Corporation obtaining approval, the name of its representative, and the address of its main office

Applicant:
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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Maize tolerant to aryloxyalkanoate herbicide (Modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (DAS40278, OECD UI: DAS-4Ø278-9)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	-

Outline of the Biological Diversity Risk Assessment Report

I. Information collected prior to assessing Adverse Effect on Biological Diversity

Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the development of maize tolerant to aryloxyalkanoate herbicide (modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (DAS40278, OECD UI: DAS-4Ø278-9) (hereinafter referred to as "this recombinant maize") are shown in Table 1 (p.3).

2) Function of component elements

(a) Functions of individual component elements of donor nucleic acid, including target gene, expression regulatory region, localization signal, and selective marker

Functions of individual component elements of transferred gene are shown in Table 1 (p.3).

The modified *aad-1* cassette contains the *RB7 MAR* sequence of the nuclear matrix attachment region. The nuclear matrix attachment region (MAR) is a region frequently detected in the genome DNA sequence, and it is considered to act to fix the DNA to the nuclear matrix for formation of DNA loop. It has been reported that the MAR helps enhance the expression of transferred gene or reduce the gene silencing or reduce suppression of the expression of genes when it exists adjacently to either side of the transferred gene (Reference 6, Reference 7).

(b) Functions of proteins produced by the expression of target gene and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity

AryloxyAlkanoate Dioxygenase (hereinafter referred to as "modified AAD-1 protein") is an enzyme which catalyzes the reaction of incorporating the oxygen into the aryloxyalkanoate herbicide (Annex 1) and transforms it to herbicidally-inactive compounds (Reference 8). For example, the modified AAD-1 protein catalyzes the reaction of incorporating the oxygen into the 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide and degrades it into herbicidally-inactive 2,4-dichlorophenol (2,4-DCP) and glyoxylic acid (Figure 1, p.3).

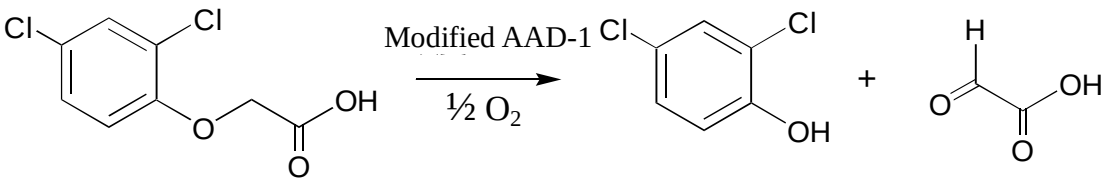

Figure 1 Action mechanism of the modified AAD-1 protein
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As a result of the amino acid sequence homology search in 2007 using the allergen database (FARRP version 7.00 Allergen Database) to investigate whether the modified AAD-1 protein shares significant sequence identity with any of the known allergens, it has been confirmed that the protein does not share any significant sequence similarity with any of the known allergens.

Table 1 Composition of the donor nucleic acid and the origins and functions of component elements

Component elements	**Origin and Function**
Modified *aad-1* cassette	
RB7 MAR	Nuclear matrix attachment region (MAR) from *Nicotiana tobacum* (Reference 9). It stabilizes the expression of the modified AAD-1 protein.
ZmUbi1	Ubiquitin promoter from *Zea mays*, including the exon and intron regions (Reference 10). This promoter initiates the expression of genes in the entire plant body.
Modified *aad-1*	Gene which has modified the aryloxyalkanoate dioxygenase gene derived from the gram-negative bacillus *Sphingobium herbicidovorans* to the codon optimized for expression in plants and which encodes the modified AAD-1 protein. Regarding the amino acid sequence, alanine is added at the second position by introducing the cloning site.
ZmPer5 3'UTR	Terminator from *Zea mays* (Reference 11). It terminates the transcription of genes.
RB7 MAR	Nuclear matrix attachment region (MAR) from *Nicotiana tobacum* (Reference 9). It stabilizes the expression of the modified AAD-1 protein.

