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Acrylamide in Foods Generated through Heating

Summary

Food Safety Commission of Japan

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment on acrylamide (AA) (CAS No. 79-06-1) in foods generated through heating, as a self-tasking risk assessment. In rodent toxicity studies, major adverse effects were observed on neurotoxicity and male reproductive toxicity. Statistically significant increases in incidences were observed in the carcinogenicity studies in Harderian gland, mammary gland, lung and forestomach in mice, and in mammary gland, thyroid and testis in rats. Positive results were also obtained from *in vitro* and *in vivo* genotoxicity studies of AA and glycidamide (GA). Therefore, FSCJ recognized AA as a genotoxic carcinogen. Judging from the dietary AA intake among Japanese people, the non-neoplastic risk is extremely low because of sufficient margins of exposure (MOEs). The neoplastic risk, however, could not be excluded due to the insufficient MOEs, although no clear evidence on human health effect have been provided from the epidemiological studies. It is important to note that no consistent relationships between AA exposure and cancer incidences have been observed even in the studies focusing on the highly exposed populations in occupational settings. FSCJ thus concluded that continual efforts are necessary to reduce dietary AA intakes in accordance with the principle of ALARA (as low as reasonably achievable) from the viewpoint of public health.

Conclusion in Brief

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment on acrylamide (AA) (CAS No. 79-06-1) in foods generated through heating, as a self-tasking risk assessment.

The data used in the assessment include toxicokinetics, acute toxicity, subacute toxicity, chronic toxicity and carcinogenicity, neurotoxicity, reproductive/developmental toxicity, developmental neurotoxicity and genotoxicity, as well as epidemiological studies and exposure surveys. Assessments reported by foreign and international organizations and published relevant research papers were reviewed.

AA ingested is distributed to various tissues, but not accumulated in the body. There are two major metabolic pathways, which lead to the formation of a highly reactive glycidamide (GA) and to the glutathione conjugate. These metabolites are further biotransformed prior to urinary excretion. Both AA and GA are reactive to form their hemoglobin and DNA adducts.

In rodent toxicity studies, major adverse effects were observed on neurotoxicity and male reproductive toxicity.

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The original full report is available in Japanese at http://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kya20160405231&fileId=200
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Statistically significant increases in incidences were observed in the carcinogenicity studies in Harderian gland, mammary gland, lung and forestomach in mice, and in mammary gland, thyroid and testis in rats. Positive results were also obtained from *in vitro* and *in vivo* genotoxicity studies of AA and GA. Therefore, FSCJ recognized AA as a genotoxic carcinogen

No consistent associations have been obtained in human studies between AA exposure and the increased incidence of cancer. Although neurological effects are reported in case of occupational exposure to AA, the relationship with the dietary exposure remains obscure. It is, thus, not feasible to conduct the quantitative assessment on health effects of AA based on the epidemiological studies on general populations and AA workers.

A point estimate of the dietary AA intake is calculated as 0.158 µg/kg bw/day among Japanese people. A probabilistic distribution of the dietary AA intake is estimated using Monte Carlo simulation where the median value is 0.154 µg/kg bw/day and the 95th percentile value is 0.261 µg/kg bw/day. Afterwards, additional data of AA contents on vegetables cooked at high temperatures became available from the Ministry of Agriculture, Forestry and Fisheries (MAFF) in November 2015. Taken into account the additional data, the revised point estimate of the AA intake is calculated as 0.240 µg/kg bw/day. (Appendix I)

Margins of exposure (MOEs) for the non-neoplastic effects of AA were estimated based on the reference point using the $BMDL_{10}$ of 0.43 mg/kg bw/day. The $BMDL_{10}$ was derived from the increased incidences of axonal degeneration in sciatic nerve observed in male rats exposed to AA in drinking water for two years (Appendix II). Following MOE values are calculated; i) 2,772 from the initial point estimate, ii) 2,792 from the median, iii) 1,648 from the 95th percentile, and iv) 1,792 from the revised point estimate.

The MOEs for neoplastic effects of AA were estimated using the $BMDL_{10}$ of 0.17 mg/kg bw/day in mice and 0.30 mg/kg bw/day in rats. The $BMDL_{10}$ of 0.17 mg/kg bw/day in mice was derived from the increased incidences of Harderian adenomas/adenocarcinomas observed in male mice exposed to AA in drinking water for two years. In addition, the $BMDL_{10}$ of 0.30 mg/kg bw/day was derived from the increased incidences of mammary fibroadenoma observed in female rats exposed to AA in drinking water for two years (Appendix II). Following MOE values are calculated; i) 1,076 (mice) and 1,899 (rats) from the initial point estimate, ii) 1,104 (mice) and 1,948 (rats) from the median, iii) 651 (mice) and 1,149 (rats) from the 95th percentile, and iv) 708 (mice) and 1,250 (rats) from the revised point estimate.

Judging from the dietary AA intake among Japanese people, the non-neoplastic risk is extremely low because of sufficient MOEs.

