Summarized data of genotoxicity tests for designated food additives in Japan

Masami Yamada1,2* and Masamitsu Honma2

Abstract

The Ministry of Health, Labour and Welfare has carried out genotoxicity tests for food additives used in Japan in cooperation with the Japan Food Additives Association since 1979. Hayashi et al. summarized these data and published a list of 337 designated additives (Shitei-tenkabutsu in Japanese) with genotoxicity test data in 2000. Thereafter, 29 items were eliminated, and 146 items were newly added. Currently, 454 designated additives are allowed to be used as food additives in Japan. This report, based on the Hayashi report, covers the addition of newly derived genotoxicity test data. Routinely, the bacterial reverse mutation test (Ames test), mammalian cell chromosomal aberration test, and in vivo rodent bone marrow micronucleus test have been used for the evaluation of genotoxicity of food additives. In addition to the data from these tests being updated in this report, it newly includes results of transgenic rodent somatic and germ cell gene mutation assays (TGR assays), incorporated in the Organisation for Economic Co-operation and Development (OECD) test guidelines after 2000. We re-evaluated the genotoxicity of 13 designated food additives considering their TGR data.

Keywords: Food additives, Designated additives, Genotoxicity test, Ames test, Transgenic rodent gene mutation assay

Background

Since 1979, as part of the safety reassessment of food additives, the Ministry of Health, Labour and Welfare (MHLW; prior to January 2001, the Ministry of Health and Welfare) has carried out mutagenicity tests annually in cooperation with the Japan Food Additives Association. In 2000, Dr. M. Hayashi (former Head of Division of Genetics and Mutagenesis at the National Institute of Health Sciences (NIHS)) and colleagues summarized the mutagenicity data for 337 designated additives, 187 existing additives (natural additives), 49 natural fragrances, and seven general food and drink additives from fiscal year (FY) 1979 to FY1998 [1] (hereafter referred to as the “Hayashi report”). Since then, concerning designated additives, 29 items have been eliminated due to abolition of form classification or for other reasons (Table 1), and 146 items have been newly added (Table 2). In this report, which is based on the Hayashi report, data on newly tested items have been added, and mutagenicity data for a total of 454 designated food additives is summarized in Table 3.

How the data were summarized

The following set of three tests has traditionally been used to evaluate mutagenicity of food additives: reverse mutation assay (Ames test) using bacteria; chromosomal aberration test using cell culture (CA); and micronucleus test using mice (MN). The Hayashi report summarized the data from the results of these three tests. Two new tests suitable for the evaluation of genotoxicity were subsequently added in the OECD Genotoxicity Test Guidelines. The two adopted test guidelines are: “Genetic mutation test using transgenic rodent somatic and germ cells (TG 488)” (the TGR test); and “In vivo mammalian alkaline comet assay (TG 489)” (the comet test). Results using these assays are included in this paper (Table 3). While both tests have advantages for the evaluation of genotoxicity in specific tissues, the TGR test is intended as a mutagenicity test (similar to the Ames test) therefore the weighting of TGR results is generally higher due to its high correlation with carcinogenicity.

* Correspondence: m-yamada@nda.ac.jp
1Department of Applied Chemistry, National Defense Academy, 1-10-20, Hashirimizu, Yokosuka-shi, Kanagawa 239-8686, Japan
2Division of Genetics and Mutagenesis, National Institute of Health Sciences, 3-25-26, Tonomachi, Kawasaki-ku, Kawasaki-shi, Kanagawa 210-9501, Japan

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When searching for items that had not been subjected to mutagenicity testing, it was felt desirable that all test outcomes were discovered without exception. Thus, in Table 3, data from journals other than Hayashi report are included; for example, data published in the Annual Report of the Tokyo Metropolitan Research Laboratory of Public Health (originally published in Japanese). Reports that were surveyed, including journals other than Hayashi report, are listed at the end of this paper. Results of the three tests used initially are mainly copied from the Hayashi report (also published in Japanese). Data reevaluated or added after the Hayashi report include results from outsourced testing laboratories accredited by the MHLW as part of the “Projects for safety of food additives.”

In the main Table, the superscript symbols “H22” and “H23” indicate results commissioned in tests conducted in Fiscal Year 2010 (FY2010) and FY2011, respectively. Test results from the Risk Assessment Reports prepared by the Food Safety Commission (FSC) are also included; these are indicated by superscript “FSC,” and the URL for the Risk Assessment Reports of the item is given in the reference list. Note that reference numbers in the text are not given in numeric order but in the order of appearance in Table 3. The eliminated and newly added substances are listed in Tables 1 and 2, respectively. In Table 3, the item numbers from Table 2 are shown underlined.

### Commentary for 13 items newly subjected to the TGR test

1) Five items positive for Ames and chromosome aberration tests while negative for in vivo micronucleus test

**Sodium nitrite (No. 6 in Table 3)**

Ames testing was performed at the highest dose of 10 mg/plate using TA1535, TA98, TA1537, TA94, TA92 in FY1979, and positive results were obtained with TA100 and TA1535 regardless of S9mix [2]. Subsequently, using TA97 and TA102, a statistically significant increase in the number of revertants (maximum dose 10 mg/plate) was reported in both strains regardless of S9mix [3]. However, the result is given as negative in Table 3 since the number of reverted colonies did not reach twice of the number for the negative control. Many positive results of Ames tests were reported in this item, thus, mutagenicity was suspected for this substance [4].

For chromosomal abnormalities, the chromosome aberration test using Chinese Hamster lung (CHL) was performed at a maximum dose of 1.0 mg/mL without S9mix, and strong induction of structural abnormalities was reported [2]. Subsequently, in vivo bone marrow micronucleus tests using ddY mice were carried out under three conditions: a single intraperitoneal (i.p.) dose of 200 mg/kg body weight; four i.p. doses at 50 mg/kg body weight at 24-h intervals; and a single oral dose of 400 mg/kg body weight [5], all results being reported as negative.

### Table 1 List of designated food additives eliminated after 2000 (As of October 6, 2016)

| Name                                           | Date       | Reason                                                                                   |
|------------------------------------------------|------------|-------------------------------------------------------------------------------------------|
| Aluminum Potassium Sulfate (dried) (syn: Burnt Alum) | June 30, 2000 | Integrated into “Aluminum Potassium Sulfate”                                           |
| Ferrous Pyrophosphate                           | June 30, 2000 | Distribution and usage records have not been confirmed                                      |
| Sodium Sulfite (anhydrous)                      | June 30, 2000 | Integrated into “Sodium Sulfite”                                                          |
| Tetrasodium Pyrophosphate (anhydrous)           | June 30, 2000 | Integrated into “Tetrasodium Pyrophosphate”                                               |
| Aluminum Ammonium Sulfate (dried) (syn: Burnt Ammonium Alum) | June 30, 2000 | Integrated into “Aluminum Ammonium Sulfate”                                               |
| Disodium Hydrogen Phosphate (anhydrous) (Disodium Phosphate (anhydrous)) | June 30, 2000 | Integrated into “Disodium Hydrogen Phosphate”                                           |
| Sodium Dihydrogen Phosphate (anhydrous) (Monosodium Phosphate (anhydrous)) | June 30, 2000 | Integrated into “Sodium Dihydrogen Phosphate”                                           |
| Tertiary Sodium Phosphate (anhydrous)           | June 30, 2000 | Integrated into “Tertiary Sodium Phosphate”                                               |
| Choline phosphate                               | June 30, 2000 | Distribution and usage records have not been confirmed                                      |
| Methyl O-Acetylricinoleate                       | June 30, 2000 | Distribution and usage records have not been confirmed                                      |
| Citric Acid (anhydrous)                         | June 30, 2000 | Integrated into “Citric Acid”                                                              |
| Ferrous Sulfate (dried)                         | June 30, 2000 | Integrated into “Ferrous Sulfate”                                                          |
| Sodium Acetate (anhydrous)                      | June 30, 2000 | Integrated into “Sodium Acetate”                                                           |
| Sodium Hydroxide (crystal)                      | June 30, 2000 | Integrated into “Sodium Hydroxide”                                                         |
| Sodium Carbonate (crystal)                      | June 30, 2000 | Integrated into “Sodium Carbonate”                                                         |
| Sodium Starch Phosphate                         | June 4, 2009 | Production and usage have not been confirmed                                              |

The date and the reasons for disappearance are indicated.
| No. | Name                                                                 |
|-----|----------------------------------------------------------------------|
| 2   | chlorous acid water                                                   |
| 4   | nitrous oxide                                                         |
| 8   | calcium L-ascorbate                                                   |
| 9   | L-ascorbic acid 2-glucoside                                           |
| 13  | asparaginase                                                          |
| 16  | acesulfame potassium                                                  |
| 17  | acetylated distarch adipate                                           |
| 18  | acetylated oxidized starch                                            |
| 19  | acetylated distarch phosphate                                         |
| 20  | acetaldehyde                                                          |
| 24  | sodium selenite pentahydrate                                          |
| 25  | azoxytribin                                                           |
| 26  | advantame                                                             |
| 28  | β-apo-8'-carotenal                                                    |
| 29  | (3-amino-3-carboxypropyl) dimethylsulfonium chloride                  |
| 30  | amylalcohol                                                           |
| 35  | ammonium alginate                                                    |
| 36  | potassium alginate                                                   |
| 37  | calcium alginate                                                     |
| 44  | ammonium isovalerate                                                 |
| 46  | ion exchange resin                                                    |
| 47  | isoamylalcohol                                                        |
| 51  | isoquinoline                                                          |
| 54  | isovaleraldehyde                                                     |
| 55  | isobutanol                                                            |
| 56  | isobutyraldehyde (isobutanal)                                         |
| 57  | isopropanol                                                           |
| 58  | isopentylamine                                                        |
| 67  | mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3, 6-dimethylpyrazine |
| 69  | 2-ethylpyrazine                                                       |
| 70  | 3-ethylpyridine                                                       |
| 71  | 2-ethyl-3-methylpyrazine                                              |
| 72  | 2-ethyl-5-methylpyrazine                                              |
| 73  | 2-ethyl-6-methylpyrazine                                              |
| 74  | 5-ethyl-2-methylpyridine                                              |
| 77  | ethers                                                                |
| 89  | octanoic acid                                                         |
| 91  | starch sodium octenyl succinate                                       |
| 94  | peracetic acid                                                        |
| 102 | canthaxanthin                                                         |
| 106 | xylitol (alias xylit)                                                 |
| 110 | triethyl citrate                                                      |
| 113 | sodium ferrous citrate (sodium iron citrate)                          |

| No. | Name                                                                 |
|-----|----------------------------------------------------------------------|
| 127 | sodium gluconate                                                      |
| 128 | glutaryl-valyl-glycine                                                |
| 130 | monoammonium L-glutamate                                              |
| 132 | monocalcium dL -L-glutamate                                           |
| 134 | monomagnesium dL-L-glutamate                                          |
| 135 | calcium silicate                                                      |
| 136 | magnesium silicate                                                   |
| 140 | ketones                                                              |
| 150 | calcium acetate                                                       |
| 156 | starch acetate                                                       |
| 165 | calcium saccharin                                                     |
| 168 | calcium oxide                                                        |
| 169 | oxidized starch                                                       |
| 172 | hypochlorous acid water                                               |
| 174 | hypobromous acid water                                                |
| 176 | 2,3-dimethylpyrazine                                                  |
| 177 | 2,3-diethyl-5-methylpyrazine                                          |
| 189 | fatty acids                                                           |
| 191 | aliphatic higher aldehydes (except those generally recognized as highly toxic) |
| 192 | aliphatic higher hydrocarbons (except those generally recognized as highly toxic) |
| 193 | 2,3-dimethylpyrazine                                                  |
| 194 | 2,5-dimethylpyrazine                                                  |
| 195 | 2,6-dimethylpyrazine                                                  |
| 196 | 2,6-dimethylpyridine                                                  |
| 201 | potassium dl-bitartrate (potassium hydrogen dl-tartrate or potassium hydrogen dl-tartrate) |
| 203 | disodium dl-tartrate (disodium dl-tartrate)                           |
| 226 | magnesium hydroxide                                                   |
| 227 | sucralose (trichlorogalactosucrose)                                   |
| 228 | calcium stearate                                                      |
| 229 | magnesium stearate                                                    |
| 231 | sodium stearoyl lactylate                                             |
| 236 | calcium sorbate                                                       |
| 248 | thiamine thiocyanate (vitamin B1 thiocyanate)                         |
| 251 | thioethers (except those generally recognized as highly toxic)        |
| 252 | thiols (thioalcohols) (except those generally recognized as highly toxic) |
| 258 | 5,6,7,8-tetrahydroquinoxaline                                         |
| 259 | 2,3,5,6-tetramethylpyrazine                                           |
| 262 | terpene hydrocarbons                                                 |
| 268 | all-racemic -α-tocopheryl acetate                                     |
| 269 | RRR -α-tocopheryl acetate                                            |
In FY2009, TGR testing using gpt delta mice was performed in the liver and glandular stomach for confirmation of in vivo mutagenicity. These organs were selected because the liver metabolizes many substances and is highly sensitive in this assay, and the glandular stomach is the organ first exposed to the substance under test with oral administration. The mutagenicity that was previously observed in the Ames test did not occur in vivo, since the TGR results were negative in both organs after 28 days of administration via drinking water at a maximum dose of 5000 mg/kg body weight [H21(FY2009)].

