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

Name: Dow Chemical Japan Ltd.
Applicant: Michiro Kurita, Representative Director
Address: 2-24 Higashi Shinagawa 2-chome, Shinagawa-ku, Tokyo

Approved Type 1 Use Regulations

Names of Types of Living Modified Organisms	Maize tolerant to aryloxyalkanoate and glyphosate herbicides (modified *cp4 epsps*, modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (NK603× DAS40278, OECD UI: MON-ØØ6Ø3-6× DAS-4Ø278-9)
Content of Type 1 Use of Living Modified Organisms	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of Type 1 Use of Living Modified Organisms	—

Outline of the Biological Diversity Risk Assessment Report

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

1. Information concerning preparation of living modified organisms

Maize tolerant to aryloxyalkanoate and glyphosate herbicides (modified *cp4 epsps*, modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (NK603× DAS40278, OECD UI: MON-ØØ6Ø3-6× DAS-4Ø278-9) (hereinafter referred to as "this stacked maize line") is the progeny line created by interbreeding with the following two modified maize lines using the conventional crossing.

- Maize tolerant to glyphosate herbicide (modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (NK603, OECD UI: MON-ØØ6Ø3-6) (hereinafter referred to as "NK603")
- Maize tolerant to aryloxyalkanoate herbicide (modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (DAS40278, OECD UI：DAS-4Ø278-9) (hereinafter referred to as "DAS40278")

The summary of the information concerning preparation of NK603 and DAS40278 is described below.

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acids and the origins of component elements used for each development of NK603 and DAS40278 is shown in Tables 1 and 2 (p. 3-4).

Table 1. Origins and functions of the component elements of PV-ZMGT32L used for the development of NK603

Component elements	Origins and functions
cp4 epsps gene cassette (1)	
P-ract1	Promoter region of the actin 1 gene derived from rice. It induces the expression of the target genes (McElroy *et al.*, 1990).
ract1 intron	Intron of the rice actin gene. It induces the expression of the target genes by improving splicing efficiency (McElroy *et al.*, 1991).
CTP 2	Nucleotide sequence coding for the N-terminal chloroplast transit peptide of the protein EPSPS in the *epsps* gene of thale cress (Klee *et al.*, 1987). It transports the target proteins from the cytoplasm to chloroplast.
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium* CP4 strain (Barry *et al.*, 1997; Padgette *et al.*, 1996a).
NOS 3'	3' untranslated region of the nopaline synthase (NOS) gene derived from *Agrobacterium tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983).
cp4 epsps gene cassette (2)	
E35S	It has the 35S promoter (Odell *et al.*, 1985) and the double enhancer region (Kay *et al.*, 1987) of the cauliflower mosaic virus (CaMV). It induces the constitutive expression of the target genes in all tissues.
ZmHsp70 Intron	Intron of the heat shock protein gene of maize. The ZmHsp 70 intron is used to increase the level of expression of foreign genes in plants (Rochester *et al.*, 1986).
CTP2	Nucleotide sequence coding for the N-terminal chloroplast transit peptide of the protein EPSPS in the *epsps* gene of thale cress (Klee *et al.*, 1987). It transports the target proteins from the cytoplasm to chloroplast.
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium* CP4 strain (Barry *et al.*, 1997; Padgette *et al.*, 1996a).
NOS 3'	3' untranslated region of the nopaline synthase (NOS) gene derived from *Agrobacterium tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983).

Table 2. Origins and functions of the component elements of pDAS1740 used for the development of DAS40278

Name	Function
Modified *aad-1* cassette	
RB7 MAR	Nuclear matrix attachment region derived from tobacco (Allen *et al.*, 1996). It stabilizes the expression of the modified AAD-1 protein.
ZmUbi1	Ubiquitin promoter derived from maize and contains the exon and intron regions (Christensen *et al.*, 1992). It initiates the transcription of genes in the entire plant body.
Modified *aad-1*	Gene modified from the aryloxyalkanoate dioxygenase gene derived from a gram-negative bacillus, *Sphingobium herbicidovorans*, to have a codon appropriate for expression in plants. It expresses the modified AAD-1 protein. As for the amino-acid sequence, alanine is added in the second position to introduce a cloning site.
ZmPer5 3'UTR	Terminator derived from maize (Dow AgroSciences LLC, 1997). It terminates gene transcription.
RB7 MAR	Nuclear matrix attachment region derived from tobacco (Allen *et al.*, 1996). 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 content remain with Dow Chemical Japan Ltd.)