The neoplastic risk, however, could not be excluded due to the insufficient MOEs, although no clear evidence on human health effect have been provided from the epidemiological studies. It is important to note that no consistent relationships between AA exposure and cancer incidences have been observed even in the studies focusing on the highly exposed populations in occupational settings. FSCJ thus concluded that continual efforts are necessary to reduce dietary AA intakes in accordance with the principle of ALARA (as low as reasonably achievable) from the viewpoint of public health.

Following researches are expected for the further risk assessment; i) the accumulation of data on comprehensive dietary intakes with cooking method information, ii) the development of methods of biological monitoring for individual exposure, and iii) the epidemiological studies on cancer among the Japanese people using biomarkers.

Appendix I

Estimation of the Dietary AA Intake

1. Data used

The data used were on food consumption and AA content in foods. Data on food consumption were obtained from 24,293 individual records with age, sex and body weight information in National Health and Nutrition Survey (2013)[1]. Data on AA content in foods were derived from various survey results reported by National Institute of Health Sciences (NIHS) (2002)[2] and MAFF (2004–2013)[3–6], and also from relevant research papers on boiled rice[7], tea[8,9], toast[10] and oven cooked potato[11]. Some data of AA contents in common Japanese foods data, such as in surface fried potato/onion for the preparation of curry or Nikujaga (Japanese meat and potato stew)[12], also in bottled Mugi-cha tea (roasted barley extracts) etc, were acquired through FSCJ additional survey.

2. Point estimation

A point estimate of the dietary AA intake was calculated as 0.158 μg/kg bw/day, after summation of the AA intake from each food item. The AA intake from each food item was calculated after multiplying the mean consumption and the mean AA content in each the food item. The AA intake from each food item is shown in **Table 2**.

The dietary AA intake was also calculated for the age groups as follows; for 1 to 6 years as 0.409 μg/kg bw /day; for 7 to 14 years as 0.290 μg/kg bw/day; for 15 to 29 years as 0.159 μg/kg bw/day; for 30 to 44 years as 0.155 μg/kg bw/day; for 45 to 59 years as 0.146 μg/kg bw/day; for over 60 years as 0.119 μg/kg/kg bw/day (NIES, 2016)[13].

3. Monte Carlo simulation

The National Institute for Environmental Studies (NIES) estimated the probabilistic distribution of dietary AA intake among Japanese people using Monte Carlo simulation in the research project granted by FSCJ. Assuming statistical distribution of food consumption and AA content in foods, the probabilistic distribution of dietary AA intake and the representative values, such as the median and the 95th percentile, can be estimated.

The estimated distribution is shown in **Fig. 1**, where the median is 0.154 μg/kg bw/day, the 95th percentile is 0.261 μg/kg bw/day and the mean is 0.166 μg/kg bw/day (**Table 1**). The estimated AA intake from each food item is shown in **Table 3** (NIES, 2016).

4. Revised point estimation

The additional data on AA contents of the vegetables (lotus root, burdock, carrot, Chinese chive, garlic, Welsh onion, bean sprout, green pepper, cabbage, asparagus, string bean and potato) cooked at high temperatures became available in November 2015[14]. Those data were obtained in a research project from 2013 to 2014 funded by MAFF. AA content data of realistic cutting size/shape and heating time were selected. The consumption data of those vegetables (Fried/Sautéed) in National Health and Nutrition Survey (2013) were used. The calculated AA intake from these vegetables is shown in **Table 4 and 5**.

The revised point estimate of dietary AA intake was calculated as 0.240 μg/kg bw/day, through adding the values on the vegetables not included in **Table 2** (lotus root, burdock, carrot, Chinese chive, garlic and Welsh onion), and substituting values on the vegetables included in **Table 2** (bean sprout, green pepper, cabbage, asparagus, string bean and potato).

The revised AA intake from each item is shown in **Table 6**.