Table 2 List of items that have been added to the designated food additives (As of October 6, 2016) (Continued)

| No. | Name                                      |
|-----|-------------------------------------------|
| 272 | trimethylamine                             |
| 273 | 2,3,5-trimethylpyrazine                    |
| 276 | nisin                                      |
| 277 | natamycin                                  |
| 284 | carbon dioxide (carbonic acid, gas)        |
| 287 | potassium lactate                          |
| 291 | neotame                                    |
| 303 | valeraldehyde                              |
| 306 | biotin                                     |
| 308 | bisbentiamine (benzoylthiamine disulfide)  |
| 311 | 1-hydroxyethylidene-1, 1-diphosphonic acid|
| 313 | hydroxyacetone dimethylacetal             |
| 314 | hydroxypropyl distarch phosphate           |
| 315 | hydroxypropyl cellulose                    |
| 316 | hydroxypropyl starch                       |
| 317 | hydroxypropyl methylcellulose              |
| 318 | piperidine                                 |
| 321 | sunflower lecithin                         |
| 323 | pyrazine                                   |
| 325 | pyrimethanil                               |
| 328 | pyrrolidine                                |
| 334 | pyrole                                     |
| 339 | 2-(3-phenylpropyl) pyridine                |
| 340 | phenethylamine                             |
| 341 | phenol ethers (except those generally recognized as highly toxic) |
| 342 | phenols (except those generally recognized as highly toxic) |
| 343 | ferrocyanides (potassium ferrocyanide (potassium hexacyanoferrate (iii), calcium ferrocyanide (calcium hexacyanoferrate (iii), sodium ferrocyanide (sodium hexacyanoferrate (iii)) |
| 344 | butanol                                    |
| 345 | butylamine                                 |
| 346 | butyraldehyde                             |
| 350 | fluodionanol                               |
| 352 | propanol                                   |
| 353 | propionaldehyde                            |
| 369 | 2-pentanol (syn: sec-amylalcohol)          |
| 370 | trans-2-pentenal                           |
| 371 | 1-penten-3-ol                              |
| 372 | aromatic alcohols                          |
| 373 | aromatic aldehydes (except those generally recognized as highly toxic) |
| 377 | polysorbate 20                             |

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L-Cysteine monohydrochloride (No. 179 in Table 3)

Ames testing was carried out using TA100, TA98, TA2637, TA94, at a maximum dose of 10 mg/plate with or without S9mix in FY1982. Positive results were reported for TA100 with S9mix, and for TA2637 with and without S9mix [6]. Chromosomal aberration tests were
Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016)

| No. | Name                                                                 | Functional classes           | CAS#         | Molecular weight | Genotoxicity tests | Remarks                                                                 |
|-----|----------------------------------------------------------------------|------------------------------|--------------|------------------|--------------------|-------------------------------------------------------------------------|
| 1   | zinc salts (limited to zinc gluconate and zinc sulfate)              | Dietary supplement           | -(13)        | -(23) c          |                    |                                                                         |
| 2   | chlorous acid water                                                 | Sterilizer                   | 13898-47-0   | 68.45            | (FSC1) d           |                                                                         |
| 3   | sodium chlorite                                                     | Bleaching agent etc.         | 7758-19-2    | 90.44            | (2) (12)           |                                                                         |
| 4   | nitrous oxide                                                       | Propellant                   | 10024-97-2   | 44.01            | (FSC1)             |                                                                         |
| 5   | adipic acid                                                        | Acidifier                    | 124-04-9     | 146.14           | (5) (22)           |                                                                         |
| 6   | sodium nitrite                                                      | Color fixative               | 7632-00-0    | 69.00            | (1) (16)           | Target organs for TG in mice: liver, stomach (glandular stomach)       |
| 7   | l-ascorbic acid (vitamin C)                                         | Antioxidant etc.             | 50-81-7      | 176.12           | (FSC10) (1)        |                                                                         |
| 8   | calcium l-ascorbate                                                 | Dietary supplement etc.      | 5743-28-2    | 426.35           | (FSC3)             |                                                                         |
| 9   | l-ascorbic acid 2-glucoside                                        | Antioxidant etc.             | 129499-78-1  | 338.26           | (26) (26) (FSC4)   |                                                                         |
| 10  | l-ascorbic stearate (vitamin C stearate)                            | Antioxidant etc.             | 25395-66-8   | 442.59           | (4)(16) (4)        |                                                                         |
| 11  | sodium l-ascorbate (vitamin C sodium)                               | Antioxidant etc.             | 134-03-2     | 198.11           | (20) (20) (20)     |                                                                         |
| 12  | l-ascorbic palmitate (vitamin C palmitate)                          | Antioxidant etc.             | 137-66-6     | 414.53           | (177) (22)         |                                                                         |
| 13  | asparaginase                                                        | Processing agent             | 9015-68-3    | 172.12           | (FSC5) (FSC5)      |                                                                         |
| 14  | monosodium l-aspartate                                             | Seasoning etc.               | 3792-50-5    | 173.10           | (26) (9)           |                                                                         |
| 15  | aspartame (o-L-aspartyl-L-phenylalanine methyl ester)               | Sweetener                    | 22839-47-0   | 294.30           | (16) (23)          |                                                                         |
| 16  | acesulfame potassium                                               | Sweetener etc.               | 55589-62-3   | 201.24           | (21) (22)          |                                                                         |
| 17  | acetylated distarch adipate                                         | Thickening agents etc.       | 68130-14-3   | 68130-14-3       | evaluated as modified starch                                           |
| 18  | acetylated oxidized starch                                          | Thickening agents etc.       | 68187-08-6   | 68130-14-3       | evaluated as modified starch                                           |
| 19  | acetylated distarch phosphate                                       | Thickening agents etc.       | 68130-14-3   | 68130-14-3       | evaluated as modified starch                                           |
| 20  | acetaldehyde                                                        | Flavoring agent etc.         | 75-07-0      | 440.5            | (FSC6) (FSC6)      |                                                                         |
| 21  | ethyl acetocetate                                                   | Flavoring agent              | 141-97-9     | 130.14           | (X12) (9)          |                                                                         |
| 22  | acetophenone                                                        | Flavoring agent              | 98-86-2      | 120.15           | (12)               |                                                                         |
| 23  | acetone                                                             | Processing agent             | 67-64-1      | 5808             | (X17) (1)          |                                                                         |
| 24  | sodium selenite pentahydrate                                       | Dietary supplement           | 10102-18-8   | 172.94           | (FSC7) (FSC7)      |                                                                         |
| 25  | azoxystrobin                                                        | Preservative etc.            | 131860-33-8  | 403.4            | (FSC8) (FSC8)      |                                                                         |
| No. | Name                                                                 | Functional classes       | CAS#         | Molecular weight | Genotoxicity tests | Remarks                                                                 |
|-----|----------------------------------------------------------------------|--------------------------|--------------|------------------|--------------------|----------------------------------------------------------------------|
|     |                                                                      |                          |              |                  | Ames                | CA | MN | TGR | Others |                        |
| 26  | advantame                                                            | Sweetener               | 714229-20-6  | 47652            | FSC9               |                |                |                  |                     |
| 27  | anisaldehyde (p-methoxybenzaldehyde)                                | Flavoring agent          | 123-11-5     | 136.15           | 2(12)              | 0)            |                |                  |                     |
| 28  | β-apo-8′-carotenal                                                   | Food color               | 1107-26-2    | 41664            | FSC10              |                |                |                  |                     |
| 29  | (3-amino-3-carboxypropyl) dimethylsulfoxonium chloride              | Flavoring agent          | 3493-12-7    | 1997             | H12                |                |                |                  |                     |
| 30  | amylicalcohol                                                        | Flavoring agent          | 71-41-0      | 88.15            | H16                |                |                |                  |                     |
| 31  | α-amylicinimataldehyde (α-amylicinamic aldehyde)                    | Flavoring agent          | 122-40-7     | 202.29           | H24                |                |                |                  |                     |
| 32  | di-alanine                                                           | Seasoning etc.           | 302-72-7     | 8909             | 3(17)              | 0)            |                |                  |                     |
| 33  | sodium sulfite                                                       | Preservative etc.        | 7757-83-7    | 12604            | 3(12)              | 1)            |                |                  |                     |
|     |                                                                      |                          |              |                  |                    |                |                |                  |                     |
| 34  | L-arginine L-glutamate                                              | Seasoning etc.           | 4320-30-3    | 32133            | 4(20)              | 4)            |                |                  |                     |
| 35  | ammonium alginate                                                   | Emulsifier etc.          | 9005-34-9    | FSC11            |                    |                |                |                  |                     |
| 36  | potassium alginate                                                  | Emulsifier etc.          | 9005-36-1    | FSC12            |                    |                |                |                  |                     |
| 37  | calcium alginate                                                    | Emulsifier etc.          | 9005-35-0    |                  |                    |                |                |                  |                     |
|     |                                                                      |                          |              |                  |                    |                |                |                  |                     |
| 38  | sodium alginate                                                     | Thickening agents        | 9005-38-3    |                  | 2(8)(18)           | 2)            |                |                  |                     |
| 39  | propylene glycol alginate                                           | Thickening agents        |              |                  |                    |                |                |                  |                     |
| 40  | benzoic acid                                                        | Preservative             | 65-85-0      | 122.12           | 2(11)              |                |                |                  |                     |
| 41  | sodium benzoate                                                     | Preservative             | 532-32-1     | 144.10           | 3(19)              | 1)            |                |                  |                     |
| 42  | methyl anthranilate                                                 | Flavoring agent          | 134-20-3     | 151.16           | 4(2)               |                |                |                  |                     |
| 43  | ammonia                                                              | Processing agent         | 7664-41-7    | 170.3            | 3(18)              |                |                |                  |                     |
| 44  | ammonium isovalerate                                                | Flavoring agent          | 1449430-58-3 | 323.43           | H17                |                |                |                  |                     |
| 45  | ionone                                                              | Flavoring agent          | 8013-90-9    | 192.30           | 2(20)              |                |                |                  |                     |
| 46  | ion exchange resin                                                  | Processing agent         |              |                  |                    |                |                |                  |                     |
| 47  | isomylalcohol                                                        | Flavoring agent          | 123-51-3     | 88.15            | H16                |                |                |                  |                     |
| 48  | isoeugenol                                                          | Flavoring agent          | 97-54-1      | 164.20           | 2(12)              |                |                |                  |                     |
| 49  | isoamyl isovalerate                                                 | Flavoring agent          | 659-70-1     | 172.26           | 3(12)              | 0)            |                |                  |                     |
| 50  | ethyl isovalerate                                                   | Flavoring agent          | 108-64-5     | 130.18           | 3(12)              | 0)            |                |                  |                     |
| 51  | isoquinoline                                                        | Flavoring agent          | 119-65-3     | 129.16           | H18                |                |                |                  |                     |

Crystalline form and anhydrous form were used for tests in (1) and (12), respectively.

See compounds with different salt

Mixture of α and β-ionone were used for the assay
Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016) (Continued)