(All the rights pertinent to the information in the table above and the responsibility for

the contents rest upon Dow Chemical Japan Ltd.）

(c) Contents of any change caused to the metabolic system of recipient organism

The modified AAD-1 protein is an enzyme which catalyzes the reaction of specifically incorporating the oxygen into the optical isomer-free compounds among those having the aryloxyalkanoate group and the dextro (Right) form of the optical isomers.

For the compounds which are present in plant body and structurally and physiological-functionally similar to the compounds having the aryloxyalkanoate group, action of the modified AAD-1 protein was examined on a laboratory scale to identify possible effects on the metabolic pathway. In the examination, the plant hormones including indole-3-acetic acid, abscisic acid, gibberellic acid (GA3) and aminocyclopropane-1-carboxylic acid and the intermediates of phenylpropanoid including cinnamic acid, coumaric acid and sinapic acid were examined as the substrates. In addition, a total of 20 L-amino acids were also examined (Annex 2).

For the 20 L-amino acids, no reaction was confirmed at a concentration of 1μM of the modified AAD-1 protein. On the other hand, as a result of application of 1μM modified AAD-1 protein to the plant hormones and the intermediates of phenylpropanoid, abscisic acid, gibberellic acid, cinnamic acid and coumaric acid exhibited slight reactions. Furthermore, as a result of application of 5μM and 10μM modified AAD-1 protein, slight reaction was observed only for the aminocyclopropane-1-carboxylic acid at a concentration of 5μM and only for the indole-3-acetic acid at a concentration of 10μM. Since no correlation was observed between the concentration of the modified AAD-1 protein and the enzyme activity as mentioned above, oxidation of the primary candidates was measured by Fourier transform mass spectrometry (FT/MS). When 10μM modified AAD-1 protein was applied, the oxidized forms of indole-3-acetic acid and cinnamic acid were detected. However, the reaction rate was very slow and the parameters *Km* and *Vmax* for the Michaelis-Menten equation could not be determined. It is considered unlikely that the identified oxidization reaction would affect *in planta* metabolic pathways, based on the findings that oxidized products were only detected utilizing the highly-sensitivity analytical technique FT/MS and that the modified AAD-1 protein reaction kinetics were not measurable even at exorbitantly high enzyme concentrations.

Additionally, compounds having the aryloxyalkanoate groups have not been identified in any plant body. It is considered that the modified AAD-1 protein would not change any other metabolic systems of plant body.

(2) Information concerning vectors

1) Name and origin

The plasmid vector used for the development of pDAS1740 is the plasmid pUC19

derived from *Escherichia coli* (*E. coli*).

2) Properties

(a) The numbers of base pairs and nucleotide sequence of vector

The total number of base pairs in the vector pDAS1740 is 8,512bp, and the total number of base pairs in the linear DNA used for the transfer is 6,236bp. The nucleotide sequence of the vector pDAS1740 is shown in Annex 3.

(b) Presence or absence of nucleotide sequence having specific functions, and the functions

The ap^r gene confers the resistance to ampicillin due to the expression and it is used for selection of the vector pDAS1740. However, the ap^r gene is not contained in the linear DNA used for the transfer and thus, this recombinant maize has no ap^r gene transferred.

Presence or absence of ap^r gene in this recombinant maize was investigated based on the Southern blotting analysis or PCR method and as a result, it has been confirmed that the ap^r gene is not present in this recombinant maize (Annex 4).

(c) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present

The linear DNA cleaved from the pDAS1740 used for the transfer by the restriction enzyme *Fsp I* does not contain any sequence which can cause infection, and then it is not known to be infectious.

(3) Method of preparing living modified organisms

1) Structure of the entire nucleic acid transferred in the recipient organism

The composition of the vector pDAS1740 is shown in Figure 2 (p.6), and the composition of the linear DNA used for the transfer is shown in Figure 3 (p.6). In addition, the process of development of pDAS1740 is provided in Annex 5.

2) Method of transferring nucleic acid transferred to the recipient organism

Transferring nucleic acid into the recipient organism was based on the Whiskers-mediated transformation (Reference 12). The immature embryo of the recipient maize line Hi-II was callused and cultured in the liquid to obtain the embryo suspension. Then, the embryo suspension was added with the linear DNA cleaved from the pDAS1740 by the restriction enzyme *Fsp I* and mixed with the needle-shaped silicon-carbide whisker fibers, then the linear DNA was transferred

into the recipient through holes in the cells produced by the silicon-carbide whisker fibers.