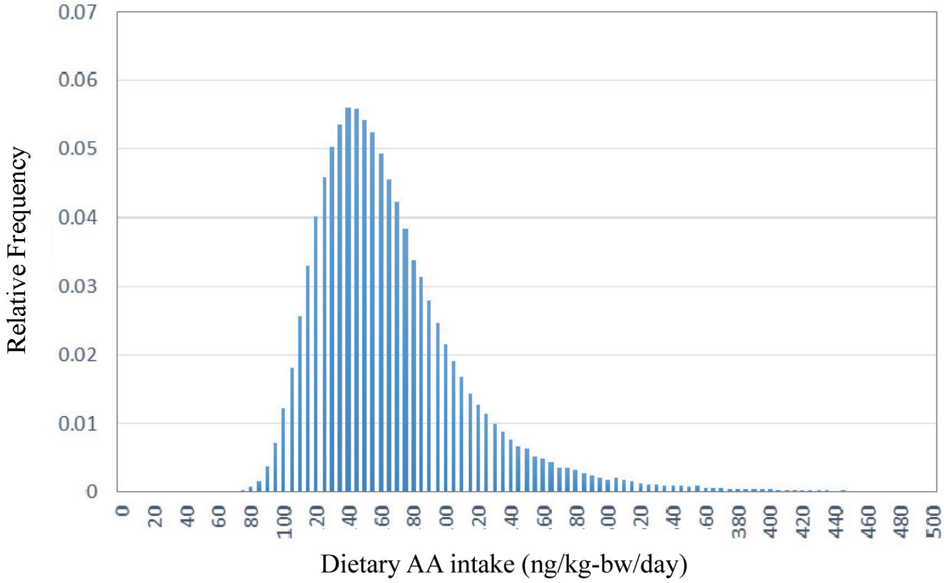

Fig. 1. Distribution of dietary AA intake

Table 1. Dietary AA intake estimated using Monte Carlo simulation (μg/kg bw/day)

Median	95th percentile	Mean
0.154	0.261	0.166

Table 2. Point estimate of AA intake from each food item

Food item	Number of people who consumed the food	Amount of the food consumption per body weight (g/kg bw/day)	AA content (ng/g)	AA intake (ng/kg bw/day)
Regular Coffee (Extracts)	4,203	0.78	16	12
Instant Coffee (Freeze-dried)	7,159	0.018	668	12
Wheat flour snack	2,273	0.058	174	10
Potato chips	568	0.020	471	9.4
Potatoes (Sautéed)	507	0.028	319	8.9
Bean sprout (Fried/Sautéed)	2,385	0.088	95	8.4
Molded mashed potato snack	171	0.0066	1187	7.8
Potatoes (Fried)	497	0.028	269	7.5
Onions (Surface fried)	3,914	0.18	37	6.7
Green pepper (Fried/Sautéed)	3,929	0.068	95	6.5
Hashed beef/Curry/Beef stew paste	2,847	0.060	101	6.1
Green tea/Oolong tea (Extracts)	12,497	4.40	1.2	5.3
Onion (Fried/Sautéed)	7,432	0.21	25	5.3
Rice cracker	2,284	0.052	99	5.1
Cooked rice	23,605	6.6	0.59	3.9
Cabbage (Fried/Sautéed)	3,739	0.18	20	3.6
Hoji-cha <Roasted tea> (Extracts)	1,329	0.41	7.6	3.1
Bread crumb for coating in frying	5,916	0.12	24	2.9
Mugi-cha tea <Roasted barley extracts infused at home>	3,593	1.3	2.0	2.6
Bottled mugi-cha tea <Roasted barley extracts>	955	0.36	7	2.5
Karinto <Fried dough cookie> (With non-centrifugal sugar)	141	0.0033	731	2.4
Roasted sesame seed	5,843	0.015	152	2.3
Coffee-based beverage	1,198	0.22	9.1	2.0
Bread roll (Untoasted/Without non-centrifugal sugar)	2,152	0.13	13	1.7
Instant noodle	1,019	0.065	26	1.7
Potatoes (Surface fried)	2,502	0.15	11	1.7
Corn flour snack	312	0.0097	142	1.4
Bread roll (Untoasted/With non-centrifugal sugar)	239	0.014	91	1.3
Eggplant (Fried/Sautéed)	1,067	0.056	20	1.1
Sliced bread (Toasted/Without non-centrifugal sugar)	5,459	0.33	3.3	1.1
Cereal	270	0.011	93	1.0

Table 2. (continued.)

Food item	Number of people who consumed the food	Amount of the food consumption per body weight (g/kg bw/day)	AA content (ng/g)	AA intake (ng/kg bw/day)
Bread roll/French bread	1,117	0.062	16	1.0
Non-centrifugal sugar (Excluding Wasanbon-to <Traditional Japanese refined sugar>)	364	0.0025	387	0.97
Almond	373	0.0029	324	0.94
Manju <Japanese steamed bun> (With non-centrifugal sugar)	100	0.0042	194	0.81
Cocoa (Powder)	679	0.0065	123	0.80
Rice miso paste	14,671	0.19	3.0	0.57
Sweetened bun	1,247	0.089	6.2	0.55
Dried fruits	685	0.011	47	0.52
Soy sauce	21,001	0.26	1.9	0.49
Sliced bread (Untoasted/Without non-centrifugal sugar)	2,340	0.14	3.4	0.48
Peanut	496	0.0060	75	0.45
Broccoli (Fried/Sautéed)	842	0.020	20	0.40
Podded pea (Fried/Sautéed)	188	0.00093	393	0.37
Imo-kenpi <Fried sweet potatoes >	67	0.0021	166	0.35
Pumpkins (Fried/Sautéed)	370	0.017	20	0.34
Kinako <Roasted soybean flour>	656	0.0039	75	0.29
Candy (With non-centrifugal sugar)	27	0.00024	1046	0.25
Asparagus (Fried/Sautéed)	92	0.0020	95	0.19
String bean (Fried/Sautéed)	417	0.0079	20	0.16
Curry powder	418	0.00033	423	0.14
White stew paste	786	0.017	8.0	0.14
English tea (Extracts)	1,880	0.39	0.29	0.11
Fried beans	19	0.00049	120	0.059
Soybean miso paste	362	0.0035	9.0	0.032
English muffin/Naan	124	0.0083	3.3	0.027
Bolo <Small round cookie>	29	0.0011	20	0.022
Mugikogashi <Roasted barley flour>	8	0.000040	236	0.0094
Wasanbon-to <Traditional Japanese refined sugar>	14	0.000066	85	0.0056
Pistachio	15	0.00011	34	0.0037
Total (ng/kg bw/day)				158

