 2006c and 2011a, a 104-week dietary administration carcinogenicity test on advantame (2,000, 10,000, 50,000 ppm, corresponding to 0, 223, 1,057, 5,693 mg/kg bw/day for male, and 0, 272, 481, 1,343, 7,351 mg/kg bw/day for female) in 6-week-old ICR mice (64 mice each in males and females in each group) was conducted. As a result, no change in survival rate or in general conditions related to the administration of the test substance was observed. Although the tendency of suppression of weight gain was observed in both sexes in the 50,000-ppm group, the test performer concluded that it did not suggest toxicity, since the change was not statistically significant and no other related toxicological finding was observed. No tumor development, no increased rate of tumor development, or no onset of non-neoplastic lesion presumably related to the administration of the test substance was observed. Hence, the test performer concluded that advantame showed no carcinogenicity. (46,50,51) FSCJ considered that the assessment by the test performer was appropriate.

##### **ii. A 52-week repeated dose study and a 104-week combined carcinogenicity/toxicity study by dietary administration to rats with an *in utero* exposure phase**

According to 2005h and 2006d, a 52-week dietary administration of advantame (2,000, 10,000, 50,000 ppm, corresponding to 0, 117, 592, 3,199 mg/kg bw/day for males, and 0, 146, 740, 4,009 mg/kg bw/day for females) was conducted in 4-week-old Wistar rats (20 or 30 rats each in male and female in each group) born from dams exposed to advantame for 4 weeks before mating. A 6-week recovery test was conducted in 10 rats each in the 0-ppm, 10,000-ppm, and 50,000-ppm groups after the administration. As a result, no death related to the administration was observed in any administration group. As for general conditions, paleness and swelling

in vent were observed in both sexes in the 50,000-ppm group in week 4 to week 32 of the administration. This change was not persistent until the end of administration, and no changes related to the administration of the test substance were observed in histopathologic examination of the same body part at the time of last sacrifice. Hence, the test performer concluded that it did not suggest toxicity. The tendency of suppression of body weight gain was observed in both sexes in the 50,000-ppm group and the change in males was significant. Although an increase in food intake was observed in both sexes in the 50,000-ppm group, the test performer concluded that this finding did not suggest toxicity, since the degree of change was minor. Increases in water intake were observed in all the administration groups, and the test performer concluded that these changes were related to the quality of the taste of advantame. Although, in a hematologic test, a decrease in blood urea value was observed in both sexes in the 50,000 ppm group, the test performer concluded that the change did not suggest toxicity, since no related histopathologic changes were observed. No other change related to the administration of the test substance was observed in ophthalmological examination, hematologic test, urine test, organ weights, autopsy, and histopathologic examination. Hence, the test performer specified the NOAEL in this study as 50,000 ppm (3,199 mg/kg bw/day for males and 4,009 mg/kg bw/day for females), which was the maximum dose applied in this study. (52, 53, 54) Also in the same report, a 104-week dietary administration carcinogenicity test on advantame (2,000, 10,000, 50,000 ppm, corresponding to 0, 97, 488, 2,621 mg/kg bw/day for males, and 0, 125, 630, 3,454 mg/kg bw/day for females) was conducted in 4-week-old Wistar rats (55 rats each in male and female in each group). As a result, no change in survival rate related to the administration of the test substance was observed. The findings similar to those observed in the 52-week repeated dose study with an *in utero* exposure phase were observed in general conditions, body weight, food intake, and in blood chemistry examination. However, no change attributable to the administration of the test substance was observed in blood chemistry examination, urine test, organ weight, and autopsy. No tumor development, no increased rate of tumor development, or no onset of non-neoplastic lesion presumably related to the administration of the test substance was observed. Hence, the test performer concluded that advantame showed no carcinogenicity. (53,55) FSCJ considered that the assessment by the test performer for paleness and swelling in vent was appropriate, which were observed in both sexes in the 50,000-ppm group in the 52-week repeated dose study with an *in utero* exposure phase. Whereas, FSCJ did not agree with the assessment by the test performer regarding the suppression of body weight gain in male rats observed in the 50,000-group and the tendency of suppression of body weight gain observed in female rats, and specified the NOAEL as 10,000 ppm

for both sexes (592 mg/kg bw/day for males and 740 mg/kg bw/day for females). FSCJ considered that the assessment for the carcinogenicity test by the test performer was appropriate.