| No. | Name                                                                 | Functional classes | CAS#              | Molecular weight | Genotoxicity tests | Remarks |
|-----|-----------------------------------------------------------------------|--------------------|-------------------|-------------------|--------------------|---------|
| 52  | Isothiocyanates (except those generally recognized as highly toxic)     | Flavoring agent    | 542-85-8          |                   | _FSC19             |         |
| 53  | Allyl isothiocyanate (volatile oil of mustard)                        | Flavoring agent    | 57-06-7           | 99.16             | _FSC12, _FSC22     | Ethyl thiocyanate was used for the assay. |
| 54  | Isovaleraldehyde                                                      | Flavoring agent    | 590-86-3          | 86.13             | _FSC13             |         |
| 55  | Isobutanol                                                            | Flavoring agent    | 78-83-1           | 74.12             | _FSC14, _H16       |         |
| 56  | Isobutyraldehyde (isobutanal)                                        | Flavoring agent    | 78-84-2           | 72.11             | _FSC15, _FSC18, _FSC15 |         |
| 57  | Isopropanol                                                          | Processing agent etc. | 67-63-0         | 60.10             | _FSC16, _FSC16     |         |
| 58  | Isopentylamine                                                       | Flavoring agent    | 107-85-7          | 87.16             | _H18, _H18, _H88   |         |
| 59  | L-isoleucine                                                         | Dietary supplement | 73-32-5           | 131.17            | _FSC19             |         |
| 60  | Disodium 5′-inosinate (sodium 5′-inosinate)                           | Seasoning etc.     | 4691-65-0         | 392.17            | _FSC17             |         |
| 61  | Imazalil                                                             | Antimolding agent  | 35554-44-0        | 297.18            | _FSC20             |         |
| 62  | Indoles and its derivatives                                          | Flavoring agent    | 120-72-9 (indole) |                   | _FSC19, _H23       |         |
| 63  | Disodium 5′-uridy late (sodium 5′-uridy late)                         | Seasoning etc.     | 3387-36-8         | 368.15            | _FSC17, _FSC19, _H22 |         |
| 64  | γ-Undecalactone (undecalactone)                                      | Flavoring agent    | 104-67-6          | 184.28            | _FSC12, _H18, _H18 |         |
| 65  | Ester gum                                                            | Chewing gum base   |                   |                   | _FSC20             |         |
| 66  | Esters                                                               | Flavoring agent    |                   |                   | _FSC20, _FSC36, _H16 |         |
| 67  | Mixture of 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine | Flavoring agent    | 55031-15-7        | 136.20            | _FSC17             |         |
| 68  | Ethylvanillin                                                        | Flavoring agent    | 121-32-4          | 166.17            | _FSC12, _H20       |         |
| 69  | 2-Ethylpyrazine                                                      | Flavoring agent    | 13925-00-3        | 108.14            | _FSC19             |         |
| 70  | 3-Ethylpyridine                                                      | Flavoring agent    | 536-78-7          | 107.15            | _FSC19             |         |
| 71  | 2-Ethyl-3-methylpyrazine                                            | Flavoring agent    | 15707-23-0        | 122.17            | _FSC19             |         |
| 72  | 2-Ethyl-5-methylpyrazine                                            | Flavoring agent    | 13360-64-0        | 122.17            | _FSC19             |         |
| 73  | 2-Ethyl-6-methylpyrazine                                            | Flavoring agent    | 13925-03-6        | 122.17            | _FSC19             |         |
| 74  | 5-Ethyl-2-methylpyridine                                            | Flavoring agent    | 104-90-5          | 121.18            | _FSC18             |         |
| 75  | Calcium disodium ethylenediaminetetraacetate (calcium disodium edta) | Antioxidant         | 62-33-9           | 410.30            | _FSC18, _FSC18, _H22 |         |
| No. | Name                                                                 | Functional classes | CAS#       | Molecular weight | Ames | CA | MN | TGR | Others                      | Remarks                                                                 |
|-----|----------------------------------------------------------------------|--------------------|------------|------------------|------|----|----|-----|-----------------------------|------------------------------------------------------------------------|
| 76  | disodium ethylenediaminetetraacetate (disodium, EDTA)                 | Antioxidant        | 6381-92-6  | 37224            |      |    |    |     |                             |                                                                        |
| 77  | ethers                                                                |                    |            |                  |      |    |    |     |                             |                                                                        |
| 78  | erythorbic acid (isocorbic acid)                                     | Antioxidant        | 89–65-6    | 176.12           |      |    |    |     |                             |                                                                        |
| 79  | sodium erythorbate (sodium isocorbate)                                | Antioxidant        | 6381–77-7  | 216.12           |      |    |    |     |                             |                                                                        |
| 80  | ergocalciferol (calciferol or vitamin D2)                              | Dietary supplement | 50–14-6  | 396.65           |      |    |    |     |                             |                                                                        |
| 81  | ammonium chloride                                                     | Raising agent      | 12125–02-9 | 5349             |      |    |    |     |                             |                                                                        |
| 82  | potassium chloride                                                    | Seasoning          | 7447-40-7  | 7455             |      |    |    |     |                             |                                                                        |
| 83  | calcium chloride                                                      | Tofu coagulator etc. | 10043–52-4 | 11098           |      |    |    |     |                             |                                                                        |
| 84  | ferric chloride                                                       | Dietary supplement | 10025–77-1 | 270.29           |      |    |    |     |                             |                                                                        |
| 85  | magnesium chloride                                                    | Tofu coagulator etc. | 7791-18-6  | 203.30           |      |    |    |     |                             |                                                                        |
| 86  | hydrochloric acid                                                     | Processing agent   | 7647–01-0  | 36.46            |      |    |    |     |                             |                                                                        |
| 87  | eugenol                                                              | Flavoring agent    | 97–53-0    | 164.20           |      |    |    |     |                             |                                                                        |
| 88  | octanal (capryl aldehyde or octyl aldehyde)                           | Flavoring agent    | 124–13-0   | 128.21           |      |    |    |     |                             |                                                                        |
| 89  | octanoic acid                                                        | Flavoring agent    | 124–07-2   | 172.26           |      |    |    |     |                             |                                                                        |
| 90  | ethyl octanoate (ethyl caprylate)                                     | Flavoring agent    | 106–32-1   |                  |      |    |    |     |                             |                                                                        |
| 91  | starch sodium octenyl succinate                                      | Thickening agents etc. | –         |                  |      |    |    |     |                             |                                                                        |
| 92  | o-phenylphenol and sodium o-phenylphenate                             | Antimolding agent  | 90–43-7    | 170.21           |      |    |    |     |                             |                                                                        |
| 93  | sodium oleate                                                         | Film-forming agent | 143–19-1   | 304.44           |      |    |    |     |                             |                                                                        |
| 94  | peracetic acid                                                       | Preservative etc.  | 79–21-0    | 48.02            |      |    |    |     |                             |                                                                        |
| 95  | hydrogen peroxide                                                     | Sterilizer         | 7722-84-1  | 3401             |      |    |    |     |                             |                                                                        |
| 96  | benzoyl peroxide                                                      | Flour treatment agent | 94–36-0  | 242.23           |      |    |    |     |                             |                                                                        |
| 97  | sodium caseinate                                                      | Processing agent   | 9005-46-3  |                  |      |    |    |     |                             |                                                                        |
| 98  | ammonium persulfate                                                  | Flour treatment agent | 7727-54-0 | 228.20           |      |    |    |     |                             |                                                                        |
| 99  | calcium carboxymethylcellulose (calcium cellulose glycolate)          | Thickening agents  | 9050-04-8  |                  |      |    |    |     |                             |                                                                        |
| 100 | sodium carboxymethylcellulose (sodium cellulose glycolate)            | Thickening agents  | 9004-32-4  |                  |      |    |    |     |                             |                                                                        |
| No. | Name | Functional classes | CAS#     | Molecular weight | Genotoxicity tests | Remarks |
|-----|------|-------------------|----------|------------------|-------------------|---------|
| 101 | β-carotene | Food color etc. | 7235-40-7 | 536.88 | (1)(13) ±(1) | |
| 102 | canthaxanthin | Food color | 514–78-3 | 564.82 | FSC22 FSC22 FSC22 | |
| 103 | isoamyl formate | Flavoring agent | 110–45-2 | 116.16 | (3)(16) | (5) |
| 104 | geranyl formate | Flavoring agent | 105–86-2 | 182.26 | (14) | (12) |
| 105 | citronellyl formate | Flavoring agent | 105–85-1 | | (20) | (12) |
| 106 | xylitol | Sweetener | 87–99-0 | 152.15 | (23) | (23) |
| 107 | disodium 5'-guanylate (sodium 5'-guanylate) | Flavoring agent etc. | 5550-12-9 | 407.18 | (3)(17) | (5) |
| 108 | citric acid | Acidifier | 77–92-9 | 192.12 | (3)(15) | (1) |
| 109 | isopropyl citrate | Antioxidant | 39413–05-3 | | (13) | |
| 110 | triethyl citrate | Sweetener | 77–93-0 | 276.28 | (2)(19) | |
| 111 | monopotassium citrate and tripotassium citrate | Flavoring agent etc. | 866–83-1 | 23021 | (17) | (8) |
| 112 | calcium citrate | Dietary supplement etc. | 813–94-5 | 57049 | (3)(13) | (5) |
| 113 | sodium ferrous citrate (sodium iron citrate) | Dietary supplement | 50717–86-7 | 52601 | (4)(12) | (2) |
| 114 | ferric citrate | Dietary supplement | 77–92-9 | 192.12 | (4)(15) | (6) |
| 115 | ferric ammonium citrate | Dietary supplement | 1185–57-5 | | (5)(15) | (6) |
| 116 | trisodium citrate (sodium citrate) | Acidifier | 68–04-2 | 25807 | (17) | (3) |
| 117 | glycine | Seasoning etc. | 56–40-6 | 7507 | (3)(19) | (3) |
| 118 | glycerol (glycerin) | Processing agent | 56–81-5 | 9209 | (2)(19) | (2) |
| 119 | glycerol esters of fatty acids | Emulsifier | | | (3) | (3) |
| 120 | calcium glycerophosphate | Dietary supplement | 27214–00-2 | 210.14 | (1)(12) | (2) |
| 121 | disodium glycyrrhizinate | Sweetener | 68797–35-3 | 899.11 | (3) | 4(1) | 6(8) |
| 122 | glucono-delta-lactone (gluconolactone) | Acidifier | 90–80-2 | 178.14 | (3)(15) | (3) |
| 123 | gluonic acid | Acidifier | 526–95-4 | 196.16 | (3)(15) | (4) |

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Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016) (Continued)

| No. | Name                                      | Functional classes | CAS#     | Molecular weight | Genotoxicity tests | Remarks |
|-----|-------------------------------------------|--------------------|----------|------------------|--------------------|---------|
|     |                                           |                    |          |                  | Ames       | CA  | MN  | TGR | Others |
| 124 | potassium gluconate                        | Acidifier          | 299-27-4 | 234.25           | (8)        |     |     |     |        |
|     |                                           |                    |          |                  | Rec assay: (8)  |       |     |     |        |
| 125 | calcium gluconate                          | Dietary supplement | 299-28-5 | 448.39           | (12)(6)    | 0   |     |     |        |
| 126 | ferrous gluconate (iron gluconate)         | Dietary supplement etc. | 299-29-6 | 446.14           | (19)       | (125) |     |     |        |
| 127 | sodium gluconate                           | Emulsifier etc.    | 527-07-1 | 218.14           | (8)        |     |     |     |        |
| 128 | glutamyl-valyl-glycine                     | Seasoning          | 38837-70-6 | 303.31           | (8)        |     |     |     |        |
| 129 | L-glutamic acid                            | Seasoning          | 56-86-0  | 147.13           | (19)       |     |     |     |        |
| 130 | monoammonium L-glutamate                   | Seasoning etc.     | 7558-63-6 | 182.18           | (24)       |     |     |     |        |
| 131 | monopotassium L-glutamate                  | Seasoning etc.     | 6382-01-0 | 203.23           | (27)       | (125) |     |     |        |
| 132 | monocalcium di-L-glutamate                 | Dietary supplement etc. | 69704-19-4 | 404.38           | (19)       |     |     |     |        |
| 133 | monosodium L-glutamate                     | Seasoning etc.     | 6106-04-3 | 187.13           | (19)       |     |     |     |        |
| 134 | monomagnesium di-L-glutamate               | Dietary supplement etc. | 129160-51-6 | 38861           | (19)       |     |     |     |        |
| 135 | calcium silicate                           | Processing agent   | 38837-70-6 |                | (25)       |     |     |     |        |
| 136 | magnesium silicate                         | Processing agent   | 1343-88-0 |                | (26)       |     |     |     |        |
| 137 | cinnamic acid                              | Flavoring agent    | 140-10-3  | 148.16           | (14)       |     |     |     |        |
| 138 | ethyl cinnamate                            | Flavoring agent    | 103-36-6  | 176.21           | (14)       |     |     |     |        |
| 139 | methyl cinnamate                           | Flavoring agent    | 103-26-4  | 162.19           | (14)       |     |     |     |        |
| 140 | ketones                                    | Flavoring agent    | 106-24-1  | 154.25           | (14)       |     |     |     |        |
| 141 | geraniol                                   | Flavoring agent    | 106-24-1  | 154.25           | (14)       |     |     |     |        |
| 142 | high test hypochlorite                     | bleaching agent etc. | 110-15-6  | 118.09           | (15)       |     |     |     |        |
| 143 | succinic acid                              | Acidifier etc.     | 110-15-6  | 118.09           | (15)       |     |     |     |        |
| 144 | monosodium succinate                       | Seasoning etc.     | 2922-54-5 | 140.07           | (19)       | (206) |     |     |        |
| 145 | disodium succinate                         | Seasoning etc.     | 150-90-3  | 162.05           | (19)       |     |     |     |        |
| 146 | cholecalciferol (vitamin D3)               | Dietary supplement | 67-97-0  | 384.64           | (20)       |     |     |     |        |
| 147 | sodium chondroitin sulfate                 | Humectant etc.     | 12678-07-8 |                | (14)       |     |     |     |        |
| 148 | isoamyl acetate                            | Flavoring agent    | 123-92-2  | 130.18           | (14)       |     |     |     |        |
| 149 | ethyl acetate                              | Flavoring agent etc. | 141-78-6  | 88.11           | (16)       |     |     |     |        |
| 150 | calcium acetate                            | Dietary supplement etc. | 62-54-4  | 158.17           | (16)       |     |     |     |        |