Table 3. Estimated AA intake from each food item using Mote Carlo simulation

Food item	Median (ng/kg bw/day)	95th percentile (ng/kg bw/day)	Mean (ng/kg bw/day)
Regular Coffee (Extracts)	11	29	13
Instant Coffee (Freeze-dried)	9.0	29	12
Potato (Sautéed)	7.0	24	9.1
Bean sprout (Fried/Sautéed)	4.9	28	8.5
Molded mashed potato snack	4.8	25	7.9
Wheat flour snack	4.5	37	10
Onions (Surface fried)	4.3	38	10
Green tea/Oolong tea (Extracts)	4.3	13	5.3
Potato (Fried)	3.9	28	8.0
Onion (Fried/Sautéed)	3.7	17	5.6
Potato chips	3.7	55	14
Green pepper (Fried/Sautéed)	3.5	22	6.6
Hashed beef/Curry/Beef stew paste	3.5	19	5.9
Cooked rice	3.5	8	3.9
Rice cracker	2.2	20	5.3
Cabbage (Fried/Sautéed)	2.0	11	3.4
Bread crumb for coating in frying	1.9	7.9	2.8
Karinto <Fried dough cookie> (With non-centrifugal sugar)	1.9	5.7	2.4
Hoji-cha <Roasted tea> (Extracts)	1.9	10	3.3
Bottled Mugi-cha tea <Roasted barley extracts>	1.9	6.5	2.5
Mugi-cha tea <Roasted barley extracts infused at home>	1.9	7.4	2.6
Coffee-based beverage	1.6	4.4	2.0
Instant noodle	1.6	2.9	1.7
Roasted sesame seed	1.4	7.5	2.4
Bread roll (Untoasted/Without non-centrifugal sugar)	1.3	3.8	1.6
Bread roll (Untoasted/With non-centrifugal sugar)	1.1	3.0	1.3
Sliced bread (Toasted/ Without non-centrifugal sugar)	0.83	2.7	1.1
Manju <Japanese steamed bun> (With non-centrifugal sugar)	0.76	1.9	0.89
Corn flour snack	0.66	5.8	1.6
Eggplant (Fried/Sautéed)	0.63	3.3	1.0
Bread roll/French bread	0.59	3.1	0.98
Cocoa (Powder)	0.56	2.3	0.81
Rice miso paste	0.49	1.4	0.60
Non-centrifugal sugar (Excluding Wasanbon-to <Traditional Japanese refined sugar>)	0.48	3.3	0.95

Table 3. (continued.)

Food item	Median (ng/kg bw/day)	95th percentile (ng/kg bw/day)	Mean (ng/kg bw/day)
Sweetened bun	0.46	1.3	0.56
Sliced bread (Untoasted/Without non-centrifugal sugar)	0.42	0.90	0.47
Soy sauce	0.37	1.4	0.51
Almond	0.34	1.3	0.46
Cereal	0.33	4.8	1.3
Peanut	0.30	1.4	0.45
Dried fruits	0.27	1.8	0.51
Potatoes (Surface fried)	0.27	0.75	0.32
Imo-kenpi (Fried sweet potatoes)	0.26	0.94	0.35
Broccoli (Fried/Sautéed)	0.22	1.2	0.37
Kinako <Roasted soybean flour>	0.19	0.90	0.30
Candy (With non-centrifugal sugar)	0.19	0.67	0.25
Pumpkin (Fried/Sautéed)	0.18	1.1	0.34
Podded pea (Fried/Sautéed)	0.15	1.4	0.38
White stew paste	0.13	0.30	0.14
Asparagus (Fried/Sautéed)	0.11	0.61	0.19
English Tea (Extracts)	0.096	0.25	0.11
Curry powder	0.079	0.46	0.14
String beans (Fried/Sautéed)	0.071	0.52	0.15
Fried beans	0.059	0.059	0.059
Soybean miso paste	0.025	0.082	0.032
English muffin/Naan	0.020	0.076	0.028
Bolo <Small round cookie>	0.015	0.060	0.021
Mugikogashi <Roasted barley flour>	0.0095	0.0095	0.0095
Wasanbon-to <Traditional Japanese refined sugar>	0.0056	0.0056	0.0056
Pistachio	0.0038	0.0038	0.0038
Total (ng/kg bw/day)	154	261	166