## **(5) Reproductive/developmental toxicity**

### **i. Reproductive toxicity study**

According to 2004g, a two-generation reproduction test was conducted in which advantame (2,000, 10,000, 50,000 ppm, corresponding to 0, 164, 833, 4,410 mg/kg bw/day for males, and 0, 204, 1,036, 5,439 mg/kg bw/day for females) was administered in mixed feeds for 10 weeks before mating in 6-week-old SD rats (F<sub>0</sub>: 30 rats each in males and females in each group) was conducted, and the resultant child animals (F<sub>1</sub>: 25 rats each in male and female in each group) were administered with advantame in the same manner (0, 184, 907, 4,776 mg/kg bw/day for male and 0, 229, 1,140, 5,920 mg/kg bw/day for female) for 10 weeks before mating, and the resultant child animals (F<sub>2</sub>) were obtained. As a result, no deaths or change in general conditions or body weight related to the administration of the test substance was observed in the parent animals (F<sub>0</sub>, F<sub>1</sub>) in any group. As for the increase in food intake observed in the administration groups, the test performer concluded that it did not suggest toxicity. In the dams (F<sub>0</sub>, F<sub>1</sub>), no effect related to the administration of the test substance was observed in estrus cycle, coitus ability, fertility, pregnancy period, birth rate, findings in autopsy, organ weights, sperm check and histopathological examination. In the child animals (F<sub>1</sub>, F<sub>2</sub>), no change related to the administration of the test substance was observed in the number of births, survival rate, sex ratio, physical and functional development, findings in autopsy, and organ weights. Hence, the test performer specified the NOAEL in this study as 50,000 ppm (4,410 mg/kg bw/day for F<sub>0</sub> males, 5,439 mg/kg bw/day for F<sub>0</sub> females, 4,776 mg/kg bw/day for F<sub>1</sub> males, and 5,920 mg/kg bw/day for F<sub>1</sub> females), which was the maximum dose applied in this study. (56, 57) FSCJ considered that the assessment by the test performer was appropriate.

### **ii. Prenatal developmental toxicity study**

According to 2002h, a test was conducted in which advantame (0, 5,000, 15,000, 50,000 ppm, corresponding to 0, 465, 1,418, 4,828 mg/kg bw/day) was administered in mixed feeds to 10-11 week-old SD rats (22 rats each in male and female in each group) at the 0th to 20th days of gestation, and Caesarean sections were conducted at the 20th day of gestation. No deaths or changes in general conditions related to the administration of the test substance were observed in any administration group. The suppression of body weight gain was observed in the 50,000-ppm group. As for food

intake, a decrease in the early stage of the administration period and an increase on and after the 3rd day of gestation were observed in the 50,000-ppm group. No effect related to the administration of the test substance was observed in gravid uterus weights, organ weights, autopsy, the number of implantations, the number of resorption embryos, the number of live fetuses, the death rate at preimplantation and postimplantation, sex ratio, weights of fetuses, and placental weights, and in fetus tests of external surfaces, organs, and skeletons. The test performer specified the NOAEL in this study as 15,000 ppm (1,418 mg/kg bw/day) based on the suppression of body weight gain observed in the 50,000-ppm group. (2,58,59) FSCJ considered that the assessment by the test performer was appropriate.

According to 2003, a test was conducted in which forced oral administration of advantame (0, 500, 1,000, 2,000 mg/kg bw/day) was conducted to 19-25 week-old New Zealand white rabbits (24 females for each group) at the 6th to 28th days of gestation, and Caesarean sections were conducted at the 29th day of gestation. As a result, as for general conditions, deteriorations (anorexia, decreases in body weight, asthenia, decreases in locomotor activity) were observed in 1 rabbit in the 1,000 mg/kg bw/day group and 5 rabbits in the 2,000 mg/kg bw/day group, and those animals were slaughtered during the administration period (between the 17th to 27th days of gestation). In autopsy in these animals, findings suggestive of digestive disorders, such as retention of intestinal tract contents, were observed in common and the test substance was found to be a highly concentrated and viscous suspension, and hence the test performer concluded that the observed digestive disorders were changes specific to rabbits caused by the physical property of the administered substance. The deaths due to digestive disorders were considered to be a non-specific change caused by osmotic activity of the test substance, since such incidence was also observed in the studies of sucralose, an existing high-sweetness sweetener. As for the spontaneous abortion observed in 1 rabbit in the 2,000 mg/kg bw/day group, the test performer considered it likely to be a secondary change due to the effects including deteriorations of general conditions. The suppression of body weight gain was observed in the early stage of administration in the 2,000 mg/kg bw/day group, but the difference was not persistent throughout the administration period. No change related to the administration of the test substance was found in food intake or in autopsy. Regarding the index of developmental toxicity, a slight increase in the number of late resorption embryos was found in the 2,000 mg/kg bw/day group. No changes related to the administration of the test substance were observed in the number of implantations, the number of live fetuses, the death rate at pre- and postimplantation, sex ratio, weights of fetuses, and placental weights, and in fetus