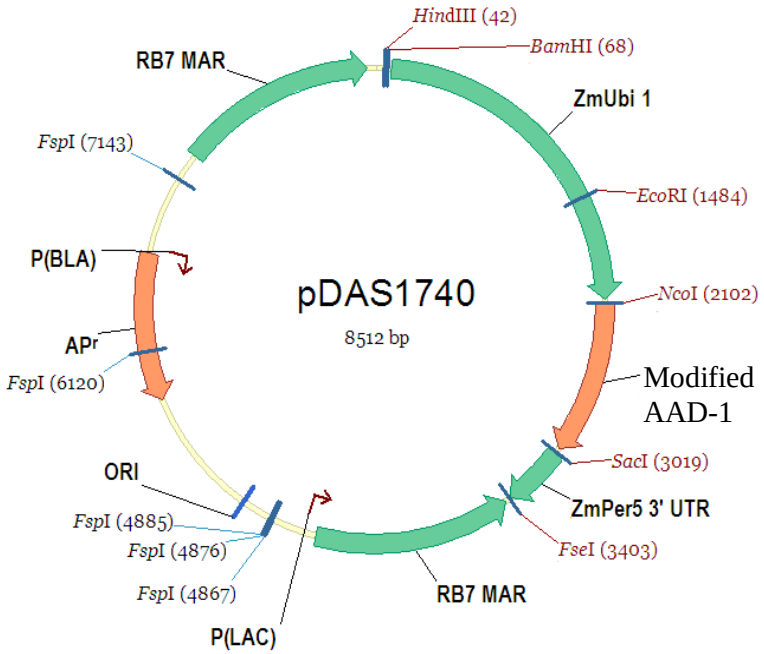

Figure 2 Composition of the vector pDAS1740 and the restriction enzyme cleavage sites

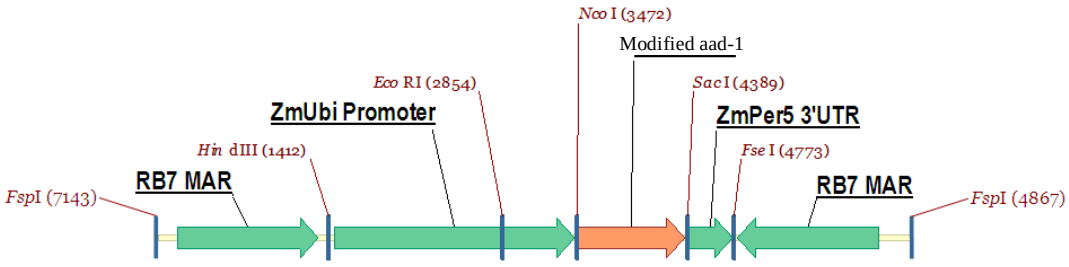

Figure 3 Composition of the linear DNA cleaved from the pDAS1740 by the restriction enzyme *Fsp I*

3) Processes of rearing of living modified organisms

(a) Mode of selecting the cells containing the transferred nucleic acid

Selection of nucleic acid-transferred cells was made based on the culture on the medium containing the Haloxyfop, one of the aryloxyalkanoate herbicides.

(b) Presence or absence of remaining *Agrobacterium* when using *Agrobacterium* method for transferring nucleic acid

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(c) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line in which the state of existence of replication products of transferred nucleic acid was confirmed, the line subjected to isolated field tests; and the line used for collection of other necessary information for assessment of Adverse Effect on Biological Diversity

As a result of spraying the Quizalofop, one of the aryloxyalkanoate herbicides, to the regenerated plant body (T0 generation), it was confirmed that the modified AAD-1 protein is produced. In addition, based on the comprehensive evaluations on the analysis of transferred gene, confirmation of protein expression, and herbicide tolerance and agronomic traits in the open-air fields in US and Canada, this recombinant maize was selected. This application includes the T1 generation and the progeny.

