A taste of the future.
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October 15, 2010

Dr. Glenn Stanley
Assistant Section Manager
Product Safety Standards
Food Standards Australia New Zealand

RE: Submission regarding the 1st Assessment Report for Application A1034

Dear Dr. Stanley

In response to your invitation for public submissions for Application A1034 – Advantame as a High Intensity Sweetener – Ajinomoto Co., Inc. wishes to make the following submission.

1. **Establishment of an ADI of 5 mg/kg bw/day from the rabbit teratology study**

In the Executive Summary (p. 2), it is stated that:

> "The only evidence of compound-related toxicity was in a rabbit developmental study, where deaths and clinical signs occurred in dams at and above 1000 mg/kg bw/day. There was no evidence that Advantame was genotoxic or carcinogenic. There was no effect on reproduction or fetal development in rats or rabbits.
>
> In human studies, doses up to 0.5 mg/kg bw were well-tolerated by volunteers with and without type-2 diabetes following a single dose or repeated dosing for up to 12 weeks.
>
> "An ADI has been set at 5 mg/kg bw, by applying a 100-fold safety factor to the no observed adverse effect level (NOAEL) of 500 mg/kg bw/day in a rabbit developmental toxicity study. The NOAEL was based on maternotoxicity at the next higher dose of 1000 mg/kg bw/day."

Based on these statements, it appears that FSANZ has considered that the deaths and clinical signs noted in the rabbit teratology study, particularly at the 2,000 mg/kg bw dose level (gavage administration), were due to systemic toxicity associated with advantame treatment. This appears to be further confirmed in the technical discussion/interpretation of the rabbit teratology study. On page 88, it is stated that:

> "Unfortunately, due to the absence of any kinetic data in rabbits it is not possible to investigate whether a difference in pharmacokinetics was the cause of an increased incidence of maternotoxicity, late resorptions, and post-implantation losses observed in rabbits."

Similarly, on page 92:

> "Other results that were unusual compared to other laboratory species were green staining of the GIT or its contents, green bladder contents and green staining of the surface of the kidneys in one mid-dose dam. Collectively, these findings suggest that rabbits are relatively sensitive to Advantame-acid relative to other laboratory animals. While there are no kinetic data available in rabbits, the green staining of the GIT, bladder contents and kidneys suggest different kinetics and possibly a different metabolite profile relative to other species."

These statements suggest that FSANZ considers the rabbit to be particularly sensitive to advantame and/or its metabolites and that this sensitivity is due to metabolic and/or pharmacokinetic differences between the rabbit and other species.

Ajinomoto, Inc. is of the opinion that the ADI should not be established on the basis of the rabbit teratology study since the data indicate that the response seen in the rabbit teratology study (*i.e.*, clinical signs and death) were not due to systemic effect of advantame, but a result of in-appetence and gastrointestinal tract distress associated with oral ingestion of large amounts of poorly absorbed material. It is our contention that there are no differences in the sensitivity of the rabbit and the other species utilized to assess the systemic toxicity of advantame. Moreover, metabolic or pharmacokinetic differences that could suggest the possibility of species-specific differences in systemic toxicity of advantame are unlikely. Data to support the above position include:

- Results of individual food consumption and bodyweights of rabbits that died or aborted during the study
- Results of individual necropsy records of rabbits that that died or aborted during the study
- Similarities between species with respect to alterations of fecal appearance and coloured staining of the tray bottoms/bedding due to contact with urine and feces
- Results of preliminary pharmacokinetic investigations in rabbits

Each of these points is expanded on and presented herein.

Food consumption and bodyweight data

The food consumption and body weight data for the individual rabbits that were killed or aborted prior to necropsy show a clear tendency for these particular animals (6 at the high-dose level of 2,000 mg/kg bw and 1 at 1,000 mg/kg bw) to essentially stop eating well before becoming moribund, necessitating euthanasia for humane reasons.

As shown in Table 1 (in Appendix A to this letter), in the rabbits that were found to be pregnant and survived to necropsy, there was considerable fluctuation in food consumption values across all groups (including the control), with decreases in food intake with time in all groups, with considerable reductions from Day 24 to the end of the study. There was no evidence of a consistent pattern or of altered food intake related relationship to advantame treatment. In fact, from gestational Day 20 onward, rabbits in the high-dose group (those pregnant and surviving to necropsy) actually tended to increase, with this increase attaining statistical significance on Days 22 to 23.