tests of external surface, organs and skeletons. Hence, the test performer specified the NOAEL in this study as 500 mg/kg bw/day for dams based on the changes in general conditions observed in the groups administered at 1,000 mg/kg bw/day or higher, and 1,000 mg/kg bw/day for fetuses based on the slight increase in the number of late resorption embryos in the 2,000 mg/kg bw/day group. (59,60) FSCJ considered that the assessment by the test performer was appropriate.

#### **(6) Allergenicity**

According to 2011b, a test was conducted in which dimethyl sulfoxide (DMSO) solutions of advantame (10, 25, 50% (w/v)) were applied to auricles of CBA/Ca mice (5 females in each group) for 3 days, and the auricular lymph nodes were collected for examination 5 days after the first treatment. As a result, a response slightly above the positive threshold value was observed in the 50 % (w/v) treatment group, and negative results were obtained in the other treatment groups. The positive threshold value was calculated to be 46.4 % (w/v). The applicant concluded that the positive response observed in this study was a weak reaction occurred in the concentration range far above the treatment concentration at which the additive “advantame” is used as a sweetener. (2,61) FSCJ considered that the food additive “advantame” was of very low concern in terms of allergenicity as long as it was appropriately used as an additive.

#### **(7) General pharmacological tests**

According to 2001d, 2001e, and 2001f, a single non-anesthesia forced oral administration test on advantame (10, 100, 1,000 mg/kg bw) in Wistar rats (male) and a single forced intraduodenally administration test on advantame (10, 100, 1,000 mg/kg bw) under anesthesia in beagle dogs (male) were conducted to examine the effect of advantame on the central nervous system, and on the respiratory and circulatory systems. As a result, no effect related to the administration of the test substance was observed in either test. (62, 63, 64)

According to 2001g, a test was conducted in which a single non-anesthesia forced oral administration of advantame (10, 100, 1,000 mg/kg bw) was conducted in Wistar rats (10 males in each group), and carbon dust was orally administered 30 minutes after the administration to examine the effect of advantame on digestive system by measuring the migration length of the carbon dust between pyloric sphincter and cecum. As a result, a decrease in the migration length of carbon dust was observed in the 1,000 mg/kg bw group. The applicant considered that the decrease was not due to a pharmacological effect of advantame but due to the high

viscosity of the test substance. (65) FSCJ considered that the result raised no concern for food safety, although the possibility could not be ruled out that the decrease in the migration length of carbon dust was due to a pharmacological effect of advantame. .

### **(8) Findings in humans**

According to 2004c, a single administration test on advantame (0.1, 0.25, 0.5 mg/kg bw/day) in healthy human (8 males in each group) was conducted. As a result, no change related to the administration of the test substance was observed. (25)

According to 2006e, a test was conducted in which placebos or capsules containing 10 mg of advantame were administered 3 times a day (30 mg/day, 0.375-0.5 mg/kg bw/day) for 4 weeks in healthy humans (6 subjects each in male and female in each group). As a result, mild pruritus was observed in 2 subjects and, for one of them, it was judged that the relation to the administration of the test substance could not be ruled out. No other change related to the administration of the test substance was observed. (66)

According to 2006f, a 12-week administration test was conducted in which placebos or capsules containing 10 mg of advantame were administered 3 times a day (30 mg/day, 0.375-0.5 mg/kg bw/day) for 12 weeks in patients of non-insulin-dependent diabetes mellitus (18 subjects each in male and female in each group). As a result, 19 adverse events were observed in 14 subjects. For dyspepsia, bloat, and nausea observed in one of which subjects, it was judged that the relation to the administration of the test substance could not be ruled out, but the subject recovered by the end of the administration. No other change related to the administration was observed. (67)