Details are shown in Figure 4 (p.8). In addition, the generations used in individual tests are listed in Table 2 (p.8).

Status of approval and application of this recombinant maize in Japan is summarized below.

July, 2009: Approval was obtained from the Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment for Type 1 Use Regulation (isolated field test) in accordance with the “Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms.”

June, 2010: Application was filed to the Ministry of Health, Labor and Welfare for approval for safety of use as food in accordance with the “Safety Evaluation Criteria for Genetically-Modified Foods (Seed Plants).”

June, 2010: Application was filed to the Ministry of Agriculture, Forestry and Fisheries for approval for safety of use as feed in accordance with the “Safety Evaluation Criteria for Feed and Additives derived from Recombinant-DNA Techniques.”

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Figure 4 Process of rearing this recombinant maize

Table 2 Generations used in individual tests

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(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid

1) Place where the replication product of transferred nucleic acid exists

The transferred nucleic acid follows the Mendel's law of inheritance once transferred in the chromosome of plant. A segregation analysis was conducted to identify how the traits transferred into this recombinant maize segregate in the populations of T1 and T2 generations. As a result of examination for presence of tolerance to the herbicide Quizalofop, it was found that nearly good agreement was obtained between the examination result and the segregation ratio expected from the Mendel's law in the nucleus and thus, it was confirmed that the transferred nucleic acid is present on the chromosome (Table 3, p.8).

Table 3 Segregation of traits between T1 and T2 generations of this recombinant maize

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2) The number of copies of replication products of transferred nucleic acid and stability of its inheritance through multiple generations

As a result of Southern blotting analysis to examine the number of copies of transferred nucleic acids in multiple generations, it was confirmed that one (1) copy of the modified *aad-1* cassette has been transferred and that it has been stably inherited through multiple generations (Annex 4).

3) The position relationship in the case of multiple copies existing in chromosome

There are no multiple copies on the chromosome.

4) Inter-individual or inter-generational expression stability under a natural environment with respect to the characteristics referred to specifically in (6)-1)

The expression level of the modified AAD-1 protein in the leaves of T3, BC1 and BC2 generations of this recombinant maize was compared based on the ELISA method. As a result, the expression level of the AAD-1 protein was found similar

for the BC1 and BC2 generations of the heterozygous genotype. On the other hand, for the T3 generation, due to the homozygous genotype, the expression level of the AAD-1 protein was found about two (2) times as high as the expression level of the AAD-1 protein in the BC1 and BC2 generations of the heterozygous genotype. Consequently, it is considered that the modified AAD-1 protein is stably expressed in multiple generations (Table 4, p.9).

Table 4 Expression level of the modified AAD-1 protein in the leaves of this recombinant maize

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5) Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants

This recombinant maize does not contain any sequence allowing transmission; therefore there is no possibility that the genes transferred to this recombinant maize might be transmitted to wild animals and wild plants.

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

For the detection and identification of this recombinant maize, the PCR method has been developed where the nucleotide sequences specific to this recombinant maize are used as primers (Annex 6). The detection limit of the PCR method is 0.04%.

(6) Difference from the recipient organism or the species to which the recipient organism belongs

1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of replication products of transferred nucleic acid

This recombinant maize is given the tolerance to aryloxyalkanoate herbicide due to the expression of the modified AAD-1 protein which is derived from the transferred modified *aad-1* gene. Cultivation of this recombinant maize given the tolerance to aryloxyalkanoate herbicide offers the cultivating farmers increased options of usable herbicides and helps control the weeds which have acquired the tolerance to the other herbicides.

In the isolated field tests conducted in 2009 at Nasu Research Station of National Institute of Livestock and Grassland Science, National Agriculture and Food Research Organization (hereinafter referred to as “National Institute of Livestock and Grassland Science”), this recombinant maize and the non-recombinant control maize were examined for tolerance to herbicide Quizalofop. About two (2) weeks after germination, twenty-five (25) individuals of each of this recombinant maize

and the non-recombinant control maize were sprayed with the herbicide Quizalofop (brand name: Portoflowable) diluted by a factor of 250-fold. One (1) week after spraying, the non-recombinant control maize was found all withered, while this recombinant maize all exhibited satisfactory herbicide tolerance without any injury ("Isolated Field Test Results Report," Figure 1, p.2).