In contrast, in the treated rabbits which were killed for humane reasons (1 at 1,000 mg/kg bw and 4 at 2000 mg/kg bw) and in the one high-dose rabbit (No. 84) that aborted the entire litter on Day 29 of gestation, food consumption was generally greatly reduced during the days prior to euthanasia. These individual animal data are shown in Table 2.

Clearly, those rabbits which did not stop eating and survived until necropsy maintained their pregnancy normally and were found to show no clinically or toxicologically significant effects of advantame treatment, including on food consumption (Table 1). On the other hand, the deaths and the poor clinical appearance of those rabbits killed prior to necropsy was directly related to the failure of these animals to feed and, in some cases, drink. It is this in-appetence which resulted in these deaths, not systemic toxicity of advantame *per se*. In-appetence, with concomitant GI tract distress, and subsequent mortality due to gavage dosing of high doses of poorly absorbed compounds has been well documented to occur in the rabbit, a species known to be sensitive to this particular effect (Kille *et al.*, 2000; SCF, 2000). The response of the rabbit to high oral doses of sucralose is one example (Kille *et al.*, 2000).

The body weight data mirror the food consumption data. The body weights of rabbits that were pregnant and survived to necropsy were found to be unaffected by treatment, even up to the 2,000 mg/kg bw dose level (see Table 3 in the Appendix to this letter). Only in those rabbits that were killed for humane reasons or which aborted their litter, did body weights either fail to increase or declined in the latter part of the gestational period (Table 4). This would be an expected outcome of the greatly reduced food intake in these animals. Since the decreased food intake cannot plausibly be associated with systemic exposure to advantame, it follows that the decreased body weights in these animals also reflect a local effect of high concentrations of advantame due to its intense sweetness and effects in the GI tract due to the absorption characteristics. This local effect is not relevant to low-dose systemic exposure to advantame as would occur in humans and, as a result, should not form the basis on which to establish the ADI.

Necropsy data

The necropsy data from the rabbits which were killed for humane reasons, or which aborted their litter prior to the end of the study, clearly demonstrate a diffuse local effect of high concentrations of advantame on the tissue of the GI tract. As shown in Table 5, there is evidence of haemorrhage of the caecal wall (animal nos. 52 and 75), production of gas (animal nos. 75, 92, and 96), congestion of the GI tract as shown by accumulation of green fluid and formation of gelatinous masses (animal nos. 52, 75, 82, 92, 96) and presence of dark fluid in the caecum of the reaming rabbits (animal nos.84, 93). A number of these rabbits also were found to have ceased to form fecal pellets (animal nos. 52, 84, 92, and 96).

All of the necropsy findings in the rabbits that were killed prior to the end of the gestation period document the GI tract distress associated with the administration of large doses of advantame by oral gavage. The suite of findings described above is consistent with a local effect on GI tract function. Similar GI tract findings, not to be confused with toxicity, due to the presence of poorly absorbed osmotically active substances in the GI tract (WHO, 1987) have been noted to occur in rabbits administered high doses of sucralose (Kille *et al*., 2000). Such findings have been considered of no relevance to the establishment of an ADI (SCF, 2000).

Fecal appearance; staining of feces, urine, and bedding

In the Technical Assessment report is stated that:

> "Other results that were unusual compared to other laboratory species were green staining of the GIT or its contents, green bladder contents and green staining of the surface of the kidneys in one mid-dose dam. Collectively, these findings suggest that rabbits are relatively sensitive to Advantame-acid relative to other laboratory animals. While there are no kinetic data available in rabbits, the green staining of the GIT, bladder contents and kidneys suggest different kinetics and possibly a different metabolite profile relative to other species."

The staining of the GI tract contents, feces, urine, and by contact the cage bedding and tray material, thought to be due to a coloured metabolite of advantame, is not unique to the rabbit. In the dog (AJO179; AJO 196), while there was no report of coloured feces or of coloured materials in the cage indicative of a coloured metabolite in the urine, a number of the studies with rats reported similar observations.