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

The functions of the component elements of the donor nucleic acids used for developing NK603 and DAS40278 are shown in Tables 1 and 2 (p. 3-4).

The modified *aad-1* cassette transferred to DAS40278 contains a nuclear matrix attachment region, the *RB7 MAR* gene. A nuclear matrix attachment region is frequently found in genomic DNA sequences and thought to have the function of attaching DNA to the nuclear matrix to form the DNA loop structure. It has been reported that when the nuclear matrix attachment region is adjacent to either end of the transferred gene, the level of expression of the transferred gene increases and gene silencing (which inhibits gene expression) decreases (Allen *et al.*, 2000; Halweg *et al.*, 2005).

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

a. Functions of proteins produced by target gene expression

--- Herbicide tolerant proteins ---

【Modified CP4 EPSPS protein】

The modified CP4 EPSPS protein expressed in NK603 exhibits tolerance to the herbicide, glyphosate. Plants treated with glyphosate die because 5-enolpyruvylshikimate 3-phosphate synthase (enzyme number: E.C.2.5.1.19, hereinafter referred to as “EPSPS protein”) is inhibited which inhibits the synthesis of aromatic amino acids essential to protein synthesis. The activity of the modified CP4 EPSPS protein is not inhibited even in the presence of glyphosate, and therefore the recombinant plants expressing this protein can grow by the normal synthesis of shikimate.

Moreover, for the modified *cp4 epsps* gene, the nucleotide sequence of the wild-type *cp4 epsps* gene is modified in to improve the level of expression in plants without changing the functional activity of the wild-type CP4 EPSPS protein. As for the amino acid sequence of the modified CP4 EPSPS protein, only serine at the second position from the N-terminal is substituted by leucine. In addition, the two modified *cp4 epsps* gene cassettes are introduced into NK603 to enhance the tolerance to glyphosate.

【Modified AAD-1 protein】

The modified AAD-1 protein expressed in DAS40278 is an enzyme, which transforms a compound into one without herbicidal activity by catalyzing the reaction of oxygen introduction to aryloxyalkanoate herbicides. For example, the modified AAD-1 protein catalyzes the reaction of oxygen introduction to 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide to transform it into 2,4-dichlorophenol (2,4-DCP) , which has no herbicidal activity, and glyoxylic acid (Dow AgroSciences LLC, 2004).

In addition, the modified *aad-1* gene has codons modified to optimize expression in plants and in its amino acid sequence alanine is added in the second position in order to introduce a cloning site.

b. Homology to known allergen proteins

The amino acid sequences of the modified CP4 EPSPS and modified AAD-1 proteins were examined for sharing the functionally important with the known allergens, using the following databases. The results showed that structurally similar sequences of those proteins were not shared with the known allergens.

AD11: Modified CP4 EPSPS protein
FARRP Allergen Database version 11: Modified AAD-1 protein

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

The EPSPS protein, functionally identical to the modified CP4 EPSPS protein, is an enzyme protein, which catalyzes the shikimate pathway for biosynthesis of aromatic amino acids. However, it is not a rate-determining enzyme in the pathway, and therefore it has been considered that the levels of the aromatic amino acids, the end products of this pathway, do not increase even with the increased activities of the EPSPS proteins (Padgette *et al.*, 1996b; Ridley *et al.*, 2002). In addition, it has been identified that the EPSPS protein specifically reacts with the substrates, phosphoenolpyruvate (hereinafter referred to as "PEP") and shikimate-3-phosphate (hereinafter referred to as "S3P") (Gruys *et al.*, 1992). Other than those substrates, only shikimic acid, an analog of S3P, is known to react with the EPSPS protein. However, the comparison of the reaction of the EPSPS proteins with shikimate and S3P by the specificity constant (k_{cat}/K_m), which represents the degree of occurrence of reaction, showed that the reaction specificity between the EPSPS protein and shikimate is one to two millionth of that between the EPSPS protein and S3P (Gruys *et al.*, 1992), and shikimate is unlikely to react as a substrate of the EPSPS protein. Therefore it is concluded that the modified CP4 EPSPS protein does not change the metabolic system of recipient organisms.