As a result of acute toxicity test for possible effects of 2,4-DCP on the aquatic organisms, a decomposition product of herbicide 2,4-D, LC_{50} (50% lethal concentration) was found 1.7 mg/L for freshwater fish and 1.4 mg/L for water flea (*Daphnia magna*), and EC_{50} (50% effective concentration) for duckweed was found 1.5 mg/L. In addition, as a result of chronic toxicity test, NOEC (no observed effect concentration) was found 0.14 mg/L for duckweed and 0.21 mg/L for *Daphnia magna*. Furthermore, regarding possible effects on the land life, LC_{50} for earthworm was found 125 mg/kg, and EC_{10} (10% effective concentration) for *Folsomia candida* was found 0.7 mg/kg (Reference 13).

On the other hand, regarding the effects of 2,4-D on the aquatic organisms, LC_{50} as a result of acute toxicity test was found 0.26 mg/L for freshwater fish and 2.2 mg/L for *Daphnia magna*, and EC_{50} for duckweed was 0.2992 mg/L. In addition, as a result of chronic toxicity test, NOEC was found 0.0476 mg/L for duckweed and 0.20 mg/L for *Daphnia magna* (Reference 14).

As can be seen from the above findings, the 2,4-DCP, a decomposition product of 2,4-D, is less toxic compared to the 2,4-D.

Furthermore, this recombinant maize was sprayed with the 2,4-D at the upper limit level of optimum dosage range to identify the residual concentration of 2,4-DCP in the grains and as a result, the residual level was found below the limit of quantitation (0.01 ppm) (Reference 15).

2) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified agricultural products and the taxonomic species to which the recipient organism belongs, and the degree of difference, if present

In 2009, isolated field test was conducted at the National Institute of Livestock and Grassland Science to examine any differences between this recombinant maize and the non-recombinant control maize (See "Isolated Field Test Results Report.").

(a) Morphological and growth characteristics

For the morphological and growth characteristics, comparison was made regarding the uniformity of germination, germination rate, time of tasseling, time of silking, culm length, plant type, tiller number, height of ear, yellow ripe stage, number of ears, number of productive ears, ear length, ear diameter, row number per ear, grain number per row, grain color, 100-kernel weight, grain shape, and

fresh weight of above ground part at harvesting time between this recombinant maize and the non-recombinant control maize. Due to the low temperatures at the late stage of growth, the recombinant and non-recombinant plants in the fields both failed to reach full maturity. For this reason, for examination regarding ear length, ear diameter, row number per ear, grain number per row, grain color, 100-kernel weight and grain shape, in November 15, 2009, two (2) individuals each of this recombinant maize and the non-recombinant control maize from each plot were transferred to a plastic greenhouse (without heating) and then their fully matured ears were used. In addition, at the point of time when this recombinant maize and the non-recombinant control maize were transferred to the plastic greenhouse, their growth was found arrested and thus, it is considered that the ear length, ear diameter, row number per ear, grain number per row, and grain shape remain unchanged even after the transfer to the greenhouse.

This recombinant maize and the non-recombinant control maize both exhibited good uniformity of germination, and no difference was observed regarding time of tasseling and time of silking between the both plants. This recombinant maize and the non-recombinant control maize both have the upright plant type, and no tiller was observed and no difference was observed in yellow ripe stage between the both plants. This recombinant maize and the non-recombinant control maize both have one (1) ear and one (1) productive ear, showing no difference between the both plants. In addition, this recombinant maize and the non-recombinant control maize both feature yellow-colored wedge-shaped grains regarding the grain color and grain shape, showing no difference. Furthermore, also regarding the germination rate, culm length, height of ear, ear length, ear diameter, row number per ear, grain number per row, 100-kernel weight and fresh weight of above ground part at harvesting time, no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize (Table 5, p.11).