In the 13-week rat study (AJO 176), the occasional animal receiving 5,000, 15,000, and 50,000 ppm was reported to have associated green or pink staining of the tray paper (either from urine or contact with feces). In the 2-year rat carcinogenicity study (AJO 195), green coloured feces were noted in the mid- and high-dose groups, and green GI tract contents were observed in high-dose females, green staining of the bedding, especially in the high-dose group, and either blue, pink or purple staining of the bedding noted in several treated animals. Finally, of note, in the teratology study (AJO 182) and 2-generation reproductive toxicity study (AJO 203) conducted in the CD rat, changes in fecal colour, largely an increase in pale feces at 50,000 ppm (parent advantame), and the presence of green staining on the undertray in all treated groups were attributed to excretion of test material and metabolites in the urine and faeces. Taken together, these data, assuming that the reported staining of the bedding/tray paper and the coloration of the feces is due to a metabolite of advantame, indicate that the metabolic profiles of rats and rabbits are similar, or at the very least, that the presumptive green coloured metabolite is not unique to the rabbit.

Toxicokinetic data in the rabbit

Toxicokinetic (TK) data for the rabbit were not included with the original submission. This was due to the fact, as explained in the submission (p.156-157), the bioanalytical method used had the effect of re-esterifying some of the advantame-acid back to parent advantame and advantame-acid conjugates back to advantame-acid. As a result, these and other data from the

rat and dog that were generated from this "original" bioanalytical method were not included in the original submission.

While the TK data generated by the "original" method strictly speaking do not provide reliable quantitative data (*i.e.*, over-estimation of the amount of parent advantame and under-estimation of advantame-acid present in plasma), these data allow a qualitative comparison between rats, dogs, and rabbits.

In the preliminary embryo-fetal toxicity study conducted in the rabbit (AJO183, included as Appendix B to this letter), conducted as a dose-range finding study for the main teratology study in rabbits (AJO 190), blood for TK analysis was drawn from animals (each of 500, 1,000, and 2,000 mg/kg bw dose groups) on gestational days 6 and 27, with sample collection intervals of 0.5, 1, 2, 4, 8, 12, and 24-hours post-dose.

In the high-dose group, on GD 6 and 27 the C_{max} values for advantame and advantame-acid were 1,070 and 18,923 ng/mL and 2,980 and 30,400 ng/mL, respectively. The C_{max} values for the acid were about 5- to 20-fold that for the parent compound. T_{max} values were generally 0.5 to 1.0 hours after dosing for both advantame and advantame-acid.

Likewise, AUC_{24} values for advantame-acid were found to be about 20 to 30-fold greater than for advantame (*e.g.*, for the 2,000 mg/kg group, at GD 27, AUC_{24} values for advantame-acid and advantame were 486,206 ng x hr/mL and 22,367 ng x hr/mL, respectively). This ratio of parent compound and metabolite, as measured by the "original" bioanalytical, is similar to that found in the rat and dog. For example, toxicokinetic studies conducted in the 13-week rat study (AJO176/014075, p. 619-620) at Week 1 in high-dose females revealed C_{max} and AUC_{24} values for advantame and advantame-acid of 978 and 3,145 ng/mL and 6,754 and 46,427 ng x hr/mL, respectively. In the 13-week dog study (AJO 179/014664, p. 347-348), corresponding C_{max} and AUC_{24} values for high dose females at Day 90 were 4,485 and 114,906 ng/mL and 81,341 and 1,983,731 ng x hr/mL, respectively.

Also, pharmacokinetic studies (gavage dosing at 150 mg/kg) in both rats (AJO 184, p. 8) and dogs (AJO 193, p. 9) demonstrate that the T_{max} for advantame-acid is in the range of 0.5 to 1.0 hours. This range is the same as that reported for advantame-acid in the rabbit.