See substrates with different salt.
| No. | Name                             | Functional classes | CAS#   | Molecular weight | Genotoxicity tests | Remarks |
|-----|----------------------------------|--------------------|--------|------------------|--------------------|---------|
| 151 | geranyl acetate                  | Flavoring agent    | 105–87-3 | 196.29          | _H(4) _H(4)       | Ames    |
| 152 | cyclohexyl acetate               | Flavoring agent    | 622–45-7 | 142.20          | _H(4) _H(25)      | CA      |
| 153 | citronellyl acetate              | Flavoring agent    | 150–84-5 | 198.30          | _H(4) _H(24)      | MN      |
| 154 | cinnamyl acetate                 | Flavoring agent    | 103–54-8 | 176.21          | _H(4) _H(24)      | TGR     |
| 155 | terpinyl acetate                 | Flavoring agent    | 8007-35-0 | 196.29          | _H(4) _H(24)      | Others  |
| 156 | starch acetate                   | Thickening agents etc. | 9045–28-7 | 8203            | _H(2)(18) _H(2)  |         |
| 157 | sodium acetate                   | Acidifier etc.     | 127–09-3 | 8203            | _H(2)(18) _H(2)  |         |
| 158 | polyvinyl acetate                | Chewing gum base etc. | 103–45-7 | 164.20          | _H(3)(14) _H(15) |         |
| 159 | phenethyl acetate (phenylethyl acetate) | Flavoring agent | 123–86-4 | 116.16          | _H(3)(15) _H(15) |         |
| 160 | butyl acetate                    | Flavoring agent    | 140–11-4 | 150.17          | _H(4)(15) _H(15) |         |
| 161 | benzyl acetate                   | Flavoring agent    | 2623-23-6 | 198.30          | _H(5)(15) _H(15) |         |
| 162 | l-menthyl acetate                | Flavoring agent    | 115–95-7 | 196.29          | _H(5)(15) _H(15) |         |
| 163 | linalyl acetate                  | Flavoring agent    | 119–36-8 | 152.15          | _H(5)(15) _H(15) |         |
| 164 | saccharin                        | Sweetener          | 81–07-2 | 183.19          | _H(2)(14) _H(2)  |         |
| 165 | calcium saccharin (saccharin)    | Sweetener          | 6381–91-5 | 467.48          | _H(2)(14) _H(2)  |         |
| 166 | methyl salicylate                | Flavoring agent    | 119–36-8 | 152.15          | _H(2)(14) _H(2)  |         |
| 167 | calcium oxide                    | Processing agent   | 1305–78-8 | 5608           | _H(2)(14) _H(2)  |         |
| 168 | oxidized starch                  | Thickening agents etc. | 1309–48-4 | 4030           | _H(2)(14) _H(2)  |         |
| 169 | magnesium oxide                  | Absorbent etc.     | 1309–37-1 | 159.69          | _H(2)(14) _H(2)  |         |
| 170 | iron sesquioxide (diiiron trioxide or iron oxide red) | Food color | 13517–11-8 | 96.91           | _H(2)(14) _H(2)  |         |
| 171 | hypochlorous acid water          | Preservative       | 7681–52-9 | 7444            | _H(2)(14) _H(2)  |         |
| 172 | sodium hypochlorite (hypochlorite of soda) | Sterilizer etc. | 13517–11-8 | 96.91          | _H(2)(14) _H(2)  |         |
| 173 | hypobromous acid water           | Sterilizer         | 7775–14-6 | 174.11          | _H(2)(14) _H(2)  |         |
| 174 | 2,3-diethylpyrazine              | Flavoring agent    | 15707–24-1 | 136.19          | _H(2)(14) _H(2)  |         |
| 175 | 2,3-diethyl-5-methylpyrazine     | Flavoring agent    | 18138–04-0 | 150.22          | _H(2)(14) _H(2)  |         |
| No. | Name                                | Functional classes | CAS#     | Molecular weight | Genotoxicity tests | Remarks                                                                 |
|-----|-------------------------------------|--------------------|----------|------------------|--------------------|--------------------------------------------------------------------------|
| 178 | allyl cyclohexylpropionate          | Flavoring agent    | 2705-87-5| 196.29           | (15)               |                                                                 |
| 179 | L-cysteine monohydrochloride        | Antioxidant etc.   | 7048-04-6| 175.64           | (4)(14)            |                                                                 |
| 180 | disodium 5′-cytidylate (sodium 5′-cytidylate) | Seasoning        | 6757-06-8| 367.16           | (1)(19)            |                                                                 |
| 181 | citral                              | Flavoring agent    | 5392-40-5| 152.23           | (5)(16)            |                                                                 |
| 182 | citronellal                         | Flavoring agent    | 106-23-0 | 154.25           | (5)                |                                                                 |
| 183 | citronellol                         | Flavoring agent    | 106-22-9 | 156.27           | (5)                |                                                                 |
| 184 | 1,8-cineole (eucalyptol)            | Flavoring agent    | 470-82-6 | 154.25           | (5)                |                                                                 |
| 185 | diphenyl (biphenyl)                 | Antimolding agent  | 92-52-4  | 154.21           | (5)                |                                                                 |
| 186 | butylated hydroxytoluene            | Antioxidant        | 128-37-0 | 220.35           | (10)               |                                                                 |
| 187 | dibenzoyl thiamine                  | Dietary supplement | 299-88-7 | 490.58           | (1)                |                                                                 |
| 188 | dibenzoyl thiamine hydrochloride    | Dietary supplement | 35660-60-7| 527.04          | (126)              |                                                                 |
| 189 | fatty acids                         | Flavoring agent    |          |                  |                    |                                                                 |
| 190 | aliphatic higher alcohols           | Flavoring agent    |          |                  |                    |                                                                 |
| 191 | aliphatic higher aldehydes (except those generally recognized as highly toxic) | Flavoring agent |          |                  |                    |                                                                 |
| 192 | aliphatic higher hydrocarbons (except those generally recognized as highly toxic) | Flavoring agent |          |                  |                    |                                                                 |
| 193 | 2,3-dimethylpyrazine                | Flavoring agent    | 5910-89-4| 108.14           | FSC30              |                                                                 |
| 194 | 2,5-dimethylpyrazine                | Flavoring agent    | 123-32-0 | 108.14           | FSC31              |                                                                 |
| 195 | 2,6-dimethylpyrazine                | Flavoring agent    | 108-50-9 | 108.14           | FSC32              |                                                                 |
| 196 | 2,6-dimethylpyridine                | Flavoring agent    | 108-48-5 | 107.15           | FSC33              |                                                                 |
| 197 | oxalic acid                         | Processing agent   | 6153-56-6| 12607            | (5)(13)            |                                                                 |
| 198 | potassium bromate                   | Flour treatment agent | 7758-01-2| 167.00          | (13)(12)          |                                                                 |
| 199 | dl-tartaric acid (dl-tartaric acid)  | Acidifier          | 133-37-9 | 15009            | (15)               |                                                                 |
| 200 | l-tartaric acid (d-tartaric acid)    | Acidifier          | 87-69-4  | 15009            | (15)               |                                                                 |
| 201 | potassium dl-bitartrate (potassium hydrogen dl-tartarate or potassium hydrogen dl-tartarate) | Processing agent etc. |          |                  |                    |                                                                 |
| 202 | potassium l-bitartrate (potassium hydrogen l-tartarate or potassium | Raising agent     | 868-14-4  | 188.18           | (15)               |                                                                 |
| No. | Name                                                                 | Functional classes | CAS#         | Molecular weight | Genotoxicity tests | Remarks                                                                 |
|-----|----------------------------------------------------------------------|-------------------|--------------|------------------|--------------------|------------------------------------------------------------------------|
|     |                                                                       |                   |              |                  | Ames                | CA | MN | TGR | Others |
| 203 | hydrogen d-tartrate                                                   | Processing agent etc. |              |                  |                    |    |    |     |        |
| 204 | disodium d-tartrate (disodium dl-tartrate)                            | Seasoning         | 6106-24-7    | 19405            |                   |    |    |     |        |
| 205 | potassium nitrate                                                     | Fermentation regulator etc. | 7757-79-1    | 101.10           |                    |    |    |     |        |
| 206 | sodium nitrate                                                        | Fermentation regulator etc. | 7631-99-4    | 8499             |                    |    |    |     |        |
| 207 | food red no.2 (amaranth) and its aluminum lake                        | Food color        | 915–67-3     | 60448            |                    |    |    |     |        |
| 208 | food red no.3 (erythrosine) and its aluminum lake                     | Food color        | 16423–68-0   | 89787            |                    |    |    |     |        |
| 209 | food red no.40 (allura red ac) and its aluminum lake                  | Food color        | 25956–17-6   | 49642            |                    |    |    |     |        |
| 210 | food red no.102 (new coccine)                                        | Food color        | 2611–82-7    | 63150            |                    |    |    |     |        |
| 211 | food red no.104 (phloxine)                                           | Food color        | 18472–87-2   | 82963            |                    |    |    |     |        |
| 212 | food red no.105 (rose bengale)                                       | Food color        | 632–69-9     | 101764           |                    |    |    |     |        |
| 213 | food red no.106 (acid red)                                           | Food color        | 3520-42-1    | 58065            |                    |    |    |     |        |
| 214 | food yellow no.4 (tartrazine) and its aluminum lake                   | Food color        | 1934-21-0    | 53437            |                    |    |    |     |        |

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Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016) (Continued)

| No. | Name                                      | Functional classes | CAS#          | Molecular weight | Genotoxicity tests | Remarks                                                                 |
|-----|-------------------------------------------|--------------------|---------------|------------------|--------------------|-------------------------------------------------------------------------|
| 215 | food yellow no.5 (sunset yellow fcf) and its aluminum lake | Food color        | 2783-94-0     | 452.37           |                   |                           |                                                                         |
| 216 | food green no.3 (fast green fcf) and its aluminum lake | Food color        | 2353-45-9     | 808.85           |                   |                           |                                                                         |
| 217 | food blue no.1 (brilliant blue fcf) and its aluminum lake | Food color        | 3844-45-9     | 792.85           |                   |                           |                                                                         |
| 218 | food blue no.2 (indigo carmine) and its aluminum lake | Food color        | 860–22-0      | 466.35           |                   |                           |                                                                         |
| 219 | sucrose esters of fatty acids              | Emulsifier        |               |                  |                   |                           |                                                                         |
| 220 | silicone resin (polymethylsiloxane)        | Antiforming agent |               |                  |                   |                           |                                                                         |
| 221 | cinnamyl alcohol (cinnamic alcohols)       | Flavoring agent   | 104–54-1      | 134.18           |                   |                           |                                                                         |
| 222 | cinnamaldehyde (cinnamic aldehyde)         | Flavoring agent   | 14371–10-9    | 132.16           |                   |                           |                                                                         |
| 223 | potassium hydroxide (caustic potash)       | Processing agent  | 1310-58-3     | 56.11            |                   |                           |                                                                         |
| 224 | calcium hydroxide (slaked lime)            | Processing agent etc. | 1305-62-0    | 74.09            |                   |                           |                                                                         |
| 225 | sodium hydroxide (caustic soda)            | Processing agent  | 1310-73-2     | 4000             |                   |                           |                                                                         |
| 226 | magnesium hydroxide                        | Dietary supplement etc. | 1309-42-8    | 5832             |                   |                           |                                                                         |
| 227 | sucralose (trichlorogalactosucrose)        | Sweetener         | 56038–13-2    | 39764            |                   |                           |                                                                         |
| 228 | calcium stearate                           | Dietary supplement | 1592–23-0     | 324.56           |                   |                           |                                                                         |
| 229 | magnesium stearate                         | Emulsifier etc.   | 557–04-0      | 591.24           |                   |                           |                                                                         |
| 230 | calcium stearoyl lactylate (calcium stearoyl lactylate) | Emulsifier | 5793-94-2     |                 |                   |                           |                                                                         |
| 231 | sodium stearoyl lactate                   | Emulsifier etc.   | 25383–99-7    | 378.53           |                   |                           |                                                                         |
| 232 | sorbitan esters of fatty acids             | Emulsifier        |               |                  |                   |                           |                                                                         |
| 233 | d-sorbitol (d-sorbit)                      | Sweetener etc.    | 50–70-4       | 182.17           |                   |                           |                                                                         |
| 234 | sorbic acid                               | Preservative      | 110–44-1      | 112.13           |                   |                           |                                                                         |
| 235 | potassium sorbate                          | Preservative      | 24634–61-5    | 150.22           |                   |                           |                                                                         |
| 236 | calcium sorbate                            | Preservative etc. | 7492-55-9     | 262.32           |                   |                           |                                                                         |
| 237 | ammonium carbonate                        | Processing agent etc. | 506–87-6     | 9609             |                   |                           |                                                                         |
| 238 | potassium carbonate (anhydrous)            | Processing agent etc. | 584–08-7     | 138.21           |                   |                           |                                                                         |
| 239 | calcium carbonate                          | Processing agent etc. | 471–34-1     | 100.09           |                   |                           |                                                                         |
| 240 | ammonium bicarbonate (ammonium carbonate) | Raising agent etc. | 1066–33-7     | 7906             |                   |                           |                                                                         |