### **III. Estimation of the daily intake**

According to the applicant, the additive “advantame” is assumed to be used as a sweetener in various foods. Under the assumption that all the present estimated intake of saccharides from foods are substituted with advantame, an estimated daily intake of this product is calculated as 2.42 mg/person/day (0.0484 mg/kg bw/day), based on the total amount of intake by food groups and the estimated amount of saccharides contained in foods from the 2008 National Health and Nutrition Survey in Japan. However, even in the current situation with many products containing high-sweetness sweeteners in the market, there are only a few products in which all saccharides are

substituted with high-sweetness sweeteners and combined use of several high-sweetness sweeteners is common. Thus, even if the high amount consumers of sweeteners are taken into account, abovementioned estimated daily intake calculated by assuming that all the saccharides in foods are substituted with advantame is considered an overestimate. (2,68)

Meanwhile, according to the applicant, under the assumption that all annual demands in Japan of the sugar, high-fructose corn syrup, sweetened processed foods (as in the equivalent amount of sugar) are substituted with advantame and that the sweetness of advantame is 20,000 times higher than that of sucrose, an estimated daily intake of the additive “advantame” is 3.57 mg/person/day (0.0714 mg/kg bw/day). (2,69)

FSCJ considered an estimated daily intake of this item as 3.57 mg/person/day (0.0714 mg/kg bw/day), taking note not to make an underestimate.

#### **IV. Discussion of intake of phenylalanine**

According to the applicant, the risk caused by an increase in phenylalanine intake by taking advantame is negligible, since ingested advantame is rapidly converted to ANS9801-acid *in vivo* and ANS9801-acid is excreted in urine or feces as a major metabolite. Assuming that all advantame is converted to phenylalanine, an estimated intake of phenylalanine in Japan is calculated as 839 µg/person/day (16.8 µg/kg bw/day) from the abovementioned estimated daily intake of advantame, which corresponds to 0.14-0.42% of the suggested intake for patients with phenylketonuria (200-220 mg/person/day for 2-year-old children and 300-600 mg/person/day for 5-year-old children). (2)

#### **V. Assessments in international organizations**

According to the applicant, no assessment has been conducted by international organizations such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), since this product is under application and assessment in countries as a new sweetener and there is no usage record in any country to date. (2)

In 2011, regarding the additive “advantame”, FSANZ specified the ADI of 5.0 mg/kg bw/day by applying a safety factor of 100 to the NOAEL of 500 mg/kg bw/day based on the result of the prenatal developmental toxicity study in rabbits. FSANZ concluded that there is no problem in using this item since the 90th percentile value of the estimated

intake in Australia is 3% of the ADI or lower. (18, 19)

## VI. Risk assessment

FSCJ reviewed the data on toxicokinetics and general pharmacology of advantame, and concluded that these data showed no matters of concern for food safety.

FSCJ concluded that advantame and its degraded-products are of no concern in terms of their genotoxicity.

FSCJ reviewed the data on acute toxicity, repeated dose toxicity, carcinogenicity, reproductive and developmental toxicity and allergenicity of advantame. In a prenatal developmental toxicity study in rabbits, digestive disorders accompanied by deterioration of general conditions were observed in dams administered with advantame at doses of 1,000 mg/kg bw/day or higher. FSCJ considered these effects are due to the administration of advantame, and regarded 500 mg/kg bw/day, which was a dose lower than that caused the abovementioned effects, as the minimum NOAEL for toxicity of advantame. In addition, FSCJ concluded that advantame showed no carcinogenicity.

FSCJ concluded from the data of advantame on humans that there is no evidence that raises any concern for food safety.

Although advantame is an L-phenylalanine compound, the amount of phenylalanine formed *in vivo* is considered to be very small, since the absorption rate of advantame is 20% at most and the major metabolite in blood, urine, and feces is ANS9801-acid. Therefore, FSCJ concluded that the risk caused by an increase in phenylalanine intake by taking advantame is negligible.