The modified AAD-1 protein is an enzyme to catalyze the reactions when oxygen is specifically introduced to the compounds with the aryloxyalkanoate group, especially R-enantiomers of chiral as well as achiral compounds. Endogenous plant compounds that share structural and physiological similarities to the compounds with an aryloxyalkanoate group were examined for the activities of the modified AAD-1 protein and the impact on the metabolic pathways were discussed. As a substrate, plant hormones (indole-3-acetic acid, abscisic acid, gibberellic acid and aminocyclopropane 1-carboxylic acid) and phenylpropanoid intermediates (trans-cinnamic acid, coumaric acid, and sinapinic acid) were examined. Twenty kinds of L-amino acids were also examined.

For the 20 L-amino acids, no reactions were observed at a concentration of 1 μM modified AAD-1 protein. Among the plant hormones and phenylpropanoid intermediates treated with 1 μM modified AAD-1 protein, abscisic acid, gibberellic acid, trans-cinnamic acid, and coumaric acid showed slight reactions. Treatment with 5 μM modified AAD-1 protein produced a slight reaction with aminocyclopropane 1-carboxylic acid and at 10 μM modified AAD-1 protein, a slight reaction was observed for indole-3-acetic acid. Since no correlation was observed between the concentrations of the modified AAD-1 protein and enzyme activity, oxides were then measured by Fourier transform mass spectrometry (FT/MS). The results showed that oxides of indole-3-acetic acid and trans-cinnamic acid were detected by the treatment with the modified AAD-1 protein at 10 μM. However, reaction rates were very slow and the parameters, *Km* and *Vmax*, of the Michaelis-Menten equation, could not be obtained. Since oxides were detected only when highly sensitive Fourier transform mass spectrometry was performed on the compounds treated with high levels of the modified AAD-1 protein and since reaction rates were extremely slow, the oxidative reactions observed are unlikely to affect the metabolic pathways of plants (Cicchillo *et al.*, 2010).

In addition, since no compounds of the aryloxyalkanoate group have been yet identified in plant bodies, the modified AAD-1 protein is not thought to change any other metabolic pathways in plant bodies.

(2) Information concerning vectors

1) Name and origin

The plasmid vectors used for the development of parent lines are as follows.

NK603: PV-ZMGT32 constructed from the vector pUC119 derived from *E. coli*
DAS40278: pDAS1740 constructed from the plasmid pUC19 derived from *E. coli*

2) Properties

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

The numbers of base pairs in the plasmid vectors used for the development of parent lines are as follows.

NK603: PV-ZMGT32; 9,308 bp
DAS40278: pDAS1740; 8,512 bp, linear DNA used for transfection; 6,236 bp

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

The antibiotic resistant genes used as selective markers are as follows. None of these antibiotic resistant genes have been transferred in the recipient organism.

NK603: *nptII* gene conferring the resistance to kanamycin
DAS40278: ap^r gene conferring the resistance to ampicillin

(c) Presence or absence of infectious characteristics of vector and, if present, the information concerning the host range

The infectivity of PV-ZMGT32 and pDAS1740 has not been known.

(3) Method of preparing living modified organisms

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

The structure diagrams of the entire nucleic acid transferred to NK603 and DAS40278 are shown in Figures 1 and 2 (p. 8).