The available pharmacokinetic data in the rabbit, and similar data in the rat and dog generated using the same bioanalytical methodology, show that that ratio of parent compound to the main metabolite advantame-acid is similar across species. Similarly absorption following gavage exposure while incomplete, appears rapid (0.5 to 1.0 hours) in each of the rat, dog, and rabbit. These findings do not indicate that the pharmacokinetics or metabolism of advantame in the rabbit is significantly different compared to either the rat or dog. As a result, kinetic differences are not likely to be involved in the maternal toxicity noted in the rabbit teratology study. This further supports the conclusion that the maternal toxicity observed in rabbits is due to a local effect of high gavage doses of advantame on the GI tract, not systemic toxicity.

Summary and Conclusion

Ajinomoto, Inc. has concluded that the maternal toxicity in noted in pregnant rabbits administered 1,000 and 2,000 mg/kg bw/day by gavage is not a systemic effect, but an effect of high concentrations of poorly absorbable, osmotically active substances in the GI tract. This condition is known to cause GI tract distress in the rabbit, leading to in-appetence, weight loss and death. The food consumption and body weight data confirm the lack of appetite only in those rabbits that either were killed or aborted prior to the end of the study, while the necropsy data on these individual animals clearly demonstrate localized effects on the GI tract. The limited pharmacokinetic data available in the rabbit do not indicate the existence of species-specific differences in the kinetics of advantame and advantame-acid. Similarly, the finding of coloured faeces/urine in the rabbit was not unique since it was also recorded in the rat studies (*i.e.*, if the colouration was due to a metabolite(s), the metabolites are likely similar between rats and rabbits).

Given that the maternal toxicity in the rabbit is not a systemic effect, and that the kinetics of advantame and the main metabolite advantame-acid do not appear to be significantly different in the rabbit in comparison to other species, it is concluded that the ADI for advantame should not be based on the results of the rabbit teratology study.

It is the opinion of Ajinomoto, Inc. that the ADI should be established on the basis of the NOAEL values obtained from the long-term rat study, as is customary, given that no species-specific toxicity (systemic) is present. In the case of Advantame, given that the NOAEL in the long-term rat study and in the other toxicity studies in rats and dogs was the highest dietary concentration tested of 50,000 ppm, the data support an ADI of “not specified”.

2. Addition of Advantame to Schedule 2 of Standard 1.3.1 – Food Additives

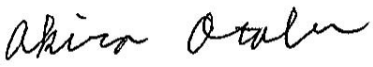
Based on the lack of evidence for any systemic toxicity for Advantame in animal studies and the high level of tolerance for Advantame demonstrated in human studies, it is the opinion of Ajinomoto, Inc. that Advantame should be included in Schedule 2 of Standard 1.3.1 – *Food Additives* of the Australia New Zealand Food Standards Code, which would enable it to be used in a wide range of foods in accordance with GMP.

The appropriate wording for the Schedule 2 entry would be: “Advantame (technological use consistent with clause 4)” which is consistent with the use of other similar high intensity sweeteners.

3. Specifications for Advantame

In order to be consistent with the specifications proposed in the USFDA petition, Ajinomoto, Inc. would like the specification value for water content to be changed from “2.5 to 5.0%” to “not more than 5%”.

Sincerely,



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References

Kille JW, Tesh JM, McAnulty PA, Ross FW, Willoughby CR, Bailey GP et al., 2000. Sucralose: Assessment of teratogenic potential in the rat and the rabbit. Food Chem Toxicol 38(Suppl. 2):S43-S52.

SCF, 2000. Opinion of the Scientific Committee on Food on Sucralose (Opinion Expressed by the SCF on 7 September 2000). Scientific Committee on Food (SCF), European Commission, Health & Consumer Protection Directorate-General, Scientific Committee on Food; Brussels, Belgium. SCF/CS/ADD/EDUL/190 Final Available from: http://www.europa.eu.int/comm/food/fs/sc/scf/out68_en.pdf.

WHO, 1987. Setting the ADI. In: *Principles for the Safety Assessment of Food Additives and Contaminants in Food*. (Environmental Health Criteria, no 70). Geneva, Switz.: World Health Organization (WHO), International Programme on Chemical Safety (IPCS), pp. 75-85. Available at: http://www.inchem.org/documents/ehc/ehc/ehc70.htm.