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| No.  | Name                                                                 | Functional classes    | CAS#          | Molecular weight | Ames | CA | MN | TGR | Others | Remarks                                                                 |
|------|----------------------------------------------------------------------|-----------------------|---------------|------------------|------|----|----|-----|--------|--------------------------------------------------------------------------|
| 241  | sodium bicarbonate (bicarbonate soda or sodium hydrogen carbonate)    | Raising agent etc.    | 144–55-8      | 8401             |      |    |    |     |        |                                                                          |
| 242  | sodium carbonate (crystal: carbonate soda, anhydrous: soda ash)       | Processing agent      | 497–19-8      | 10599            |      |    |    |     |        |                                                                          |
| 243  | magnesium carbonate                                                  | Processing agent      | 546–93-0      | 8431             |      |    |    |     |        |                                                                          |
| 244  | thiabendazole                                                        | Antimolding agent     | 148–79-8      | 20125            |      |    |    |     |        |                                                                          |
| 245  | thiamine hydrochloride (vitamin B1 hydrochloride)                    | Dietary supplement    | 67–03-8       | 33727            |      |    |    |     |        |                                                                          |
| 246  | thiamine mononitrate (vitamin B1 mononitrate)                        | Dietary supplement    | 532–43-4      | 32736            |      |    |    |     |        |                                                                          |
| 247  | thiamine dicetyl sulfate (vitamin B1 dicetyl sulfate)                | Dietary supplement    |               |                  |      |    |    |     |        |                                                                          |
| 248  | thiamine thiocyanate (vitamin B1 thiocyanate)                        | Dietary supplement    | 130131–60-1   | 34145            |      |    |    |     |        |                                                                          |
| 249  | thiamine naphthalene-1,5-disulfonate (vitamin B1 naphthalene-1,5-disulfonate) | Dietary supplement |               |                  |      |    |    |     |        |                                                                          |
| 250  | thiamine dilauryl sulfate (vitamin B1 dilauryl sulfate)              | Dietary supplement etc.|               |                  |      |    |    |     |        |                                                                          |
| 251  | thioethers (except those generally recognized as highly toxic)       | Flavoring agent       |               |                  |      |    |    |     |        |                                                                          |
| 252  | thiois (thioalcohols) (except those generally recognized as highly toxic) | Flavoring agent    |               |                  |      |    |    |     |        |                                                                          |
| 253  | L-theanine                                                           | Seasoning             | 3081-61-6     | 17420            |      |    |    |     |        |                                                                          |
| 254  | decanal (decyl aldehyde)                                            | Flavoring agent       | 112–31-2      | 15627            |      |    |    |     |        |                                                                          |
| 255  | decanol (decyl alcohol)                                              | Flavoring agent       | 112–30-1      | 15828            |      |    |    |     |        |                                                                          |
| 256  | ethyl decanoate (ethyl caprate)                                      | Flavoring agent       | 110–38-3      | 20032            |      |    |    |     |        |                                                                          |
| 257  | sodium iron chlorophyllin                                            | Food color            |               |                  |      |    |    |     |        |                                                                          |
| 258  | 5,6,7,8-tetrahydroquinoline                                          | Flavoring agent       | 34413–35-9    | 13418            |      |    |    |     |        |                                                                          |
| 259  | 2,3,5,6-tetramethylpyrazine                                           | Flavoring agent       | 1124–11-4     | 13620            |      |    |    |     |        |                                                                          |
| 250  | sodium dehydroacetate                                                | Preservative          | 4418–26-2     | 20814            |      |    |    |     |        |                                                                          |
| 261  | terpineol                                                            | Flavoring agent       | 8000–41-7     | 15425            |      |    |    |     |        |                                                                          |
| 262  | terpene hydrocarbons                                                 | Flavoring agent       |               |                  |      |    |    |     |        |                                                                          |
| 263  | sodium carboxymethylstarch                                           | Thickening agents     |               |                  |      |    |    |     |        |                                                                          |
| No. | Name                                                                 | Functional classes            | CAS#             | Molecular weight | Genotoxicity tests | Remarks                        |
|-----|----------------------------------------------------------------------|-------------------------------|-----------------|------------------|--------------------|--------------------------------|
| 264 | copper salts (limited to copper gluconate and cupric)               | Dietary supplement            |                 |                  |                    |                                |
| 265 | sodium copper chlorophyllin                                         | Food color                    | 28302-36-5     | 722.13           | (3)(13)            |                                |
| 266 | copper chlorophyll                                                   | Food color                    | 15739-09-0     |                  | (1)                |                                |
| 267 | dl-α-tocopherol                                                     | Antioxidant                   | 50-02-9         | 430.71           | (3)(10)            |                                |
| 268 | all-racemic-α-tocopheryl acetate                                    | Dietary supplement etc.       |                 |                  | (3)                |                                |
| 269 | RRR-α-tocopheryl acetate                                            | Dietary supplement etc.       |                 |                  |                    |                                |
| 270 | α-tryptophan                                                        | Dietary supplement etc.       | 54-12-6         | 204.23           | (2)(15)            |                                |
| 271 | l-tryptophan                                                        | Dietary supplement etc.       | 73-22-3         | 204.23           | (2)(15)            |                                |
| 272 | trimethylamine                                                      | Flavoring agent               | 75-50-3         | 591.11           | (11)               |                                |
| 273 | 2,3,5-trimethylpyrazine                                             | Flavoring agent               | 14667-55-1     | 122.17           | (15)               |                                |
| 274 | δ-threonine                                                         | Dietary supplement etc.       | 80-68-2         | 119.12           | (15)               |                                |
| 275 | L-threonine                                                         | Dietary supplement etc.       | 72-19-5         | 119.12           | (15)               |                                |
| 276 | nisin                                                               | Preservative etc.             | 1414-45-5       | 335407           | (37)               |                                |
| 277 | natamycin                                                           | Preservative                  | 7681-93-8       | 665.73           | (38)               |                                |
| 278 | sodium methoxide (sodium methylate)                                 | Processing agent              | 124-41-4        | 5402             | (4)                |                                |
| 279 | nicotinic acid (niacin)                                             | Dietary supplement etc.       | 59-67-6         | 123.11           | (11)               |                                |
| 280 | nicotinamide (niacinamide)                                          | Dietary supplement etc.       | 98-92-0         | 122.12           | (11)               |                                |
| 281 | sulfur dioxide (sulfurous acid, anhydride)                          | Preservative etc.             | 7446-09-5       | 6406             |                    |                                |
| 282 | chlorine dioxide                                                    | Flour treatment agent         | 10049-04-4      | 6745             | (3)                |                                |
| 283 | silicon dioxide (silica gel)                                        | Filtration aid                | 14464-46-1      | 6008             | (20)               |                                |
| 284 | carbon dioxide (carbonic acid, gas)                                 | Preservative                  | 124-38-9        | 4401             | (19)               |                                |
| 285 | titanium dioxide                                                    | Food color                    | 13463-67-7      | 7987             | (12)               |                                |
| 286 | lactic acid                                                         | Acidifier                     | 50-21-5         | 9008             | (18)               |                                |
| 287 | potassium lactate                                                   | Seasoning etc.                | 996-31-6        | 128.17           | (10)               |                                |
| No. | Name                          | Functional classes       | CAS#       | Molecular weight | Ames assay | CA | MN | TGR | Others | Remarks                                                                 |
|-----|-------------------------------|--------------------------|------------|------------------|------------|----|----|-----|--------|-----------------------------------------------------------------------|
| 288 | calcium lactate               | Sweetener etc.           | 814-80-2   | 218.22           | +(+13)     |    |    |     |        |                                                                                                                                  |
| 289 | iron lactate                  | Dietary supplement       | 5905-52-2  | 233.99           | +(+18) +(-4) | +(+5) | -(-7) | +(+123) | Target organs for TGR in mouse liver, kidneys                          |
| 290 | sodium lactate                | Acidifier etc.           | 72-17-3    | 112.06           | -(-19) +(-4) |    |    |     |        |                                                                                                                                  |
| 291 | neotame                       | Sweetener etc.           | 165450-17-9| 378.46           | -(-18)     |    |    |     |        |                                                                                                                                  |
| 292 | y-nonalactone (nonalactone)   | Flavoring agent          | 104-61-0   | 233.99           | +(+18)     |    |    |     |        |                                                                                                                                  |
| 293 | potassium norbixin            | Food color               | 33261-80-2 | 456.66           | -(-19)     |    |    |     |        |                                                                                                                                  |
| 294 | sodium norbixin               | Food color               | 33261-81-3 | 424.45           | +(+18)     |    |    |     |        |                                                                                                                                  |
| 295 | vanillin                      | Flavoring agent          | 121-33-5   | 152.15           | -(-16)     |    |    |     |        |                                                                                                                                  |
| 296 | isobutyl p-hydroxybenzoate    | Preservative             | 4247-02-3  | 194.23           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 297 | isopropyl p-hydroxybenzoate   | Preservative             | 4191-73-5  | 180.20           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 298 | ethyl p-hydroxybenzoate       | Preservative             | 120-47-8   | 166.17           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 299 | butyl p-hydroxybenzoate       | Preservative             | 94-26-8    | 194.23           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 300 | propyl p-hydroxybenzoate      | Preservative             | 94-13-3    | 180.20           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 301 | p-methylacetophenone          | Flavoring agent          | 122-00-9   | 134.18           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 302 | L-valine                      | Dietary supplement etc.  | 72-18-4    | 117.15           | +(+1)     |    |    |     |        |                                                                                                                                  |
| 303 | valeraldehyde                 | Flavoring agent          | 110-62-3   | 861.3            | +(+16)     |    |    |     |        |                                                                                                                                  |
| 304 | calcium pantothenate          | Dietary supplement       | 137-08-6   | 476.53           | +(+1)     |    |    |     |        |                                                                                                                                  |
| 305 | sodium pantothenate           | Dietary supplement       | 75033-16-8 | 244.31           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 306 | biotin                        | Dietary supplement       | 58-85-5    | 244.31           | +(+1)     |    |    |     |        |                                                                                                                                  |
| 307 | L-histidine monohydrochloride | Dietary supplement       | 7048-02-4  | 209.63           | +(+1)     |    |    |     |        |                                                                                                                                  |
| 308 | bisbentiamine (benzoylthiamine disulfide) | Dietary supplement | 2667-89-2  | 770.92           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 309 | vitamin A (retinol)           | Dietary supplement       | 68-26-8    | 286.45           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 310 | vitamin a fatty acids esters (retinol esters of fatty acids esters) | Dietary supplement | 68-26-8    | 286.45           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 311 | L-hydroxyethylidene-1, 1-diphosphonic acid | Processing agent | 2809-21-4  | 286.45           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 312 | hydroxycitronellal            | Flavoring agent          | 107-75-5   | 172.26           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 313 | hydroxydehydrogenone dimethylacetal | Flavoring agent     | 141-92-4   | 218.33           | +(+16)     |    |    |     |        |                                                                                                                                  |
| 314 | hydroxypropyl starch phosphate | Thickening agents etc.  | 5324-00-8  | 218.33           | +(+16)     |    |    |     |        | Evaluated as modified starch                                                                                                 |
| No. | Name                                      | Functional classes              | CAS#          | Molecular weight | Genotoxicity tests | Remarks                                                                 |
|-----|--------------------------------------------|--------------------------------|---------------|------------------|-------------------|-------------------------------------------------------------------------|
| 315 | hydroxypropyl cellulose                    | Emulsifier etc.                | 9004-64-2    |                  | Ames CA MN TGR Others |                                                          |
| 316 | hydroxypropyl starch                       | Thickening agents etc.         | 68130-14-3   |                  | J112 J112 J112 J112 | Evaluated as modified starch                                          |
| 317 | hydroxypropyl methylcellulose              | Emulsifier etc.                | 9004-65-3    |                  | FSC44 J117 J117 J117 |                                                          |
| 318 | piperidine                                 | Flavoring agent                | 110-89-4     | 85.15            |                   |                                                          |
| 319 | piperonal (heliotropine)                   | Flavoring agent                | 120-57-0     | 150.13           |                   | Target organs for TGR in miceliver, kidneys                            |
| 320 | piperonyl butoxide                         | Insecticide                    | 51-03-6      | 338.44           |                   |                                                          |
| 321 | sunflower lecithin                         | Emulsifier                     | 8002-43-5    |                  |                   |                                                          |
| 322 | acetic acid, glacial                       | Acidifier                      | 64-19-7      | 6005             |                   |                                                          |
| 323 | pyrazine                                   | Flavoring agent                | 290-37-9     | 8009             |                   |                                                          |
| 324 | pyridoxine hydrochloride (vitamin B6)      | Dietary supplement             | 58-56-0      | 205.64           |                   |                                                          |
| 325 | pyrimethanil                               | Preservative etc.              | 131341-86-1  | 199.26           |                   |                                                          |
| 326 | potassium pyrosulfite (potassium hydrogen sulfite or potassium metabisulfite) | Preservative etc.              | 16731-55-8   | 222.33           |                   |                                                          |
| 327 | sodium pyrosulfite (sodium metabisulfite, acid sulfate of soda) | Preservative etc.              | 7681-57-4    | 190.11           |                   | Described as sodium bisulfite, anhydrous                             |
| 328 | pyrrolidine                                | Flavoring agent                | 123-75-1     | 71.12            |                   |                                                          |
| 329 | potassium pyrophosphate (tetrapotassium pyrophosphate) | Processing agent              | 7320-34-5    | 330.34           |                   |                                                          |
| 330 | calcium dihydrogen pyrophosphate (acid calcium pyrophosphate) | Dietary supplement etc.        | 14866-19-4   | 216.04           |                   |                                                          |
| 331 | disodium dihydrogen pyrophosphate (acid disodium pyrophosphate) | Processing agent              | 7758-16-9    | 221.94           |                   |                                                          |
| 332 | ferric pyrophosphate                       | Dietary supplement etc.        | 1332-96-3    | 745.21           |                   |                                                          |
| 333 | sodium pyrophosphate (tetrasodium pyrophosphate) | Dietary supplement etc.        | 7722-88-5    | 265.90           |                   |                                                          |
| 334 | pyrrole                                    | Flavoring agent                | 109-97-7     | 67.09            |                   |                                                          |
| 335 | L-phenylalanine                            | Dietary supplement etc.        | 63-91-2      | 165.19           |                   |                                                          |
| 336 | isoamyl phenylacetate                      | Flavoring agent                | 102-19-2     | 206.28           |                   |                                                          |
| 337 | isobutyl phenylacetate                     | Flavoring agent                | 102-13-6     | 192.25           |                   |                                                          |
| 338 | ethyl phenylacetate                        | Flavoring agent                | 101-97-3     | 164.20           |                   |                                                          |
| 339 | 2-(3-phenylpropyl)pyridine                 | Flavoring agent                | 2110-18-1    | 197.28           |                   |                                                          |
Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016) (Continued)