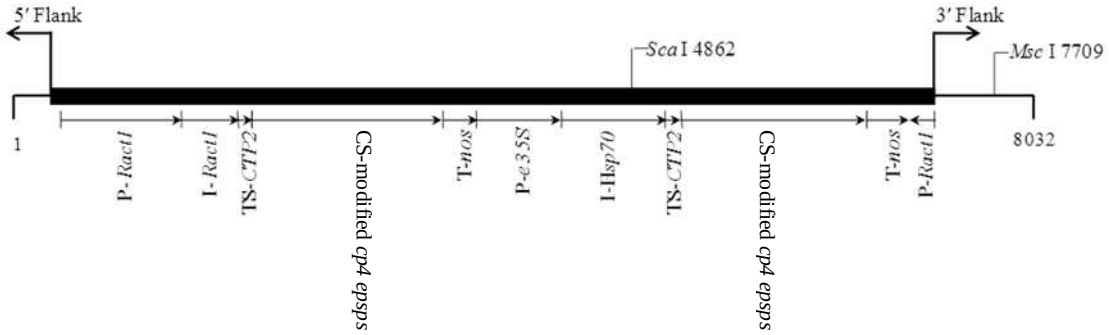

Figure 1. Structure diagram of the entire nucleic acid transferred to NK603

The angled arrows in the structure diagram show the 5'- and 3'-terminal regions of the transferred genes and the subsequent adjacent endogenous sequences of maize. The positions of the component elements and restriction endonuclease cleavage sites are shown as estimated approximate positions.

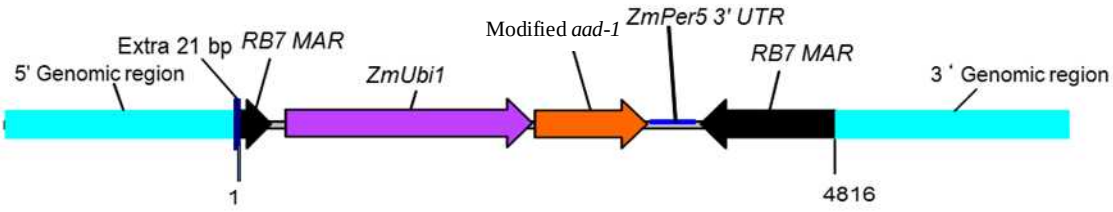

Figure 2. Structure diagram of the entire nucleic acid transferred to DAS40278

(All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

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

The transferring of nucleic acids to the recipient organism was performed using the following method.

NK603: Particle gun method

DAS40278: Whisker method[1]

3) Process of rearing of living modified organisms

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

The transformed cells were selected in the media added with the following substance.

NK603: Glyphosate

DAS40278: Haloxyfop

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

The nucleic acids were transferred to the recipient organisms of NK603 and DAS40278 using the particle gun method and the whisker method, respectively. The agrobacterium method was not used.

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[1]The embryonic suspension was obtained by liquid culture of the callus from immature embryos of Hi-II, the recipient maize. The linear DNA cut from the pDAS1740 with the restriction enzyme, *Fsp I*, and needle-like silicon carbide whisker fibers were added to the embryonic suspension and stirred, and then the silicon carbide whisker fibers created holes in the cells, which resulted in the transfer of the linear DNA to the recipient organism (Thompson *et al*., 1995).

(c) Process 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 Effects on Biological Diversity

NK603 was crossed with commercial cultivars of yellow dent corn and other various cultivars. The evaluation of NK603 for selecting lines was started in 1997, and its morphological and growth characteristics were examined in 103 fields in total from 1997 to 1999. In addition, expression of the modified CP4 EPSPS protein and the transgenes were analyzed and finally an excellent line was selected.

As for DAS40278, production of the modified AAD-1 protein was confirmed by spraying quizalofop, an aryloxyalkanoate herbicide to the regenerated plants (T0 generation). DAS40278 was selected based on comprehensive manner by evaluation of transgene analysis, confirmation of protein expression, the herbicide tolerance and the agronomic characteristics in outdoor field trials conducted in US and Canada.

The status of application and approval of NK603, DAS40278, and this stacked maize line in Japan is described below (Table 3, p. 10).

Table 3. The status of application and approval of NK603, DAS40278, and this stacked maize line in Japan

	Food[1)]	Feed[2)]	Environment[3)]
NK603	March 2001 Confirmed safety	March 2003 Confirmed safety	November 2004 Approved for Type I Use
DAS40278	May 2012 Confirmed safety	September 2012 Confirmed safety	December 2012 Approved for Type I Use
This stacked maize line	December 2012 Applied	January 2013 Notified	September 2012 Applied

[1)] Food Sanitation Act

[2)] Act on Safety Assurance and Quality Improvement of Feeds

[3)] Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms

【Process of rearing of this stacked maize line】

This stacked maize line was developed from NK603 and DAS40278 by crossing (Figure 3, p. 11).