APPENDIX A

TABLES OF SUPPORTING DATA

Table 1 Mean food consumption values (g/animal/day) during gestation in rabbits given Advantame daily by gavage from days 6 to 28

Dosage (mg/kg bw/day)	Day											
	1-5	**6-7**	**8-9**	**10-11**	**12-13**	**14-15**	**16-17**	**18-19**	**20-21**	**22-23**	**24-25**	**26-28**
0	213±50	204±62	200±61	216±47	185±65	187±68	192±76	194±85	169±101	135±78	120±69	125±56
500	205±36	168±62	189±53	200±39	199±41	201±49	201±36	209±43	189±56	170±61	140±60	130±50
1000	220±38	191±44	206±37	191±48	188±55	181±71	176±88	176±85	166±63	163±58	157±70	151±72
2000	227±45	173±55	210±55	197±52	188±46	194±51	206±79	199±93	199±73	191[a]±77	166±59	139±48

Results are means±SD(standard deviation)
n: number of females with live young on Day 29 of gestation (*i.e.*, data do not include animals that died, aborted their litter, or were not pregnant), where n=19 in the controls, 18 in the 500 mg/kg group, 21 in the 1000 mg/kg group, and 12 in the 2000 mg/kg group
[a]Statistically significant difference compared to vehicle control ($p<0.05$)

Table 2 Food consumption (g/rabbit/day) of rabbits given Advantame daily by gavage from days 6 to 28 that were killed for humane reasons or aborted their litter

Treatment group (Animal No. and day of kill)	Day											
	1-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-28
1000 mgkg (52, killed day 27)	237	129	145	236	290	80	141	227	238	246	172	52[a]
2000 mg/kg (75, killed day 24)	225	197	179	219	189	210	250	236	219	78	-	-
2000 mg/kg (82, killed day 26)	271	236	246	209	120	35	27	7	3	3	2	-
2000 mg/kg (92, killed day 23)	166	123	119	86	112	102	32	9	2	5[a]	-	-
2000 mg/kg (93, killed day 17)	200	80	33	11	3	1	0[a]	-	-	-	-	-
2000 mg/kg (96, killed day 26)	197	104	113	198	205	138	28	9	0	13	2	-
2000 mg/kg (84, aborted on day 29)	246	181	238	215	181	209	233	110	32	63	45	18

[a] The number of days food consumption is calculated over where less than entire period,

Table 3 Mean body weights (kg) during gestation of rabbits given Advantame daily by gavage from days 6 to 28

Dose (mg/kg bw/day)	Day										
	0	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**24**	**28**
0	3.98±0.36	4.15±0.40	4.20±0.41	4.22±0.45	4.34±0.45	4.36±0.45	4.45±0.48	4.47±0.50	4.50±0.51	4.48±0.54	4.57±0.58
500	3.96±0.39	4.12±0.39	4.14±0.40	4.20±0.41	4.25±0.41	4.32±0.40	4.38±0.40	4.42±0.40	4.46±0.39	4.52±0.40	4.57±0.38
1000	3.99±0.33	4.19±0.36	4.23±0.33	4.27±0.33	4.30±0.35	4.33±0.33	4.40±0.38	4.40±0.38	4.40±0.38	4.45±0.36	4.52±0.42
2000	4.09±0.42	4.32±0.43	4.32±0.46	4.32±0.43	4.40±0.48	4.44±0.44	4.54±0.48	4.55±0.47	4.60±0.50	4.65±0.53	4.69±0.53

Results are means±SD(standard deviation)
n: number of females with live young on Day 29 of gestation (i.e., data do not include animals that died, aborted their litter, or were not pregnant), where n=19 in the controls, 18 in the 500 mg/kg group, 21 in the 1000 mg/kg group, and 12 in the 2000 mg/kg group
No statistically significant differences ($p>0.05$)

Table 4 Individual body weights (kg) of rabbits given Advantame daily by gavage from days 6 to 28 that were killed for humane reasons or aborted their litter