Table 4. Point estimate of dietary AA intake from some vegetables not included in **Table 2**

Food item	Number of people who consumed the food	Amount of the food consumption per body weight (g/kg bw/day)	AA content (ng/g)	AA intake (ng/kg bw/day)	Experimental conditions (Heating time on 200 °C hot plate, cutting size/shape, etc)
Lotus root (Fried/Sautéed)	848	0.0231	455	10.5	Heating time: 6 min. Sliced 1-2 mm thick Repeated 4 times × 2 ways of pretreatment (with/without soaked in water)
Burdock (Fried/Sautéed)	1,684	0.0344	80	2.8	Heating time: 7 min. Thinly sliced Repeated 4 times
Carrot (Fried/Sautéed)	9,691	0.1443	16	2.3	Heating time: 10 min. Half circle slice, 2 mm thick Repeated 3 times
Chinese chive (Fried/Sautéed)	738	0.0114	30	0.3	Heating time: 3 min. Cut 4 cm long Repeated 3 times
Garlic (Fried/Sautéed)	2,332	0.0031	173	0.5	Heating time: 7 min. 1–2 mm thick Repeated 3 times
Welsh onion (Fried/Sautéed)	1,979	0.0221	43	1.0	Heating time: 6 min. Diagonal cut, 4 cm long and 5 mm thick Repeated 3 times

Table 5. Point estimate of dietary AA intake from some vegetables included in **Table 2**

Food item	Number of people who consumed the food	Amount of the food consumption per body weight (g/kg bw/day)	AA content (ng/g)	AA intake (ng/kg bw/day)	Experimental conditions (Heating time on 200 °C hot plate, cutting size/ shape, etc)
Bean sprout (Fried/Sautéed)	2,385	0.088	752[※1]	66.1	Heating time: 2 min. /7 min. Whole (without removing fibrous root) Repeated 3 times
Green peppers (Fried/Sautéed)	3,929	0.068	43	2.9	Heating time: 6 min. Sliced 2 mm thick Repeated 3 times
Cabbage (Fried/Sautéed)	3,739	0.18	50	9.0	Heating time: 5 min. Cut 3 cm x 3 cm size Repeated 3 times
Asparagus (Fried/Sautéed)	92	0.0020	400	0.8	Heating time: 5 min. Cut 4.5 cm long Repeated 3 times
String bean (Fried/Sautéed)	417	0.0079	63	0.5	Heating time: 6 min. Cut 4.5 cm long Repeated 3 times
Potato (Sautéed)	507	0.028	463[※2]	13.0	Heating time: 10 min. Half circle slice, 5 mm thick Reservation ways: room temperature/cooled Repeated 4 times × 2 ways of reservation

※1 The AA content is average of the data on 2 min. and 7 min. ※2 The weight of reservation ways (room temperature or cooled) was estimated from FSCJ survey data.

Table 6. Revised point estimate of AA intake from each food item

Food item	Number of people who consumed the food	Amount of the food consumption per body weight (g/kg bw/day)	AA content (ng/g)	AA intake (ng/kg bw/day)	Revised item from **Table 2** (Add or Substitute)
Bean sprout (Fried/Sautéed)	2,385	0.088	752	66	Substitute
Potato (Sautéed)	507	0.028	463	13	Substitute
Regular Coffee (Extracts)	4,203	0.78	16	12	
Instant Coffee (Freeze-dried)	7,159	0.018	668	12	
Lotus root (Fried/Sautéed)	848	0.0231	455	11	Add
Wheat flour snacks	2,273	0.058	174	10	
Potato chips	568	0.020	471	9.4	
Cabbage (Fried/Sautéed)	3,739	0.18	50	9.0	Substitute
Molded mashed potato snack	171	0.0066	1187	7.8	
Potato (Fried)	497	0.028	269	7.5	
Onion (Surface fried)	3,914	0.18	37	6.7	
Hashed beef/Curry/Beef stew paste	2,847	0.060	101	6.1	
Green tea/Oolong tea (Extracts)	12,497	4.40	1.2	5.3	
Onion (Fried/Sautéed)	7,432	0.21	25	5.3	
Rice cracker	2,284	0.052	99	5.1	
Cooked rice	23,605	6.6	0.59	3.9	
Hoji-cha <Roasted tea> (Extracts)	1,329	0.41	7.6	3.1	
Green pepper (Fried/Sautéed)	3,929	0.068	43	2.9	Substitute
Bread crumb for coating in frying	5,916	0.12	24	2.9	
Burdock (Fried/Sautéed)	1,684	0.0344	80	2.8	Add
Mugi-cha tea <Roasted barley extracts infused at home>	3,593	1.3	2.0	2.6	
Bottled Mugi-cha tea <Roasted barley extracts>	955	0.36	7	2.5	
Karinto <Fried dough cookie> (With non-centrifugal sugar)	141	0.0033	731	2.4	
Roasted sesame seed	5,843	0.015	152	2.3	
Carrot (Fried/Sautéed)	9,691	0.1443	16	2.3	Add
Coffee-based beverage	1,198	0.22	9.1	2.0	
Bread roll (Untoasted/Without non-centrifugal sugar)	2,152	0.13	13	1.7	
Potatoes (Surface fried)	2,502	0.15	11	1.7	
Instant noodle	1,019	0.065	26	1.7	
Corn flour snack	312	0.0097	142	1.4	
Bread roll (Untoasted/With non-centrifugal)	239	0.014	91	1.3	
Sliced bread (Toasted/Without non-centrifugal sugar)	5,459	0.33	3.3	1.1	
Cereal	270	0.011	93	1.0	
Bread roll/French bread	1,117	0.062	16	1.0	
Welsh onion (Fried/Sautéed)	1,979	0.0221	43	1.0	Add