Confidential information: not disclosed to unauthorized persons

Figure 3. Process of rearing this stacked maize line

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

(a) Place where the replication product of transferred nucleic acid exists

It was confirmed that the transferred genes in NK603 and DAS40278 existed on the chromosome.

(b) The number of copies of replication products of transferred nucleic acid and stability of its transmission across multiple generations

As a result of Southern blot analysis on the parent lines, it was confirmed that one copy of respective target genes existed at a site on the chromosomes for NK603 (Deng *et al.*, 1999) and DAS40278 (In-house report 1). In the evaluation of the parent lines, Southern blot analysis on multiple generations also showed that the transferred genes were stably inherited to subsequent generations.

In NK603, Southern blot analysis and the nucleotide sequence analysis of the 3'-terminal revealed that a 217 bp fragment of P-*Ract1* existed in the reverse direction near the 3'-terminal of the transferred gene. As for the 217 bp fragment of P-*Ract1* near the 3'-terminal of the transferred gene in NK603, the strand-specific RT-PCR revealed a transcription product, which was thought to initiate from either the P-*Ract1* or the P-*e35S* of the transferred gene and to read through the NOS 3' terminator. However, since only the modified CP4 EPSPS protein was detected in NK603, it was thought that a stop codon upstream of the terminator was preserved in the read through transcription product (It was concluded that this reading through did not affect the safety evaluation and therefore it was approved for the Type I Use Regulation (provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them) by the Ministry of Agriculture, Forestry and Fisheries and the

Ministry of the Environment of Japan in November 2004, based on the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms). In addition, in the transferred gene of NK603, the bases at positions 456 and 641 from the 5'-terminal of the coding region in the modified *cp4 epsps* gene induced by the P-*e35S* were changed from thymine (T) to cytosine (C), compared to the bases in the plasmid for expression in plants. It was revealed that the change of the base at position 456 was not associated with the change of the amino acid. However, in the modified CP4 EPSPS protein expressed by P-*e35S*, the change of the base at position 641 caused the amino acid change, leucine to proline, at position 214 from the N-terminal in the original CP4 EPSPS protein (this protein is hereinafter referred to as "L214P protein"). Regarding L214P protein, it is concluded that the structures and functions of L214P protein and the modified CP4 EPSPS protein are comparable, because 1) proline at position 214 from the N-terminal was not one of the seven amino acids essential for activity of the EPSPS protein family, 2) this change of the amino acid did not affect the active site and the three-dimensional structure of the EPSPS protein, and 3) enzyme activity and immunoreactivity of the L214P and modified CP4 EPSPS proteins are comparable (Astwood *et al.*, 2001). The comparison of the database, in order to evaluate whether the L214P protein shared functionally important amino acid sequences with known contact allergens, revealed that the L214P protein did not share structurally similar sequences with the known contact allergens. It was concluded that the changes of the bases were observed in multiple generations and stably inherited to subsequent generations.

(c) The position relationship in the case of multiple copies existing in a chromosome

This item is not applicable because there is only one copy each of NK603 and DAS40278.

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

The stability of expression of the parent lines was identified by the evaluation of the parent lines as follows.

NK603: The expression of the modified CP4 EPSPS protein was confirmed in multiple generations by the glyphosate-herbicide spraying test during the growth.

DAS40278: Confirming the expression of proteins by ELISA

(e) 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

The sequences of the nucleic acids transferred to NK603 and DAS40278 do not contain any sequences that allow gene transmission. Therefore, it is unlikely that these genes would be transmitted through virus infection and/or any other routes to wild animals and wild plants.

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

In order to detect and identify NK603, the DNA sequences of the transferred genes and the nearby regions of the plant genomes are used as primers. As a result, NK603 is specifically detectable (Cavato *et al.*, 2001).