FSCJ concluded that it is necessary to specify ADI for the additive "advantame", taking account of the observed toxicological effects and the estimated intake of the additive "advantame" (3.57 mg/person/day (0.0714 mg/kg bw/day)) after its approval in Japan (3.57 mg/person/day (0.0714 mg/kg bw/day)). FSCJ specified the ADI of 5.0 mg/kg bw/day, applying a safety factor of 100 to the NOAEL of 500 mg/kg bw/day specified in the prenatal developmental toxicity study in rabbits.

ADI	5.0 mg/kg bw/day
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(Referred data for setting the ADI)	Prenatal developmental toxicity study
(Animal species)	Rabbit
(Administration method)	Forced oral administration
(Observations for setting the NOAEL)	Digestive disorders accompanied by deterioration of general conditions
(NOAEL)	500 mg/kg bw/day
(Safety factor)	100

◀Appendix 1: Abbreviations▶

Abbreviation	Name, etc.
ANS9801	Advantame
ANS9801-acid	<i>N</i> -[ <i>N</i> -[3-(3-Hydroxy-4-methoxyphenyl)propyl]- <i>L</i> - $\alpha$ -aspartyl]- <i>L</i> -phenylalanine
ANS9801-imide	<i>N</i> -(3-(3-Hydroxy-4-methoxyphenyl)propyl)- <i>L</i> -aspartimide- <i>L</i> -phenylalaninate methyl ester
$\beta$ -ANS9801	<i>N</i> -[ <i>N</i> -[3-(3-Hydroxy-4-methoxyphenyl)propyl]- <i>L</i> - $\beta$ -aspartimide]- <i>L</i> -phenylalaninate methyl ester
$\beta$ -ANS9801-acid	<i>N</i> -[ <i>N</i> -[3-(3-Hydroxy-4-methoxyphenyl)propyl]- <i>L</i> - $\beta$ -aspartyl]- <i>L</i> -phenylalanine
FSANZ	Food Standard Australia New Zealand
HF-1	<i>N</i> -[3-(3-Hydroxy-4-methoxyphenyl)propyl]- <i>L</i> -aspartic acid
HU-1	3-(3-hydroxy-4-methoxyphenyl)-1-Propylamine
JECFA	Joint FAO/WHO Expert Committee on Food Additives

◁Appendix 2: Results of toxicity studies▷

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
Genotoxicity	Reverse mutation test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, <i>E. coli</i> WP2uvrA/pKM101	-	<i>in vitro</i>	-	Advantame	Maximum dose: 5,000 µg/plate	A negative result was obtained, regardless of the presence or absence of the metabolic activation system.	(2001a) 31, 32
Genotoxicity	Mouse lymphoma TK assay	L5178Y mouse lymphoma cells	-	<i>in vitro</i>	-	Advantame	Maximum concentrations: 5,000 µg/mL for short-term method, 1,500 µg/mL for 24-hour continuous method	A negative result was obtained, regardless of the presence or absence of the metabolic activation system.	(2002g) 32, 33
Genotoxicity	Micronucleus assays using rodents	ICR mouse	Single	Forced oral administration	5 males in negative control group and 7 males each in advantame groups	Advantame	500, 1,000, 2,000 mg/kg bw	A negative result was obtained.	(2001c) 34
Genotoxicity	Reverse mutation test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, <i>E. coli</i> WP2uvrA	-	<i>in vitro</i>	-	β-ANS9801, β-ANS9801-acid, HF-1 and ANS9801-imide	Maximum dose: 5,000 µg/plate	A negative result was obtained, regardless of the presence or absence of the metabolic activation system.	(2009d, 2009e, 2009f, 2009g) 35, 36, 37, 38

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
Genotoxicity	Mouse lymphoma TK assay	L5178Y mouse lymphoma cells	-	<i>in vitro</i>	-	$\beta$ -ANS9801	Maximum concentration: 4,600 $\mu\text{g}/\text{mL}$ for both short-term and 24-hour continuous methods	A negative result was obtained, regardless of the presence or absence of the metabolic activation system.	(2009h) 39
Genotoxicity	Mouse lymphoma TK assay	L5178Y mouse lymphoma cells	-	<i>in vitro</i>	-	$\beta$ -ANS9801-acid	Maximum concentration: 4,500 $\mu\text{g}/\text{mL}$ for both short-term and 24-hour continuous methods	A negative result was obtained, regardless of the presence or absence of the metabolic activation system.	(2009i) 40
Genotoxicity	Mouse lymphoma TK assay	L5178Y mouse lymphoma cells	-	<i>in vitro</i>	-	HF-1	Maximum concentration: 1,000 $\mu\text{g}/\text{mL}$ for both short-term and 24-hour continuous methods	A negative result was obtained, regardless of the presence or absence of the metabolic activation system.	(2009j) 41