Table 6. (continued.)

Food item	Number of people who consumed the food	Amount of the food consumption per body weight (g/kg bw/day)	AA content (ng/g)	AA intake (ng/kg bw/day)	Revised item from **Table 2** (Add or Substitute)
Non-centrifugal sugar (Excluding Wasanbon-to <Traditional Japanese refined sugar>)	364	0.0025	387	0.97	
Almond	373	0.0029	324	0.94	
Manju <Japanese steamed bun> (With non-centrifugal sugar)	100	0.0042	194	0.81	
Coccoa (Powder)	679	0.0065	123	0.80	
Asparagus (Fried/Sautéed)	92	0.0020	400	0.80	Substitute
Eggplant (Fried/Sautéed)	1,067	0.056	12	0.67	Substitute
Pumpkin (Fried/Sautéed)	370	0.017	34	0.58	Substitute
Rice miso paste	14,671	0.19	3.0	0.57	
Sweetened bun	1,247	0.089	6.2	0.55	
Dried fruits	685	0.011	47	0.52	
String bean (Fried/Sautéed)	417	0.0079	63	0.50	Substitute
Garlic (Fried/Sautéed)	2,332	0.0031	173	0.5	Add
Soy sauce	21,001	0.26	1.9	0.49	
Sliced bread (Untoasted/Without non-centrifugal sugar)	2,340	0.14	3.4	0.48	
Peanut	496	0.0060	75	0.45	
Broccoli (Fried/Sautéed)	842	0.020	20	0.40	Substitute
Podded pea (Fried/Sautéed)	188	0.00093	393	0.37	
Imo-kenpi (Fried sweet potato)	67	0.0021	166	0.35	
Chinese chive (Fried/Sautéed)	738	0.0114	30	0.3	Add
Kinako <Roasted soybean flour>	656	0.0039	75	0.29	
Candy (With non-centrifugal sugar)	27	0.00024	1046	0.25	
Curry powder	418	0.00033	423	0.14	
White stew paste	786	0.017	8.0	0.14	
English tea (Extracts)	1,880	0.39	0.29	0.11	
Fried beans	19	0.00049	120	0.059	
Soybean miso paste	362	0.0035	9.0	0.032	
English muffin/Naan	124	0.0083	3.3	0.027	
Bolo <Small round cookie>	29	0.0011	20	0.022	
Mugikogashi <Roasted barley flour>	8	0.000040	236	0.0094	
Wasanbon-to <Traditional Japanese refined sugar>	14	0.000066	85	0.0056	
Pistachio	15	0.00011	34	0.0037	
Total (ng/kg bw/day)				240	

Appendix II

Dose-response Assessment

Since AA is recognized as a genotoxic carcinogen, FSCJ considered it appropriate to estimate the Margin of Exposure (MOE) for the assessment rather than to set a threshold value. In order to derive the reference point for MOE approach, the benchmark dose (BMD) method was applied to the dose-response relationship for both neoplastic and non-neoplastic effects. BMD method is an acceptable alternate of classical NOAEL method for non-neoplastic effect.

1. Selection of appropriate toxicity studies

The toxicity studies, where clear dose-response relationship were observed, and where animal species, number of animals, administration method and dose settings were appropriate, were selected for BMD analysis.

Thus, the chronic toxicity and carcinogenicity studies done by NTP (2012)[15], by Johnson et al. (1986)[16] and by Friedman et al. (1995)[17] were selected.

2. Selection of data on critical endpoints

Quantal (dichotomous) data obtained from critical endpoints, such as cancer, degenerative diseases and inflammatory diseases etc, were selected for the BMD analysis.

3. BMR setting

The benchmark response (BMR) of 10% was used for the assessment.

4. Estimation of BMD and BMDL

BMD and the benchmark dose lower confidence limit (BMDL) of the BMD approach were estimated by using “BMDS ver 2.5” from US-EPA. Models used were Gamma, Logistic, Log-Logistic, Multistage, Probit, Log-Probit, Quantal-Linear and Weibull. In order to exclude biologically unrealistic dose-response curve, models were restricted where possible.