In order to detect and identify DAS40278, the PCR using the DNA sequences of the transferred genes and the nearby regions of the plant genomes as primers has been developed (Dow AgroSciences LLC, 2009).

In order to detect and identify this stacked maize line, the above-mentioned methods must be applied to each grain of maize seeds.

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

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

This stacked maize line contains the following characteristics derived from individual parent lines.

NK603: Tolerance to glyphosate due to modified CP4 EPSPS protein derived from the transferred gene
DAS40278: Tolerance to aryloxyalkanoate herbicides due to modified AAD-1 protein derived from the transferred gene

Modified CP4 EPSPS and modified AAD-1 proteins have high substrate specificity, and therefore, are not thought to change the metabolic system of the recipient organism. Each protein substrate is different and each involves an independent metabolic pathway . Therefore, it is difficult to think that these proteins will interact to produce an unexpected metabolite.

Based on the above, it is unlikely that the expressed proteins derived from respective parent lines interact with one another in this stacked maize line.

Therefore, differences in physiological and ecological properties between this stacked maize line and a maize line, the species of the taxonomy to which the recipient organism belongs were evaluated, based on the results of the individual examination of the parent lines, NK603 and DAS40278.

(b) 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

The Biological Diversity Risk Assessment Report of respective parent lines was completed and confirmed that the following physiological or ecological properties were not different between the respective parent lines and their controls, non-recombinant maize. As for the information on the physiological or ecological properties, see the website of the Japan Biosafety Clearing House[2].

a. Morphological and growth characteristics
b. Cold-resistance and heat-resistance at the early stage of growth
c. Wintering ability and summer survival of the mature plant
d. Fertility and size of the pollen
e. Production, shedding habit, dormancy, and germination rate of the seed
f. Crossability
g. Productivity of harmful substances

[2] NK603
https://ch.biodic.go.jp/bch/OpenDocDownload.do?info_id=88&ref_no=1

DAS40278
https://ch.biodic.go.jp/bch/OpenDocDownload.do?info_id=1584&ref_no=1

II. Review by persons with specialized knowledge and experience concerning Adverse Effects on Biological Diversity

A review was made by persons with specialized knowledge and experience concerning Adverse Effects on Biological Diversity (called Experts) for possible Adverse Effects on Biological Diversity caused by the use in accordance with the Type 1 Use Regulation for Living Modified Organism based on the Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms. Results of the review are listed below.

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

Maize resistant to aryloxyalkanoate and glyphosate (hereinafter referred to as “this stacked line”) was developed with the following lines by crossing.

(a) Maize tolerant to glyphosate, to which the modified *cp4 epsps* gene coding the modified CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) was transferred (NK603)
(b) Maize tolerant to aryloxyalkanoate herbicide, to which the modified *aad-1* gene coding the modified AAD-1 protein (aryloxyalkanoate dioxygenase) was transferred (DAS40278)

As for the modified CP4 EPSPS and modified AAD-1 proteins, which are both herbicide tolerant proteins derived from the genes transferred to this stacked line, their substrates and actions are different and their involved metabolic pathways are independent. In addition, both of the modified CP4 EPSPS and modified AAD-1 proteins are highly substrate-specific. Therefore, it was concluded that these proteins did not interact to change the metabolic system of the recipient organism to produce an unexpected metabolite in this stacked line.

Based on the above, it was unlikely that these proteins derived from respective parent lines functionally interact with one another in the plant body of this stacked maize line. Therefore, it was concluded that there were no trait changes to be evaluated, except having the traits which the parent line had.

The examination of the respective evaluation items has already been completed*. Based on the results of the examination, the conclusion described in the Biological Diversity Risk Assessment Report that the use of the respective parent lines in accordance with the Type I Use Regulation causes no Adverse Effects on Biological Diversity in Japan has been judged to be reasonable.

a. Competitiveness

b. Productivity of harmful substances

c. Crossability

* The results of the evaluation of the respective parent lines are available as described below

- NK603

https://ch.biodic.go.jp/bch/OpenDocDownload.do?info_id=88&ref_no=2

- DAS40278

https://ch.biodic.go.jp/bch/OpenDocDownload.do?info_id=1584&ref_no=2

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

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this stacked maize line, in accordance with the Type 1 Use Regulation, causes Adverse Effects on Biological Diversity in Japan. It was judged that the conclusion above made by the applicant is reasonable.