| No. | Name                                                                 | Functional classes         | CAS#                  | Molecular weight | Genotoxicity tests | Remarks |
|-----|----------------------------------------------------------------------|-----------------------------|-----------------------|------------------|--------------------|---------|
|    |                                                                      |                             |                       |                  | Ames CA MN TGR Others |         |
| 340 | phenethylamine                                                      | Flavoring agent             | 64-04-0               | 121.18           | $^{+H17}$         |         |
| 341 | phenol ethers (except those generally recognized as highly toxic)   | Flavoring agent             |                       |                  |                    |         |
| 342 | phenols (except those generally recognized as highly toxic)        | Flavoring agent             |                       |                  |                    |         |
| 343 | ferrocyanides (potassium ferrocyanide (potassium hexacyanoferrate (ii)), calcium ferrocyanide (calcium hexacyanoferate (ii)), sodium ferrocyanide (sodium hexacyanoferate (ii))) | Processing agent etc.      | 13943–58-3, 13821–08-4, 13601–19-9 | 42239, 508.29, 48406 | $^{–}$ | $^{–}$ |
|     |                                                                      |                             |                       |                  |                    |         |
| 344 | butanol                                                             | Processing agent etc.       | 71—36–3              | 74.12            | $^{+H15}$         |         |
| 345 | butylamine                                                          | Flavoring agent             | 109–73–9             | 73.14            | $^{+H15}$         |         |
| 346 | butyraldehyde                                                       | Flavoring agent             | 123–78–8             | 72.11            | $^{+H15}$         |         |
| 347 | butylated hydroxyanisole                                           | Antioxidant                 | 25013–16–5           | 180.24           | $^{–}$ $^{–}$      |         |
| 348 | fumaric acid                                                        | Acidifier                   | 110–17–8             | 116.07           | $^{–}$ $^{–}$      |         |
| 349 | monosodium fumarate (sodium fumarate)                              | Acidifier                   | 5873–57–4            | 138.05           | $^{–}$ $^{–}$      |         |
| 350 | fludioxonil                                                         | Preservative etc.           | 131341–86–1          | 248.19           | $^{–}$ $^{–}$      |         |
| 351 | furfurals and its derivatives (except those generally recognized as highly toxic) | Flavoring agent             |                       |                  |                    |         |
| 352 | propanol                                                            | Processing agent etc.       | 71–23–8              | 6009             | $^{–}$ $^{–}$      |         |
| 353 | propionaldehyde                                                     | Flavoring agent             | 123–38–6             | 5808             | $^{–}$ $^{–}$      |         |
| 354 | propionic acid                                                      | Flavoring agent etc.        | 79–09–4              | 7408             | $^{–}$ $^{–}$      |         |
| 355 | isoamyl propionate                                                  | Flavoring agent             | 105–68–0             | 144.21           | $^{–}$ $^{–}$      |         |
| 356 | ethyl propionate                                                    | Flavoring agent             | 105–37–3             | 102.13           | $^{–}$ $^{–}$      |         |
| 357 | calcium propionate                                                  | Preservative                | 4075–81–4            | 186.22           | $^{–}$ $^{–}$      |         |
| 358 | sodium propionate                                                   | Preservative                | 137–40–6             | 96.06            | $^{–}$ $^{–}$      |         |
| 359 | benzyl propionate                                                   | Flavoring agent             | 122–63–4             | 164.20           | $^{–}$ $^{–}$      |         |
| 360 | propylene glycol                                                    | Quality sustainer etc.      | 57–55–6              | 7609             | $^{–}$ $^{–}$      |         |
| 361 | propylene glycol esters of fatty acids                             | Emulsifier                  |                       |                  |                    |         |
| 362 | hexanoic acid (caproic acid)                                        | Flavoring agent             | 142–62–1             | 116.16           | $^{–}$ $^{–}$      |         |
| 363 | allyl hexanoate (allyl caproate)                                    | Flavoring agent             | 123–68–2             | 156.22           | $^{–}$ $^{–}$      |         |
| No. | Name                              | Functional classes   | CAS#         | Molecular weight | Genotoxicity tests  | Remarks                                                                 |
|-----|-----------------------------------|----------------------|--------------|------------------|---------------------|-------------------------------------------------------------------------|
| 364 | ethyl hexanoate (ethyl caproate)  | Flavoring agent      | 123–66-0    | 144.21           | *H16 (17) (4)         | **H16 (17) (4)**                                                       |
| 365 | ethyl heptanoate (ethyl eranthen) | Flavoring agent      | 106–30-9    | 158.24           |                      | **H22 (12)**                                                          |
| 366 | 1-pentylaldehyde                  | Flavoring agent      | 18031–40-8  | 150.22           | *(19) (3) (17) (4)    | **H16 (17) (4) (19) (3)**                                             |
| 367 | benzyl alcohol                    | Flavoring agent      | 100–51-6    | 108.14           | *(17) (3) (19) (3)    | **H16 (17) (4) (19) (3)**                                             |
| 368 | benzaldehyde                      | Flavoring agent      | 100–52-7    | 106.12           |                      | **H22 (17)**                                                          |
| 369 | 2-pentanol (sec-amyl alcohol)     | Flavoring agent      | 6032-29-7   | 88.15            | **H16 (17) (4)         | **H16 (17) (4)**                                                       |
| 370 | trans-2-pentenal                  | Flavoring agent      | 1576-87-0   | 84.12            |                      | **H16 (17) (4)**                                                       |
| 371 | 1-penten-3-ol                     | Flavoring agent      | 616–25-1    | 86.13            | *(17) (3) (19) (3)    | **H16 (17) (4) (19) (3)**                                             |
| 372 | aromatic alcohols                 | Flavoring agent      |             |                  |                     | **H16 (17) (4)**                                                       |
| 373 | aromatic aldehydes (except those  | Flavoring agent      |             |                  |                     | **H16 (17) (4)**                                                       |
|      | generally recognized as highly    |                      |             |                  |                     | **H16 (17) (4)**                                                       |
|      | toxic)                            |                      |             |                  |                     | **H16 (17) (4)**                                                       |
| 374 | propyl gallate                    | Antioxidant          | 121–79-9    | 212.20           | *(17) (3) (19) (3)    | **H16 (17) (4) (19) (3)**                                             |
| 375 | sodium polyacrylate               | Thickening agents etc.| 9003–04-7  |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 376 | polysobutylene (butyl rubber)     | Chewing gum base     | 9003–27-4   |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 377 | polysorbate 20                    | Emulsifier etc.      | 9005-64-5   | 122772           |                     | **H16 (17) (4) (19) (3)**                                             |
| 378 | polysorbate 60                    | Emulsifier etc.      | 9005-67-8   | 131190           |                     | **H16 (17) (4) (19) (3)**                                             |
| 379 | polysorbate 65                    | Emulsifier etc.      | 9005-71-4   | 1842             |                     | **H16 (17) (4) (19) (3)**                                             |
| 380 | polysorbate 80                    | Emulsifier etc.      | 9005-65-6   | 130968           |                     | **H16 (17) (4) (19) (3)**                                             |
| 381 | polyvinylpyrrolidone              | Thickening agents etc.| 9003–39-8  |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 382 | polyvinylpyrrolidone              | Processing agent     | 25249–54-1  |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 383 | polybutene (polybutylene)         | Chewing gum base     | 9003–28-5   |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 384 | potassium polyphosphate           | Processing agent     | 68956–75-2  |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 385 | sodium polyphosphate              | Processing agent     | 68915–31-1  |                  |                     | **H16 (17) (4) (19) (3)**                                             |
| 386 | 2-borneol                         | Flavoring agent      | 464–43-7    | 154.25           | *(10) (17) (4)         | **H16 (17) (4) (19) (3)**                                             |
| 387 | maltol                            | Flavoring agent      | 118–71-8    | 126.11           | *(10) (17) (4)         | **H16 (17) (4) (19) (3)**                                             |

* Ames showed positive only in T1537, whose maximum number of the revertants was within the range of negative control.
| No.  | Name                                    | Functional classes           | CAS#      | Molecular weight | Genotoxicity tests | Remarks                                      |
|------|-----------------------------------------|------------------------------|-----------|------------------|--------------------|-----------------------------------------------|
| 388  | D-mannitol (D-mannite)                  | Antisticking agent etc.      | 69-65-8   | 182.17           | _3X(13) _0         | Micellifer, stomach (glandular stomach)       |
| 389  | potassium metaphosphate                 | Processing agent             | 7790-53-6 | 118.07           | _3X(15) _0         |                                               |
| 390  | sodium metaphosphate                    | Processing agent             | 10361-03-2 | 101.96           | _3X(15) _0         |                                               |
| 391  | α-methionine                            | Dietary supplement etc.      | 59-51-8   | 149.21           | _18 _22            |                                               |
| 392  | L-methionine                            | Dietary supplement           | 63-68-3   | 149.21           | _18 _23            |                                               |
| 393  | methyl n-methylanthranilate             | Flavoring agent              | 85-91-6   | 165.19           | _17 _22            |                                               |
| 394  | 5-methylquinoxaline                     | Flavoring agent              | 13708-12-8 | 144.17           | _15 _15 _85        |                                               |
| 395  | 6-methylquinoline                       | Flavoring agent              | 91-62-3   | 143.19           | _16 _16 _86        |                                               |
| 396  | 6,7-dihydro-5-methyl-5 h-cyclopentapyrazine | Flavoring agent       | 23747-48-0 | 134.18           | _16 _16 _87        |                                               |
| 397  | methyl cellulose                        | Thickening agents etc.       | 9004-67-5 | 142.20           | _FSC58 _17 _88 _FSC38 |                                               |
| 398  | 1-methyl naphthalene                    | Flavoring agent              | 90-12-0   | 142.20           | _FSC58 _17 _88 _FSC38 |                                               |
| 399  | methyl β-naphthyl ketone                | Flavoring agent              | 93-08-3   | 170.21           | _17 _24            |                                               |
| 400  | 2-methylpyrazine                        | Flavoring agent              | 109-08-0  | 94.11            | _FSC59 _16 _86     |                                               |
| 401  | 2-methylbutanol                         | Flavoring agent              | 137-32-6  | 88.15            | _15 _15 _85        |                                               |
| 402  | 3-methyl-2-butanol                      | Flavoring agent              | 598-75-4  | 88.15            | _17 _17            |                                               |
| 403  | 2-methylbutyraldehyde                   | Flavoring agent              | 96-17-3   | 86.13            | _FSC60 _16 _87     |                                               |
| 404  | trans –2-methyl-2-butenal, (e)-2-methyl-2-butenal | Flavoring agent       | 497-03-0  | 84.12            | _16 _16 _87        |                                               |
| 405  | 3-methyl-2-butenol                      | Flavoring agent              | 107-86-8  | 84.12            | _16 _16 _87        |                                               |
| 406  | 3-methyl-2-butenol                      | Flavoring agent              | 556-82-1  | 86.13            | _16 _16 _87        |                                               |
| 407  | methyl hesperidin (soluble vitamin P)   | Dietary supplement           | 11013-97-1 | 624.59           | _3X(12) _0         |                                               |
| 408  | dl-menthol (dl-peppermint camphor)       | Flavoring agent              | 89-78-1   | 156.27           | _3X(20) _1         |                                               |
| 409  | l-menthol (peppermint camphor)          | Flavoring agent              | 2216-51-5 | 156.269          | _1X(17) _4         |                                               |
| 410  | morpholine salts of fatty acids         | Coating agent                |           |                  | _2 _2             |                                               |
| 411  | folic acid                              | Dietary supplement           | 59-30-3   | 441.04           | _2X(11) _5         |                                               |
| 412  | butyric acid                            | Flavoring agent              | 107-92-6  | 88.06            | _2X(17) _5         |                                               |
| 413  | isoamyl butyrate                        | Flavoring agent              | 106-27-4  | 158.241          | _2X(17) _5         |                                               |
| 414  | ethyl butyrate                          | Flavoring agent              | 105-54-4  | 116.16           | _2X(17) _5         |                                               |
Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016) (Continued)