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
Genotoxicity	Mouse lymphoma TK assay	L5178Y mouse lymphoma cells	-	<i>in vitro</i>	-	ANS9801-imide	Maximum concentrations: 400 µg/mL for short-term method without metabolic activation system, 178 µg/mL for short-term method with metabolic activation system, and 500 µg/mL for 24-hour continuous method	A positive result was obtained.	(2009k) 42
Genotoxicity	Micronucleus assays using rodents	ICR mouse	Single	Forced oral administration	5 males each in negative control group, positive control group, and advantame group	ANS9801-imide	500, 1,000, 2,000 mg/kg bw	A negative result was obtained.	(2009l) 43
Acute toxicity	Acute toxicity study	Rat	Single	Forced oral administration	-	Advantame	5,000 mg/kg bw	The approximate lethal dose of advantame is above 5,000 mg/kg bw.	(2001c) 44

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
Repeated dose toxicity	13-week study	Rat	13 weeks for administration and 4 weeks for recovery test	Mixed feed	20 or 25 rats each in male and female in each group	Advantame	0, 1,500, 5,000, 15,000, 50,000 ppm: 0, 118, 415, 1,231, 4,227 mg/kg bw/day for males; 0, 146, 481, 1,487, 5,109 mg/kg bw/day for females	FSCJ considered the assessment by the test performer appropriate, in which the NOAEL in this study was specified as 50,000 ppm (4,227 mg/kg bw/day for male and 5,109 mg/kg bw/day for female), which is the maximum dose applied in this study.	(2004b) 45, 46
Repeated dose toxicity	13-week study	Beagle	13 weeks for administration and 4 weeks for recovery test	Mixed feed	4 or 6 dogs each in male and female in each group	Advantame	0, 5,000, 15,000, 50,000 ppm: mean doses are 0, 205, 667, 2,230 mg/kg bw/day for males; 0, 229, 703, 2,416 mg/kg bw/day for females	FSCJ supported the assessment by the test performer in which the NOAEL was specified as 15,000 ppm based on the suppression of body weight gain observed in the 50,000-ppm group, and regarded 15,000 ppm (667 mg/kg bw/day for males and 703 mg/kg bw/day for females) as the NOAEL.	(2005f) 2, 46, 47
Repeated dose toxicity	52-week study	Beagle	52 weeks for administration and 6 weeks for recovery test	Mixed feed	4 or 6 dogs each in male and female in each group	Advantame	0, 2,000, 10,000, 50,000 ppm: 0, 83, 421, 2,058 mg/kg bw/day for males; 0, 82, 406, 2,139 mg/kg bw/day for females	FSCJ considered the assessment by the test performer appropriate in which the NOAEL in this study was specified as 50,000 ppm (2,058 mg/kg bw/day for males and 2,139 mg/kg bw/day for females), which is the maximum dose applied in this study.	(2005g) 48, 49

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
Carcinogenicity	104-week study	ICR mouse	104 weeks	Mixed feed	64 mice each in male and female in each group	Advantame	2,000, 10,000, 50,000 ppm: 0, 223, 1,057, 5,693 mg/kg bw/day for males; 0, 272, 481, 1,343, 7,351 mg/kg bw/day for females	FSCJ considered the assessment by the test performer appropriate, which stated advantame showed no carcinogenicity.	(2006c, 2011a) 46, 50, 51
Combined repeated dose / carcinogenicity study	52-week with an <i>in utero</i> exposure phase study, 104-week study	Wistar rat	52 weeks for administration and 6 weeks for recovery test	Mixed feed	20 or 30 rats each in male and female in each group	Advantame	2,000, 10,000, 50,000 ppm: 0, 117, 592, 3,199 mg/kg bw/day for males, 0, 146, 740, 4,009 mg/kg bw/day for females	FSCJ considered the assessment by the test performer appropriate, in which the paleness and swelling in vent observed in both sexes in the 50,000-ppm group did not suggest toxicity. Meanwhile, FSCJ did not agree to the assessment by the test performer which stated the suppression of body weight gain observed in males and the tendency of suppression of body weight gain observed in females in the 50,000-group did not suggest toxicity. FSCJ regarded 10,000 ppm (592 mg/kg bw/day for males and 740 mg/kg bw/day for females) as the NOAEL for both sexes.	(2005h, 2006d) 52, 53, 54, 55