References

Allen, G.C., Hall, Jr. G., Michalowski, S., Newman, W., Spiker, S., Weissinger, A.K., Thompson, W.F. (1996) High-level transgene expression in plant cells：Effects of a strong scaffold attachment region from tobacco. *The Plant Cell* **8**, 899-913.

Allen, G.C., Spiker, S., Thompson, W.F. (2000) Use of matrix attachment regions (MARs) to minimize transgene silencing. *Plant Mol. Biol.* **43**, 361-376.

Astwood, J. D., George, C., Alibhai, M., McCoy, R., Lahman, L., Hammond, B. G., Leach J. N., Silvanovich, A. (2001) Safety Assessment of Roundup Ready Corn Event NK603 Containing Genes Encoding CP4 EPSPS and CP4 EPSPS L241P. Monsanto Technical Report MSL 17600. (In-house report)

Barry, G.F., Kishore, G.M., Padgette, S.R., Stallings, W.C. (1997) Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. Patent 5,633,435, U.S. Patent Office, Washington, D.C.

Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B., Schaller, H. (1982) Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn*5*. *Gene* **19**, 327-336.

Bevan, M., Barnes, W.M., Chilton, M-D. (1983) Structure and transcription of the nopaline synthase gene region of T- DNA. *Nucleic Acids Research* **11**, 369-385.

Cavato, T. A., Deng, M. Y., Lirette, R. P. (2001) Confirmation of the Genomic DNA Sequences Flanking the 5’ and 3’ Ends of the Insert in Roundup Ready Corn Event NK603. Monsanto Technical Report MSL 17617 (In-house report)

CFIA (1994) Biology Document BIO1994-11: The Biology of *Zea mays* (L.) (Maize).

Christensen, A.H., Sharrock, R.A., Quail, P.H. (1992) Maize polyubiquitin genes：Structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. *Plant Mol. Biol.* **18**, 675-689.

Cicchillo R., Godbey J., Wright T. (2010) Substrate Specificity of Aryloxyalkanoate Dioxygenase-1 (AAD-1). Dow AgroSciences LLC (In-house report)

Deng, M. Y., Lirette, R. P., Cavato T. A., Sidhu, R. S. (1999) Molecular Characterization of Roundup Ready (CP4 EPSPS) Corn Line NK603. Monsanto Technical Report MSL 16214. (In-house report)

Dickson, R.C., Abelson, J., Barnes, W.M., Reznikoff, W.S. (1975) Genetic regulation: The lac control region. *Science* **187**, 27-35.

Doebley, J.F. (1984) Maize introgression into teosinte - a reappraisal. *Ann.Missouri Bot.Gard*. **71**, 1100-1113.

Dow AgroSciences LLC (1997) Regulatory Sequences from Transgenic Plants. U.S. Patent Application Number 60/049,752.

Dow AgroSciences LLC (2004) Novel Herbicide Resistance Genes. U.S. Patent Application Number 60/567,052.

Dow AgroSciences LLC (2009) Protocol for event-specific quantitation of DAS-40278-9 maize & Report on the development and validation of an event-specific real-time PCR system for the quantitative detection of DAS-40278-9 maize. (In-house report)

FAOSTAT (2012) http://faostat.fao.org (See February 22, 2012)

Farabaugh, P.J. (1978) Sequence of the *lacI* gene. *Nature* **274**, 765-769.

Fraley, R.T., Rogers, S.G., Horsch, R.B., Sanders, P.R., Flick, J.S., Adams, S.P., Bittner, M.L., Brand, L.A., Fink, C.L., Fry, J.S., Galluppi, G.R., Goldberg, S.B., Hoffman, N.L., Woo, S.C. (1983) Expression of bacterial genes in plant cells. *Proc. Natl. Acad. Sci. USA* **80**, 4803-4807.