5. Model selection

Models were examined from the following points; 1) whether the estimated values do not extremely deviate from the data obtained in animal studies, 2) whether the confidence interval of estimated BMD is small enough, and 3) whether the estimated BMDL is close to the lowest dose administered in the animal studies.

Thus, the following criteria were applied for model selection.

1) The p-value for a goodness of fit test >0.1

2) BMDL/BMD >0.1

3) BMDL/the lowest dose of each test >0.1

6. Determination of the reference point

The reference points were determined according to the following procedure; firstly, the endpoints with lower BMD_{10} values among the models were examined. Then, the most appropriate endpoint with the lowest BMD_{10} was selected in considering the model fitting. Finally, $BMDL_{10}$ of the selected endpoint was chosen as the reference point.

a. Non-neoplastic effects

Table 7 shows the endpoints on non-neoplastic effects with lower BMD_{10} values.

The endpoint with the lowest BMD_{10} was ovarian atrophy in rats observed in NTP (2012). Considerable number of ovarian atrophy, however, were observed also in the control animals. In addition, the association of AA with the ovarian atrophy was not clear. Thus the endpoint of ovarian atrophy in rats was not adopted.

The endpoint with the next lower BMD_{10} was the axonal degeneration of sciatic nerves in male rats observed in NTP (2012), where BMD_{10} of 0.61 mg/kg bw/day, and $BMDL_{10}$ of 0.43 mg/kg bw/day were obtained. The $BMDL_{10}$ of 0.43 mg/kg bw/day was determined as the reference point. **Fig. 2** shows the dose-response curve of the selected model.

b. Neoplastic effects

Table 8 shows the endpoints on neoplastic effects with lower BMD_{10} values in rats and mice.

<Mice> The endpoint with the lowest BMD_{10} was Harderian adenomas in male mice (NTP 2012). Although humans have no Harderian gland, this organ is highly sensitive to genotoxicity and carcinogenicity in rodents. As AA exerts

Table 7. Endpoint with lower BMD_{10} value (Non-neoplastic effects)

Endpoint	Animal species (M/ F) *1	Model	Restrict	P-value	BMD_{10} mg/kg bw/day	$BMDL_{10}$ mg/kg bw/day	$BMDL_{10}$ /BMD_{10}	$BMDL_{10}$ /lowest dose	Source
Overian atrophy	Rats (F)	Log-Logistic	ON	0.63	0.30	0.08	0.3	0.2	NTP 2012
Axonal degeneration of sciatic nerves	Rats (M)	Quantal-Linear	*2	0.64	0.61	0.43	0.7	1.3	NTP 2012
Retinal degeneration	Rats (F)	Log-Logistic	ON	0.90	1.02	0.49	0.5	1.1	NTP 2012
Prepuce thymus pipe expansion	Rats (M)	Log-Logistic	ON	0.15	1.23	0.60	0.5	1.8	NTP 2012
Mouth vagina mucous membrane epithelium hyperplasia	Rats (M)	Log-Logistic	ON	0.45	2.08	1.07	0.5	107.1	Johnson et al. 1986

※1) M: male; F: female ※2) Restrict option is not provided in the Quantal-Linear model.

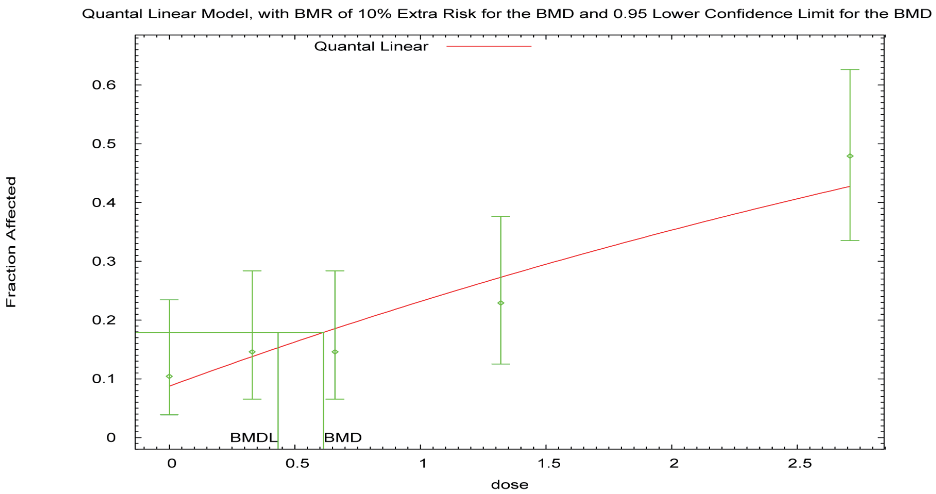

Fig. 2. Dose-response curve by Quantal-Linear model on axonal degeneration of sciatic nerve in male rats (NTP 2012)

carcinogenicity in various organs, the endpoint could not be ignored in the human risk assessment. Since both adenomas and adenocarcinomas are equally important endpoints on the Harderian gland, both Harderian adenomas and adenocarcinomas were selected as the endpoints. BMD_{10} of 0.37 mg/kg bw/day and $BMDL_{10}$ of 0.17 mg/kg bw/day were obtained. The $BMDL_{10}$ of 0.17 mg/kg bw/day was determined as the reference point. **Fig. 3** shows the dose-response curve of the selected model.