| No.  | Name                                      | Functional classes | CAS#      | Molecular weight | Genotoxicity tests | Remarks          |
|------|-------------------------------------------|--------------------|-----------|------------------|--------------------|------------------|
| 415  | cyclohexyl butyrate                        | Flavoring agent    | 1551-44-6 | 170252           | _ (17)            | _ (125)         |
| 416  | butyl butyrate                             | Flavoring agent    | 109-21-7  | 144214           | _ (17)            | _ (124)         |
| 417  | lactones (except those generally recognized as highly toxic) | Flavoring agent |           |                  |                   |                  |
| 418  | L-lysine L-aspartate                       | Dietary supplement etc. |           |                  | _ (5)            | _ (5)           |
| 419  | L-lysine monohydrochloride                 | Dietary supplement etc. | 657-27-2  | 18265           | _ (5)(18)         | _ (5)           |
| 420  | L-lysine L-glutamate                       | Dietary supplement etc. |           |                  | _ 123            | _ 123           |
| 421  | linalool                                   | Flavoring agent    | 78-70-6   | 15425            | _ (3)(17)         | _ (3)           |
| 422  | calcium 5'-ribonucleotide                  | Seasoning          |           |                  | _ 122            | _ 122           |
| 423  | disodium 5'-ribonucleotide (sodium 5'-ribonucleotide) | Seasoning |           |                  |                   |                  |
| 424  | riboflavin (vitamin B2)                    | Dietary supplement etc. | 83-88-5  | 376369           | _ (10)(17)        | _ (10)          |
| 425  | riboflavin tetrabutyrate (vitamin B2 tetrabutyrate) | Dietary supplement etc. | 752-56-7  | 656733           | _ (10)(18)        | _ (10)          |
| 426  | riboflavin 5'-phosphate sodium (riboflavin phosphate sodium, vitamin B2 phosphate sodium) | Dietary supplement etc. | 130-40-5  | 47833            | _ (10)(18)      | _ (10)          |
| 427  | sulfuric acid                              | Processing agent   | 7664-93-9 | 98072            | _ (16)            |                  |
| 428  | aluminum ammonium sulfate (crystal: ammonium alum, desiccated: burnt ammonium alum) | Raising agent etc. | 7784-25-0  | 23715            | _ (14)           | _ 120           |
| 429  | aluminum potassium sulfate (crystal: alum or potassium alum, desiccated: burnt alum) | Raising agent etc. | 10043-67-1  | 25821            | _ (14)           | _ 120           |
| 430  | ammonium sulfate                           | Processing agent   | 7783-20-2 | 13214            | _ (18)           | _ 123           |
| 431  | potassium sulfate                          | Processing agent   | 7778-80-5 | 17425            |                   |                  |
| 432  | calcium sulfate                            | Tofu coagulator etc. | 7778-18-9  | 17217            | _ (13)           | _ 123           |
| 433  | ferrous sulfate                            | Dietary supplement etc. | 13463-43-9 | 15191            | _ (19)(10)       | _ (10)          |
| 434  | sodium sulfate                             | Processing agent   | 7757-82-6 | 142036           | _ (18)           | _ 123           |
| 435  | magnesium sulfate                          | Tofu coagulator etc. | 7487-88-9  | 120361           | _ (18)           | _ (3)           |
| 436  | dl-malic acid (dl -malic acid)             | Acidifier          | 6915-15-7 | 134087           | _ (17)           | _ (2)           |
| 437  | sodium dl-malate (sodium dl -malate)       | Acidifier etc.     | 138-09-0  | 178051           | _ (19)(10)       | _ (10)          |
## Table 3 List of the results in genotoxicity tests for the 454 designated food additives (As of October 6, 2016) (Continued)

| No. | Name                                      | Functional classes          | CAS#       | Molecular weight | Genotoxicity tests | Remarks                                      |
|-----|-------------------------------------------|----------------------------|------------|------------------|--------------------|------------------------------------------------|
| 438 | phosphoric acid                           | Acidifier                  | 7664-38-2 | 97994            | (18)               |                                                |
| 439 | distarch phosphate                        | Thickening agents etc.     | –          | –                | (18)               |                                                |
| 440 | monostarch phosphate                      | Thickening agents etc.     | –          | –                | (18)               |                                                |
| 441 | tripotassium phosphate (potassium phosphate, tribasic) | Processing agent           | 7778-53-2 | 212264           | (19)               |                                                |
| 442 | tricalcium phosphate (calcium phosphate, tribasic) | Processing agent etc.     | 7758-87-4 | 310.18           | (13)               |                                                |
| 443 | trimagnesium phosphate                    | Processing agent           | 10233–87-1 | 262.86           |                    | See substances with different salt             |
| 444 | diammonium hydrogen phosphate (diammonium phosphate or ammonium phosphate, dibasic) | Processing agent           | 7783-28-0 | 132056           | (18)               |                                                |
| 445 | ammonium dihydrogen phosphate (ammonium phosphate,monobasic or monoammonium phosphate) | Processing agent           | 7722-76-1 | 115025           | (18)               |                                                |
| 446 | dipotassium hydrogen phosphate (dipotassium phosphate or potassium phosphate, dibasic) | Processing agent           | 7758-11-4 | 174.174          | (19)               |                                                |
| 447 | potassium dihydrogen phosphate (monopotassium) | Processing agent           | 7778-77-0 | 136084           | (18)               |                                                |
| 448 | calcium monohydrogen phosphate (calcium phosphate) | Processing agent           | 136.06    | 13606            | (12)               |                                                |
| 449 | calcium dihydrogen phosphate (calcium phosphate) | Processing agent           | 7758-23-8 | 23405            | (14)               |                                                |
| 450 | disodium hydrogen phosphate (disodium phosphate) | Processing agent           | 7558-79-4 | 141958           | (19)               |                                                |
| 451 | sodium dihydrogen phosphate (monosodium phosphate) | Processing agent           | 7558-80-7 | 119976           | (18)               |                                                |
| 452 | magnesium monohydrogen phosphate           | Processing agent etc.      | 7782–75-4 | 17433            |                    | See compounds with different salt             |
| 453 | trisodium phosphate (sodium phosphate, tribasic) | Processing agent           | 7601-54-9 | 16394            | (19)               | Anhydrous substrate was also used in the assay |
| 454 | phosphated distarch phosphate              | Thickeners agents etc.     | –          | –                |                    | See substrate with different salt             |

**Notes:**
- Numbers are consistent with those shown in Tables 1 and 2.
- Underlined bold style means that the items are added as indicated in Table 2.
- (H12) means Heisei era 12, and indicates that it referred to the report on food inspection expenses implemented in 2000 (=Heisei 12). The same applies to H13, H16, H17, H18, H20, H21, H22, H23, H24, H25, H26.
- (FSC) indicates Risk assessment reports in Food Safety Commission of Japan. See [http://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=000](http://www.fsc.go.jp/fsciis/evaluationDocument/list?itemCategory=000) (Additional file 1)
performed using CHL cells without S9mix and structural abnormalities were induced (maximum dose was 2 mg/mL) [6]. Subsequently, the Ames test was conducted at Tokyo Metropolitan Research Laboratories of Public Health (TMRL) using TA97 and TA102 with and without S9mix at a maximum dose of 10 mg/plate, and positive results were reported under all conditions [7]. Since genotoxicity was detected in vitro, bone marrow micronucleus test using ddY mice was conducted in 1986. The test results were negative for single i.p. doses of 125, 250 and 500 mg/kg body weight [5].

In FY2009, TGR testing using gpt delta mice was performed in the liver and glandular stomach for confirmation of in vivo mutagenicity; the results were negative in both organs following oral gavage for 28 days at a maximum dose of 1000 mg/kg body weight [H22(FY2010)].

Despite positive in vitro results, it was concluded that L-cysteine hydrochloride is not genotoxic in living organisms because results were negative in vivo MN and TGR tests.

**Cinnamaldehyde (No. 222 in Table 3)**

Ames tests were performed at a maximum dose of 0.5 mg/plate with and without S9mix using TA100, TA1535, TA98, TA1537, TA92, TA97 in FY1981. Only TA100 showed positive results regardless of the metabolic activation in this report [8]. In the chromosome aberration test simultaneously carried out using CHL cells, structural abnormalities were induced without S9mix (maximum dose 0.015 mg/mL) [8]. Subsequently, Ames tests were carried out at TMRL using TA97 and TA102 at a maximum dose of 0.1 mg/plate; results were negative regardless of metabolic activation [7]. Since genotoxicity was detected in vitro, in vivo bone marrow MN testing was conducted in FY1986. Mice (ddY) were given a single i.p. injection at 125, 250 and 500 mg/kg body weight and the results were negative [5].

We conducted TGR tests in the liver and small intestine (jejunum) using gpt delta mice to confirm in vivo mutagenicity in FY2010 and FY2011. The reason for choosing the small intestine as the target organ is that it is the first in the gastrointestinal tract to be exposed to substances administered orally. Mice were dosed by oral gavage at 125, 250, 500 and 1000 mg/kg body weight for 28 days, and mutagenicity was investigated for the animals dosed at 500 and 1000 mg/kg body weight. Negative results were obtained in both organs [H22(FY2010)].

Despite showing genotoxicity in vitro, it was concluded that cinnamaldehyde does not show genotoxicity in living organisms because results were negative in vivo MN and TGR tests.

**Iron lactate (No. 289 in Table 3)**

Ames testing was carried out in FY1983 and the results were positive without S9mix in TA97, TA102 and TA2637 at the highest dose of 5.0 mg/plate, and negative in TA100 and TA98 with and without S9mix [9]. Chromosomal aberration testing using CHL cells conducted in the same year induced structural abnormalities without S9mix (maximum dose 2.5 mg/mL) [9]. Subsequently, Ames tests were conducted at TMRL using TA97 and TA102, yielding negative results with and without S9mix [10]. The maximum dose in this study, 1.0 mg/plate, is considered to be insufficient. In vivo MN testing using ddY mice was conducted in FY1986 (single i.p. administration of 30 mg/kg body weight, and four separate i.p. doses of 7.5 mg/kg body weight/day), with negative results [11].

In FY2011, TGR tests in the liver and kidney were carried out using gpt delta mice to confirm in vivo mutagenicity. The reason for using the kidney as the target organ is that nephrotoxicity was observed in macroscopic examinations. After doses of 250, 500 and 1000 mg/kg body weight for 28 days by oral gavage, mutation was investigated at doses of 500 and 1000 mg/kg body weight. Since the results were negative in both organs [H23(FY2011)], it was concluded that iron lactate does not induce mutation in vivo.

**Propyl gallate (No. 374 in Table 3)**

Ames testing was carried out in TA100, TA98, TA1537 at 500 μg/plate in FY1979, and negative results were obtained regardless of metabolic activation [2]. Subsequently, Ames tests were carried out at TMRL using TA97 and TA102, and at the maximum dose of 0.1 mg/plate it was found that TA102 showed a statistically significant increase in the number of revertants regardless of metabolic activation [12]. TA100 and TA1535 mainly detect base substitution occurring in GC base pairs, while TA102 mainly reveals substitution in AT base pairs. Thus, these results (negative in TA100 and TA1535, positive in TA102) suggest that propyl gallate is reactive with AT base pairs. The negative results for TA98, TA1537 and TA97 indicate that the probability of inducing a frameshift mutation is low. Five out of six tests showed positive results at doses higher than 50 μg/mL, but an unusual pattern was shown regarding dose correlation for which the mechanism is unknown.

Since the above results suggested that propyl gallate induces base substitution with AT base pairs in vitro, TGR testing was performed in liver and glandular stomach using gpt delta mice in FY2009 [H21(FY2009)]. Repeated administration over 28 days produced negative results for both organs at the highest dose of 1000 mg/kg body weight. Thus, the mutagenicity of propyl gallate was detected in vitro, but not considered to be detected in vivo.

After chromosomal aberration testing in FY1979 it was reported that structural abnormalities were induced
in CHL after 24 h treatment at a dose of 0.04 mg/mL without S9mix [2]. To investigate the risk of chromosomal aberration, in vivo bone marrow MN testing was conducted in FY2009, with negative results at the maximum dose of 1000 mg/kg body weight (administered twice) [H21(FY2009)]. Therefore, although chromosomal abnormalities were detected in vitro, they were not in vivo.

From the results detailed above, propyl gallate was considered to be non-genotoxic to living bodies.

2) Two items negative for chromosome aberration and in vivo micronucleus tests while positive for Ames test Erythorbic acid (isoadsorbic acid)(No. 78 in Table 3)

This substance was positive only in TA100 regardless of S9mix (highest dose 50 mg/plate) in the Ames test using the strains TA100, TA98, TA1535, TA98, TA1537, TA92 and TA94 in FY1980 [13]. In chromosomal aberration tests using CHL cells, a negative result was reported at the highest dose of 0.25 mg/mL without S9mix [13]. In the Ames test conducted at TMRL using TA97 and TA102, a statistically significant increase in the number of revertants was reported in both strains regardless of S9mix (maximum dose 10 mg/plate) [14]. However, the result is given as negative in Table 3 since the number of revertants did not reach twice of the number of the negative control. Subsequently, an in vivo bone marrow MN test using ddY mice was performed, and this substance showed negative when administered in a single dose of 1500 mg/kg body weight (at the maximum) or as four treatments (at 24-h intervals) at 750 mg/kg body weight (at the maximum).

Thereafter, TGR testing using gpt delta mice was conducted for liver and glandular stomach (maximum dose 1000 mg/kg body weight for 28 days by gavage) in FY2009 in order to investigate in vivo mutagenicity. Neither point mutation nor deletion mutation was induced in either organ [H21(FY2009)]. It was concluded that there are no concerns for genotoxicity of erythorbic acid to living bodies.