Table 5 Comparison of morphological and growth characteristics between this recombinant maize and the non-recombinant control maize

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(b) Cold-tolerance and heat-tolerance at the early stage of growth

This recombinant maize and the non-recombinant control maize were examined for cold-tolerance at the early stage of growth. Twenty-five (25) individuals each of this recombinant maize and the non-recombinant control maize raised up to 2- to 3-leaf stage were left in the open air (in the premises of isolated field) in December 30, 2009. As a result, all the individuals of both of this recombinant maize and the non-recombinant control maize withered in about four (4) days, showing no difference between the both plants (“Isolated Field Test Results Report,” Figure 5, p.8).

(c) Wintering ability and summer survival of the mature plant

Maize is a summer type annual plant, and after ripening it usually dies out. Actually, in the isolated field, this recombinant maize and the non-recombinant control maize were both found withered ("Isolated Field Test Results Report," Figure 6, p.9).

(d) Fertility and size of the pollen

Fertility and size of the pollen of this recombinant maize and the non-recombinant control maize were examined with the pollens stained with acetocarmine solution. As a result, no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize (Table 6, p.12).

Table 6 Fertility and diameter of the pollen of this recombinant maize and the non-recombinant control maize

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(e) Production, shedding habit, dormancy and germination rate of the seed

Regarding seed production, the differences between this recombinant maize and the non-recombinant control maize were examined in the number of productive ears, row number per ear, grain number per row and 100-kernel weight. As a result, no statistically significant difference was observed in all the items examined. Consequently, it was judged that no difference exists in seed production between this recombinant maize and the non-recombinant control maize (Table 5, p.11).

Regarding shedding habit of the seed, no seed shedding was observed because the ears of this recombinant maize and the non-recombinant control maize were covered with bracts at the time of harvesting.

Regarding dormancy of the seed, it was judged that this recombinant maize and the non-recombinant control maize both have very low level of seed dormancy based on the findings that the seeds of the parent plants of this recombinant maize and the non-recombinant control maize and the harvested seeds examined immediately after harvesting both exhibited high germination rate (Table 7, p.12). For examination on the germination rate of harvested seeds, the seeds matured in the plastic greenhouse were used.

Table 7 Comparison of germination rate between this recombinant maize and the non-recombinant control maize

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(f) Crossability

In Japan, no wild relatives that can be crossed grow voluntarily. Thus, crossability test of this recombinant maize was not performed.

(g) Productivity of harmful substances

For comparison of productivity of harmful substances between this recombinant maize and the non-recombinant control maize, succeeding crop test, plow-in test and soil microflora test were conducted.

<Succeeding crop tests>

Soil in the root zone of this recombinant maize and the non-recombinant control maize was collected from four (4) spots in each plot, mixed with each other then mixed with compound fertilizer, and packed into a seedlings-raising vat having 5 holes × 5 holes. One (1) grain of radish seed was sown in each cell in the seedlings-raising vat. Examination was conducted for germination rate 7 days after sowing, and for plant height and dry weight 14 days after sowing. As a result, regarding the germination rate, plant height and dry weight of radish (test plant), no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize (Table 8, p.13).

Table 8 Succeeding crop test result using the radish

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<Plow-in tests>

Five (5) grams of dried powder of stems and leaves of this recombinant maize and the non-recombinant control maize collected at the time of harvesting and 850 g of commercially available soil for raising of seedlings were mixed with each other and the mixed soil was packed into a seedlings-raising vat having 5 holes × 5 holes. One (1) grain of radish seed was sown in each cell in the seedlings-raising vat. Examination was conducted for germination rate 7 days after sowing, and for plant height and dry weight 14 days after sowing. As a result, regarding the germination rate, plant height, fresh weight and dry weight of radish (test plant), no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize (Table 9, p.13).

Table 9 Plow-in test result using the radish

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<Soil microflora tests>

Soil after harvesting was collected from four (4) spots in each plot of cultivation fields of this recombinant maize and the non-recombinant control maize and mixed with each other. Based on the dilution plate technique, the number of bacteria, the number of

actinomycetes, and the number of filamentous fungus were examined. As a result, no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize (Table 10, p.14).