<Rats> The endpoint with the lowest BMD_{10} was clitoral gland adenomas in female rats reported by Johnson et al. (1986). In the study, the histological examination of the adenomas was, however, performed only on rats with the macroscopical lesion. Thus, the endpoint was not adopted.

The endpoint with the next lower BMD_{10} was mammary fibroadenoma in female rats reported by NTP (2012), where BMD_{10} of 0.55 mg/kg bw/day and $BMDL_{10}$ of 0.30 mg/kg bw/day were obtained. The $BMDL_{10}$ of 0.30 mg/kg bw/day was determined as the reference point. **Fig. 4** shows the dose-response curve of the selected model.

Table 8. Endpoint with lower BMD_{10} value (Neoplastic effects)

Endpoint	Animal species (M/F) *1	Model	Restrict	P-value	BMD_{10} mg/kg bw/day	$BMDL_{10}$ mg/kg bw/day	$BMDL_{10}$ /BMD_{10}	$BMDL_{10}$ /lowest dose	Sources
Clitoral gland adenoma	Rats (F)	Log-Logistic	ON	0.24	0.02	0.002	0.1	0.2	Johnson et al. 1986
Hardarian adenoma	Mice (M)	Log-Logistic	ON	0.34	0.36	0.17	0.5	0.2	NTP 2012
Hardarian adenoma/ adenocarcinoma	Mice (M)	Log-Logistic	ON	0.30	0.37	0.17	0.5	0.2	NTP 2012
Hardarian adenoma	Mice (F)	Log-Logistic	ON	0.43	0.47	0.28	0.6	0.3	NTP 2012
Mammary fibroadenoma	Rats (F)	Log-Logistic	ON	0.61	0.55	0.30	0.5	0.7	NTP 2012

※1) M: male; F: female

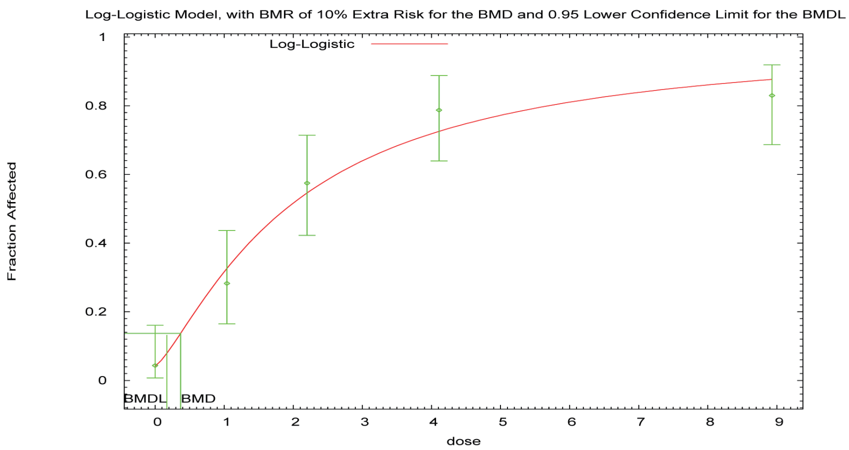

Fig. 3. Dose-response curve by Log-Logistic model on Harderian adenomas/adenocarcinomas in male mice (NTP 2012)

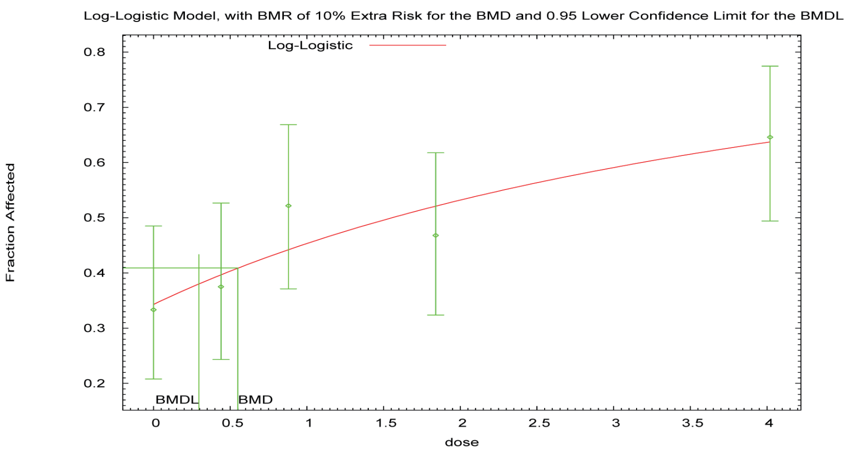

Fig. 4. Dose-response curve by Log-Logistic model on mammary fibroadenoma in female rats (NTP 2012)

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