Gruys, K.J., Walker, M.C., Sikorski, J.A. (1992) Substrate synergism and the steady-state kinetic reaction mechanism for EPSP synthase from *Escherichia coli*. *Biochemistry* **31**, 5534-5544.

Halweg, C., Thompson, W.F., Spiker, S. (2005) The Rb7 matrix attachment region increases the likelihood and magnitude of transgene expression in tobacco cells：A flow cytometric study. *The Plant Cell* **17**: 418-429.

Kay, R., Chan, A., Daly, M., McPherson, J. (1987) Duplication of CaMV 35*S* promoter sequences creates a strong enhancer for plant genes. *Science* **236**, 1299-1302.

Klee, H.J., Muskopf, Y.M., Gasser, C.S. (1987) Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: Sequence analysis and manipulation to obtain glyphosate-tolerant plants. *Molecular and General Genetics* **210**, 437-442.

Mazodier, P., Cossart, P., Giraud, E., Gasser, F. (1985) Completion of the nucleotide sequence of the central region of Tn*5* confirms the presence of three resistance genes. *Nucl. Acids. Res.* **13**, 195-205.

McElroy, D., Zhang, W., Cao, J., Wu, R. (1990) Isolation of an efficient actin promoter for use in rice transformation. *The Plant Cell* **2**, 163-171.

McElroy, D., Blowers, A.D., Jenes, B., Wu, R. (1991) Construction of expression vectors based on the rice actin 1 (*Act1*) 5' region for use in monocot transformation. *Molecular and General Genetics* **231**, 150-160.

Odell, J.T., Nagy, F., Chua, N-H. (1985) Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature* **313**, 810-812.

OECD (2003) Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 27: Consensus Document on the Biology of Zea Mays subsp. Mays (Maize).

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T. (1996a) New weed control opportunities: Development of soybeans with a Roundup Ready™ gene. Pages 53-84 in Herbicide-Resistant Crops: Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. S.O. Duke (ed.). CRC Press, Boca Raton, Florida.

Padgette, S.R., Taylor N.B., Nida D.L., Bailey M.R., MacDonald J., Holden L.R., Fuchs R.L. (1996b) The Composition of Glyphosate-Tolerant Soybean Seeds is Equivalent to That of Conventional Soybeans. *Journal of Nutrition* **126**, 702-716.

Pleasants, J. M., Hellmich, R. L., Dively, G. P., Sears, M. K., Stanley-Horn, D. E., Mattila, H. R., Foster, J. E., Clark, P., Jones, G. D. (2001) Corn pollen deposition on milkweeds in and near cornfields. *Proc. Natl. Acad. Sci. USA* **98**, 11919-11924.

Ridley, W.P., Sidhu R.S., Pyla P.D., Nemeth M.A., Breeze M.L., Astwood, J.D. (2002) Comparison of the Nutritional Profile of Glyphosate-Tolerant Corn Event NK603 with That of Conventional Corn (*Zea mays* L.). *Journal of Agricultural and Food Chemistry* **50**, 7235-7243.

Rochester, D.E., Winer, J.A., Shah, D.M. (1986) The structure and expression of maize genes encoding the major heat shock protein, *hsp70*. *The EMBO Journal* **5**, 451-458.

Shuman, H.A., Silhavy, T.J. (2003) The art and design of genetic screens: *Escherichia coli*. *Nature Reviews Genetics* **4**, 419-431.

Sutcliffe, J.G. (1979) Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Pages 77-90 in Cold Spring Harbor Symposia on Quantitative Biology,

Cold Spring Harbor, New York.

Thompson, J.A., Drayton, P.R., Frame, B.R., Wang, K., Dunwell, J.M. (1995) Maize transformation utilizing silicon carbide whiskers：A review. *Euphytica* **85**, 75-80.

Shigeyuki Sendo. (2001). Maize - Cultivar and ecology of maize. IV Collection. Compendium of crop rotation Volume 3. Miscellaneous grain. Rural Culture Association Japan (ed), Rural Culture Association Japan

Hideo Tozawa (2005). “Maize - History and culture, characteristics and cultivation, and processing and utilization”. Rural Culture Association Japan

In-house report 1: DAS40278, Number of the transferred genes and their stability between generations and in one generation (In-house report)