Table 10 Number of soil microorganisms after harvesting in the cultivation of this recombinant maize and the non-recombinant control maize

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Based on the above results, regarding the productivity of harmful substances, it is considered that this recombinant maize could not produce any unexpected harmful substances.

Assessment Result by the Committee for Review on the Biological Diversity Risk Assessment

1 Name of the type of Living Modified Organism: Maize tolerant to aryloxyalkanoate herbicide (modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis.) (DAS40278, OECD UI: DAS-4Ø278-9)

Content of the Type 1 Use of Living Modified Organism: Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them

Applicant: Dow Chemical Japan Ltd.

(1) Item-by-item assessment of Adverse Effect on Biological Diversity

1) Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis), the biological species to which the recipient organism belongs, has been long used in Japan, including for cultivation, etc., though there is no report that it has become self-seeding in Japan.

This recombinant maize is given traits to be tolerant to aryloxyalkanoate herbicide. However, it is considered unlikely that the tolerance to aryloxyalkanoate herbicide enhances the competitiveness of this recombinant maize in the natural environment less expected to suffer spraying of aryloxyalkanoate herbicide.

As a result of isolated field tests conducted in 2009 at the National Institute of Livestock and Grassland Science for various characteristics referring to competitiveness of this recombinant maize, no difference was observed between this recombinant maize and the non-recombinant control maize.

Based on the above understanding, it was judged that the following conclusion made by the applicant is valid: The wild animals and wild plants likely to be affected cannot be specified and thus this recombinant maize would pose no risk of Adverse Effect on Biological Diversity that is attributable to competitiveness.

2) Productivity of harmful substances

Regarding maize (*Zea mays* subsp. *mays* (L.) Iltis), the biological species to which the recipient organism belongs, there is no report that it produces a harmful substance to wild animals and wild plants.

There has been no report that the modified AAD-1 protein produced by this recombinant maize would be any harmful substance, and no homology with any known allergens has been identified.

The modified AAD-1 protein catalyzes the reaction of incorporating the oxygen into the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and transforms it into herbicidally-inactive 2,4-dichlorophenol (2,4-DCP), though it has not been considered that the modified AAD-1 protein could affect any other metabolic systems.

The 2,4-DCP is found less toxic compared to 2,4-D, and it is considered not to have any worse effects than offered by 2,4-D sprayed even at the highest estimate of concentration of 2,4-DCP produced by spraying of 2,4-D. In addition, based on the findings that as a result of examination for residual concentration of 2,4-DCP in grains after spraying of the upper limit of optimum dosage range of 2,4-D to this recombinant maize, the residual concentration is found below the limit of quantitation (0.01 ppm), it is considered that imported seeds of this recombinant maize would not affect any wild animals.

In the isolated field tests conducted in 2009 at the National Institute of Livestock and Grassland Science, this recombinant maize was investigated for productivity of any harmful substances (the substances secreted from the roots which can affect other plants and microorganisms in soil, and the substances existing in the plant body which can affect other plants after dying) based on the plow-in test, succeeding crop test and soil microflora test, and as a result, no significant difference was observed between this recombinant maize and the non-recombinant control maize.

Based on the above understanding, it was judged that the following conclusion made by the applicant is valid: The wild animals and wild plants likely to be affected cannot be specified and thus this recombinant maize would pose no risk of Adverse Effect on Biological Diversity that is attributable to productivity of harmful substances.

3) Crossability

In the Japanese natural environment, there are no wild plants which can cross with maize. Therefore, it was judged that there are no specific wild plants that are possibly affected by this recombinant maize, and that the use of such maize poses no risk of Adverse Effect on Biological Diversity that is attributable to crossability. It was judged that the conclusion above made by applicant is valid.

(2) Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, it was judged that the conclusion made by the Biological Diversity Risk Assessment Report is valid: The use of this recombinant maize in accordance with Type 1 Use Regulation would pose no risk on the Adverse Effect on Biological Diversity in Japan.

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Maize tolerant to aryloxyalkanoate herbicide

(Modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis.)
(DAS40278, OECD UI: DAS-4Ø278-9)

Biological Diversity Risk Assessment Report

Annex List

Dow Chemical Japan Ltd.