Piperonal (No. 319 in Table 3)

In Ames testing at TMRL using TA97 and TA102 this substance showed positive results in TA97 without S9mix at the highest dose of 1 mg/plate [3]. It is reported that a statistically significant increase was observed with S9mix, but the level did not reach twice that of the negative control. There are no reports on chromosomal aberration tests. MN testing using ICR mice was carried out in FY2010, and the results were negative in bone marrow after oral administration of 250, 500 and 1000 mg/kg body weight (two doses at 24-h intervals) [H22(FY2010)]. In FY2010–11, TGR testing using gpt delta mice was performed in the liver and kidney in order to confirm in vivo mutagenicity at doses of 250, 500 and 1000 mg/kg body weight for 28 days by gavage. The results were negative for both organs at doses of 500 and 1000 mg/kg body weight [H23(FY2011)].

From the above results it was concluded that piperonal does not show genotoxicity in living organisms.

3) One item positive in all three tests (Ames, chromosomal aberration and in vivo micronucleus tests) Maltol

In 1982, Ames testing using TA100, TA98, TA2637, and TA94 was carried out for maltol at a maximum dose of 10.0 mg/plate, and the results were negative both with and without S9mix [6]. Chromosomal aberration testing was conducted in the same year, and it was reported that structural abnormalities were induced in CHL cells at the highest dose (0.075 mg/mL) without S9mix [6]. Subsequently, Ames testing was performed at TMRL with TA97 and TA102, at the highest dose of 10.0 mg/plate with and without S9mix. Induction of colony formation at a reversion level almost double that of the negative control was observed in TA97 at a dose of 1 mg/plate without S9mix. Positive judgment has been reported in a micronucleus test using bone marrow of ddY mice, 24 h after single i.p. administration of 125, 250 and 500 mg/kg body weight [5]. Since the usage of this item is limited to fragrances, there is no possibility of exposure in vivo at a concentration equivalent to the dose at which chromosomal abnormality was detected in vitro.

In FY2009, TGR testing using gpt delta mice was performed in the liver and glandular stomach for confirmation of in vivo gene mutagenicity. The results were negative in both organs at doses of 400, 200, 100 and 50 mg/kg body weight for 28 days by gavage [H21(FY2009)]. From the above, it seems that there is no concern of genotoxicity in maltol for living bodies.

4) Five items for which the Ames test was negative 1-Methylnaphthalene

In FY2005 this substance was reported to have induced structural abnormalities in a chromosome aberration test using CHL cells [H17(FY2005)] while in vivo bone marrow micronucleus testing conducted in FY2006 reported negative results in a two-dose study of 1000 mg/kg body weight at the maximum [H18(FY2006)].

Regarding mutagenicity, in Ames tests using several strains of Salmonella typhimurium conducted from 1980 to 2002, all results were negative, whereas a weak positive result was reported in the forward mutation test using S. typhimurium (maximum dose 0.992 mg/mL, 2-h exposure) [FSC58]. In theory, the Ames test, which is a reverse mutation test, can detect only specific point mutations while a forward mutation test can detect mutations of any type. Thus, it would be problematic for the
negative results of the Ames tests to be taken as completely eliminating the concerns about mutagenicity arising from the result of the forward mutation tests. Subsequently, a TGR test in gpt delta mice (males and females) was performed on the lungs. The reason that the lungs were selected as the target organ was that weak carcinogenicity was observed in the lungs of mice in the 81-week chronic toxicity–carcinogenicity combination test reported in 1993. TGR tests were conducted at doses of 170 and 280 mg/kg body weight for females and 120 and 220 mg/kg body weight for males by dietary administration for 13 weeks, the results being negative in all conditions ([15], FSC58).

From the above, 1-methylnaphthalene is considered to have no concerns of genotoxicity for living bodies.

Food Red No. 40
In FY1995 at TMRL, negative results (maximum dose 10 mg/plate) in Ames tests with TA97 and TA102 with and without S9mix were reported [16]. Chromosomal aberration tests have not been carried out. Subsequently, in vivo micronucleus tests using CD1 mice was performed in FY2008, and results of single oral gavage of 500, 1000 and 2000 mg/kg body weight were reported to be negative in bone marrow [H20(FY2008)].

In FY2008 and FY2011, comet and TGR tests using mice were conducted to examine in vivo DNA damage inducibility and mutagenicity, respectively. In the comet test, CDF1 mice were administered two doses of 500, 1000 and 2000 mg/kg body weight by oral gavage with a 24-h interval. The results were negative for both liver and glandular stomach (H20(FY2008), [17]). In addition, another comet assay using ICR mice was carried out with two oral gavage administrations (24-h interval) at doses of 500, 1000 and 2000 mg/kg body weight. The results were negative in both stomach and colon, while an increase without dose correlation was observed in liver [H23(FY2011)]. TGR testing was conducted using the Muta™Mouse, orally gavaged at doses of 250, 500 and 1000 mg/kg body weight for 28 days; mutagenicity in the liver and glandular stomach was not observed [H20(FY2008)]. Furthermore, TGR tests using gpt delta mice were carried out and the results were negative for mutagenicity in the large intestine following oral gavage for 28 days at doses of 250, 500 and 1000 mg/kg body weight [H23(FY2011)].

From the above, it seems that there is no concern of genotoxicity of Food Red No. 40 for living bodies.

Food Red No. 102
In 1979 negative results were reported following Ames tests carried out with and without S9mix conditions using TA100, TA1535, TA98, TA1537, TA92 and TA94 (maximum dose 5.0 mg/plate) [2]. In chromosomal aberration tests using CHL cells carried out in the same year, induction of structural abnormalities was observed with S9mix (maximum dose of 4.0 mg/mL) [2]. Subsequently, Ames tests were conducted at TMRL using TA97 and TA102, and the results were negative with and without S9mix (maximum dose 10 mg/plate) [10]. Since chromosomal abnormalities were induced in vitro, micronucleus tests using ddY mice were carried out in FY1980. Results were negative for two sets of conditions in bone marrow: single i.p. administration of 300, 600 and 1200 mg/kg body weight; and four i.p. doses of 300 mg/kg body weight [13].

In FY2008, comet and TGR tests using mice were carried out to examine in vivo DNA damage inducibility and mutagenicity, respectively [H20(FY2008)]. Comet tests were carried out by oral gavage (twice, at 24-h intervals) at doses of 500, 1000 and 2000 mg/kg body weight using CDF1 mice, and the results were judged as negative in both liver and glandular stomach. The TGR tests were carried out in Muta™Mouse using oral gavage at 250, 500 and 1000 mg/kg body weight for 28 days, and the results were negative in both liver and glandular stomach.

From the above, Food Red No.102 is considered not to have concerns of genotoxicity to living bodies.

Food Red No. 104
In 1979, negative results in Ames tests carried out using TA100, TA1535, TA98, TA1537, TA92 and TA94 with and without S9mix (maximum dose 5 mg/plate) were reported [2]. In the same year, chromosomal aberration tests using CHL cells were conducted without S9mix, and the results were negative (maximum dose 0.25 mg/mL) [2]. Ames tests were also conducted at TMRL with TA97 and TA102, and the results were negative with and without S9mix (maximum dose 1 mg/plate) [10]. Micronucleus testing using mice was not performed because both in vitro tests were negative.

In FY2008, comet and TGR tests using mice were carried out to confirm in vivo DNA damage inducibility and mutagenicity, respectively. Comet tests for liver and glandular stomach were performed by oral gavage (twice, 24-h interval) at doses of 250, 500 and 1000 mg/kg body weight on CDF1 mice. Results showed false positive in the liver and positive in glandular stomach [H20(FY2008)]. TGR tests were conducted using Muta™Mouse with oral gavage at 250, 500 and 1000 mg/kg body weight for 28 days and liver and glandular stomach were examined for mutation induction; the results were negative in both [H20(FY2008)].

Based on the above results, it is likely that the DNA damage detected in the comet tests would not reach the level necessary to produce mutation. The negative results in liver and glandular stomach in TGR tests support this view, and it seems likely that the DNA damage is repaired in mouse body. Therefore, Food Red No. 104 is considered not to induce genotoxicity (mutagenicity) in vivo.
Food Red No. 105
In FY1978 results were negative in Ames tests (maximum dose 5.0 mg/plate) with and without S9mix using TA100, TA1535, TA98, TA1537, TA92 and TA94 [2]. In the same year, chromosomal aberration tests using CHL cells were also carried out (S9mix only) and the results were negative (maximum dose 0.25 mg/mL) [2]. Subsequently, Ames tests were carried out at TMRL using TA97 and TA102 (maximum dose 1 mg/plate) with and without S9mix, with negative results [10]. Micronucleus tests using mice were not carried out because both in vitro tests were negative.

In FY2008, in order to examine in vivo DNA damage inducibility and mutagenicity, comet and TGR tests were conducted, respectively, in mice. The comet test was positive in both liver and glandular stomach for oral administration (twice, 24-h interval) at doses of 250, 500 and 1000 mg/kg body weight for CDF1 mice, and were examined [H20(FY2008)]. The TGR test was conducted using Muta™Mouse with oral gavage at 250, 500 and 1000 mg/kg body weight for 28 days. Mutation induction in the liver and glandular stomach was tested for, both results being negative [H20(FY2008)].

Since the TGR tests performed in mouse liver and glandular stomach were negative, the DNA damage detected in the comet test is considered to have been repaired in vivo. Thus, there is a high possibility that such DNA damage would not lead to mutation. In conclusion, there are no concerns that Food Red No. 105 induces genotoxicity (mutagenicity) in vivo.

Discussion
The standard genotoxicity tests are carried out to detect gene mutation by Ames test using bacteria, and to detect chromosomal abnormalities by an in vitro chromosomal aberration test using cell culture and an in vivo micronucleus test using mice. Chromosomal abnormalities in chromosomal aberration tests are observed as morphological abnormalities in chromosomes during the interphase of cell division because damaged DNA is not normally replicated and the abnormalities persist. Such structural abnormalities are lethal for cells in many cases, and the majority of chromosomal abnormalities are not inherited by the next generation. Similarly, micronuclei in the micronucleus test also transiently appear in daughter cells after cell division, and disappear after the next cell division. Therefore, chromosomal abnormalities and micronuclei are indicators that DNA has been exposed to genotoxic substances, not a cause of cancer in cells. The fragmentation of DNA observed in the comet test is also transient, thus the comet test is also an indicator test. On the other hand, gene mutation is irreversible and permanent. Gene mutations arising in oncogenes or tumor suppressor genes have the possibility to cause cell transformation and initiate cancer-forming cells. Therefore, genetic mutation is a direct trigger of cancer, and it is highly correlated with carcinogenicity in rodents compared to other genotoxic end points [18], while the chromosome aberration test and micronucleus test have high false positive rates and low correlation with carcinogenicity tests [19].

The TGR test, which is an in vivo gene mutation test, is thus recommended when chemical substances have shown positive results in chromosome aberration tests, micronucleus tests, and comet tests. In particular, when comet and TGR tests are carried out on the same target tissue, if the results differ between the two, the results of the TGR tests should be given priority. The TGR test is also useful for follow-up of the same gene mutation test, the Ames test. A false positive reaction sometimes occurs in the Ames test because of bacteria-specific conditions such as drug metabolism, in vitro test-specific reactions using rat S9, as well as nonspecific reactions due to non-physiological conditions differing from the in vivo situation. Confirmation of an indication of mutagenicity with the Ames test by the TGR test in the living body is important on both scientific and safety grounds.

Among the 13 designated food additives covered in the Commentary section, eight items were positive for the Ames test, but the TGR test showed negative results for all of them. As a result, the possibility that these eight food additives exhibit genetic toxicity (especially mutagenicity, which is problematic for living bodies) is eliminated. This knowledge is important to ensure human safety. The TGR test took effect with publication of the OECD Guideline TG488 in 2011 and therefore was not available for implementation at the time of the Hayashi report (2000). We expect the safety of other food additives to be confirmed as TGR test results are accumulated.

Postscript
In this report, we summarized the data for the most widely used substances in the classification of designated food additives in Japan. Currently we are summarizing the results of genotoxicity tests conducted at MHLW for existing food additives, a group of the next most widely used food additives, in the same way. The reports will be updated from time to time since additions and deletions of items are considered likely in the future.

Additional file
Additional file 1: References Reports in Japanese. (DOCX 14 kb)

Abbreviations
CA: Chromosomal aberration test; CHL: Chinese hamster lung; FSC: Food Safety Commission; FY: Fiscal year; MHLW: The Ministry of Health, Labour and Welfare; MN: Micronucleus test; NIH: National Institute of Health Sciences; OECD: The Organisation for Economic Co-operation and Development;
TGR: Transgenic rodent gene mutation assay; TMRL: Tokyo Metropolitan Research Laboratories of Public Health

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Availability of data and materials

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Authors’ contributions

MH conceived of the study and participated in its design and coordination. MY collected the data, created a detailed table and wrote the manuscript. MH conceived of the study and participated in its design and coordination.

Ethics approval and consent to participate

Both authors read and approved the final manuscript.

Consent for publication

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Competing interests

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