Treatment group (Animal No. and day of kill)	Day											
	0	6	8	10	12	14	16	18	20	24	28	29
1000 mg/kg (52, killed day 27)	4.10	4.46	4.35	4.36	4.36	4.63	4.36	4.41	4.51	4.64	-	-
2000 mg/kg (75, killed day 24)	4.01	4.23	4.22	4.24	4.29	4.37	4.49	4.55	4.53	4.22	-	-
2000 mg/kg (82, killed day 26)	4.50	4.63	4.95	4.74	4.79	4.62	4.68	4.68	4.44	4.24	-	-
2000 mg/kg (92, killed day 23)	3.61	3.88	3.89	3.94	3.84	4.02	4.01	3.93	3.82	-	-	-
2000 mg/kg (93, killed day 17)	4.10	4.20	4.12	4.05	3.88	3.82	3.59	-	-	-	-	-
2000 mg/kg (96, killed day 26)	4.00	4.32	4.25	4.26	4.34	4.30	4.51	4.35	4.21	3.99	-	-
2000 mg/kg (84, aborted on day 29)	4.56	4.72	4.68	4.71	4.64	4.72	4.82	4.86	4.75	4.56	4.61	4.39

Table 5 Individual necropsy findings in rabbits given Advantame daily by gavage from days 6 to 28 that were killed for humane reasons or aborted their litter

Treatment group	Animal No.	Necropsy Findings
1000 mg/kg	52 (killed on day[a] 27)	Kidneys: multiple punctuate foci on surfaces Caecum and stomach: caecal contents fluid, thick green gelatinous material at ileo-caecal junction. Caecal wall haemorrhagic, stomach wall thickened adjacent to pyloric sphincter Colon: contained pale green/brown fluid Internal structures: Tinged green Rectum: devoid of contents Uterus: no implantations
2000 mg/kg	75 (killed on day 24)	Coat ungroomed. Green staining on nares, urino-genital region, ventral body surfaces and all paws. Kidneys: multiple clear punctate cysts on both kidneys Caecum: entire wall haemorrhagic, green gelatinous material at ileo/caecal junction Duodenum, ileum and jejunum: contained bright green material Colon and rectum: contained green frothy material Uterus: 12 implantations comprising 2 early resorptions and 10 live fetuses
2000 mg/kg	82 (killed on day 26)	Green staining on ventral abdominal region, urinogenital region, all paws and tail Caecum: distended with dark green fluid Duodenum: contained pale green viscous fluid Ileum and jejunum: contained pale green creamy material Colon and rectum: contained small amount of dark green fluid Urinary bladder: contents green Uterus: no implantations
2000 mg/kg	92 (killed on day 23)	Green staining on head, ventral body surfaces and all paws. Matted green staining on urino-genital region and tail Thin build Gall bladder: enlarged Stomach: contained dark green matted material, serosa of stomach tinged green Caecum: contained dark green fluid and large amount of air Gastro-intestinal tract: contains dark green viscous fluid Rectum: no faecal pellet formation present Uterus: 11 implantations comprising 3 late resorptions and 8 live fetuses.
2000 mg/kg	93 (killed on day 17)	Green staining on all paws and urino-genital region Caecum: contents dark and fluid Ileum and jejunum: devoid of contents Colon and rectum: contained dark amorphous material with soft pale granular material Uterus: 12 implantations comprising 3 early resorptions and 9 live fetuses

Treatment group	Animal No.	Necropsy Findings
2000 mg/kg	96 (killed on day 26)	Dark green matted fur around tail and urino-genital region. Green staining on all paws, red staining around vagina. Slight hairloss on ventral body surface. Caecum: aerated and contained dark green fluid Liver: appeared pale with accentuated lobular pattern on all lobes Rectum: contained few soft faecal pellets Uterus: 12 implantations comprising 12 live fetuses
2000 mg/kg	84 (aborted on day 29)	Yellow/brown staining on all paws, red staining on urino-genital region and tail 1 placenta and 1 dead fetus found in cage undertray Liver: pale Caecum: contents dark and fluid Rectum: no faecal pellet formation Uterus and cervix: contained large amount of dark red serous material. 7 implantations comprising 1 empty site, 3 dead fetuses, 2 early resorptions and 1 live fetus. All placentae* appeared pale with dark mottled areas

[a] "day" refers to the number of days after mating

APPENDIX B

AJO 183 PRELIMINARY EMBRYO-FETAL TOXICITY STUDY IN NEW ZELAND WHITE RABBITS