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
			104 weeks	Mixed feed	55 rats each in male and female in each group	Advantame	2,000, 10,000, 50,000 ppm: 0, 97, 488, 2,621 mg/kg bw/day for males and 0, 125, 630, 3,454 mg/kg bw/day for females	FSCJ considered the assessment by the test performer appropriate which stated advantame showed no carcinogenicity.	
Reproductive and developmental toxicity	Reproductive toxicity study	SD rat	Mixed feed to dams for 10 weeks before mating and mixed feed to their child animals in the same manner for 10 weeks before mating	Mixed feed	F <sub>0</sub> : 30 rats each in male and female in each group F <sub>1</sub> : 25 rats each in male and female in each group	Advantame	2,000, 10,000, 50,000 ppm: 0, 164, 833, 4,410 mg/kg bw/day for F <sub>0</sub> males; 0, 204, 1036, 5,439 mg/kg bw/day for F <sub>0</sub> females, 0, 184, 907, 4,776 mg/kg bw/day for F <sub>1</sub> males; 0, 229, 1,140, 5,920 mg/kg bw/day for F <sub>1</sub> females	FSCJ considered the assessment by the test performer appropriate, in which the NOAEL in this study was specified as 50,000 ppm (4,410 mg/kg bw/day for F <sub>0</sub> males and 5,439 mg/kg bw/day for F <sub>0</sub> females, 4,776 mg/kg bw/day for F <sub>1</sub> males and 5,920 mg/kg bw/day for F <sub>1</sub> females), which is the maximum dose applied in this study.	(2004g) 56, 57
Reproductive and developmental toxicity	Prenatal developmental toxicity study	SD rat	From 0th to 20th day of gestation	Mixed feed	22 female rats in each group	Advantame	0, 5,000, 15,000, 50,000 ppm: 0, 465, 1,418, 4,828 mg/kg bw/day	FSCJ considered the assessment by the test performer appropriate, in which the NOAEL was specified as 15,000 ppm (1,418 mg/kg bw/day) based on the suppression of body weight gain observed in dams in the 50,000-ppm group.	(2002h) 2, 58, 59

Test item	Type of test	Animal species, etc.	Test period	Administration route	Group setting	Test substance	Doses	Summary of test results	References
Reproductive and developmental toxicity	Prenatal developmental toxicity study	New Zealand White Rabbit	From 6th to 28th day of gestation	Forced oral administration	24 female rats in each group	Advantame	0, 500, 1,000, 2,000 mg/kg bw/day	FSCJ considered the assessment by the test performer was appropriate, in which the NOAEL for dams in this study was specified as 500 mg/kg bw/day based on the changes in general conditions observed in the groups administered at 1,000 mg/kg bw/day or higher, and the NOAEL for fetuses as 1,000 mg/kg bw/day based on the slight increase in the number of late resorption embryos in the 2,000-mg/kg bw/day group.	(2003) 59, 60
Allergenicity	Allergenicity test	CBA/Ca mouse	3 days	Skin-painting on auricles	5 female mice in each group	Advantame	10, 25, 50%(w/v)	FSCJ considered that an additive “advantame” was of very low concern in terms of allergenicity as long as it was used appropriately as an additive.	(2011b) 2, 61
General pharmacology	General pharmacological test	Wistar rat	Single	Forced oral administration	Male	Advantame	10, 100, 1,000 mg/kg bw	No effect of advantame was observed in either test.	(2001d, 2001e, 2001f) 62, 63, 64
		Beagle	Single	Forced oral administration	Male	Advantame	10, 100, 1,000 mg/kg bw		
General pharmacology	General pharmacological test	Wistar rat	Single	Forced oral administration	10 male rats in each group	Advantame	10, 100, 1,000 mg/kg bw	FSCJ concluded that the data raised no concern for food safety, although the possibility could not be ruled out that the decrease in migration length of carbon dust observed in this study was due to a pharmacological effect of advantame.	(2001g) 65

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