Extended Determination[1] of Nonregulated Status for Okanagan Specialty Fruits Non-Browning Arctic Apple (16-004-01p)

In response to a request from Okanagan Specialty Fruits Inc. (hereinafter referred to as OSF) to extend a determination of nonregulated status to OSF non-browning Arctic® apple event NF872 (hereinafter referred to as NF872 apple) with non-browning phenotype via suppression of four genes for polyphenol oxidase (petition number 16-004-01p), the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has determined, based on similarity to its antecedent organisms, that NF872 apples and progeny derived from them are unlikely to pose plant pest risks and are no longer to be considered regulated articles under APHIS' Biotechnology Regulations (Title 7 of Code of Federal Regulations (CFR), part 340). This extension request is based upon APHIS' determination of nonregulated status of its antecedent organisms: OSF apple events GD743 Golden Delicious and GS784 Granny Smith apples (hereinafter referred to as OSF antecedent apple events), with non-browning phenotype. OSF antecedent apple events from petition number 10-161-01p were deregulated on February 18, 2015. APHIS-approved permits or acknowledged notifications that were previously required for environmental release, interstate movement, or importation under those regulations will no longer be required for NF872 apples and their progeny. Importation of NF872 seeds, other propagative material, and bulk or table stock, will still be subject to APHIS foreign quarantine notices at 7 CFR part 319 and the Federal Seed Act regulations at 7 CFR parts 201 and 361.

The same genetic construct GEN-03, used to transform the OSF antecedent apple events (GD743 and GS784) with non-browning characteristics was also used to transform and generate the NF872 apple event. APHIS evaluated the plant pest risk of NF872 apples by assessing its similarity to the deregulated OSF antecedent apple events.

APHIS previously conducted a Plant Pest Risk Assessment on the antecedent organisms and found them unlikely to pose risks as plant pests. Based on a plant pest similarity assessment (see the Similarity Table) of NF872 apple to the antecedents, APHIS concludes that NF872 apples are unlikely to pose a plant pest risk and should no longer be regulated under 7 CFR part 340. From the similarity assessment, APHIS concludes the following with respect to NF872 apples and their progeny:

(1) No plant pest risk was identified from the transformation process, the insertion and/or expression of new genetic material, or from changes in metabolism in NF872 apples.

(2) Disease and pest incidence and/or damage are not expected to be increased or atypical for NF872 apples. No plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.

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[1] This extended determination is not effective until officially signed and published.

(3) Based on an evaluation of the gene products, and their similarity to the antecedents, NF872 apples are unlikely to adversely impact non target organisms beneficial to agriculture.

(4) NF872 apples are no more likely to become weedier or more difficult to control as a weed than the antecedents, which are not weedy.

(5) NF872 apples are not likely to increase the weed risk potential of other species with which they can interbreed in the U.S. or its territories. Gene flow, hybridization and/or introgression of inserted genes from NF872 apples to other sexually compatible relatives with which it can interbreed is unlikely to occur.

(6) Significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, etc.) from adoption of NF872 apples are not expected.

(7) Horizontal gene transfer of the new genetic material inserted into the GE plant to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

In addition to our finding that NF872 apples are unlikely to pose a plant pest risk, APHIS prepared and reached a Finding of No Significant Impact (FONSI) for this action based on an Environmental Assessment completed for the antecedents GD743 and GS784 in 2014. NF872 apples will have no significant impacts, individually or collectively, on the quality of the human environment and will have no effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitats (http://www.aphis.usda.gov/biotechnology/not_reg.html).

Based on my review and consideration of all of the scientific and environmental data, analyses, information, and previous conclusions regarding the plant pest risk assessment for the antecedent organisms, the plant pest risk similarity assessment, and FONSI, and my knowledge and experience as APHIS' Deputy Administrator for APHIS Biotechnology Regulatory Services, I have determined and decided that this determination of nonregulated status of NF872 apples is the most scientifically sound and appropriate regulatory decision.

M J. Firko

23 September 2016

Michael J. Firko, Ph.D.
APHIS Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date

APPENDIX A

Okanagan Specialty Fruit Inc. Request (16-004-01p) for Extension of Determination of Nonregulated Status of Non-Browning Arctic Apple NF872.

OECD Unique Identifier: OKA-NB003-1

Plant Pest Risk Similarity Assessment

February 2016

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A. Introduction

The Animal and Plant Health Inspection Service (APHIS) of the United States Department Agriculture (USDA) has received an extension request (petition number 16-004-01p) from Okanagan Specialty Fruits Inc. (hereafter referred to as OSF). In accordance with §340.6(e)(2), OSF requested that APHIS extend the nonregulated status of OSF antecedent apple events GD743 and GS784, with non-browning characteristics to a genetically engineered (GE) NF872 apple event (non-browning) and any progeny derived from crosses of the NF872 event either with conventional apple varieties or with deregulated/nonregulated GE apple varieties according to the regulations at 7 CFR part 340. The USDA announced its determination of nonregulated status for two non-browning apple events GD743, Golden Delicious variety currently marketed as Arctic Golden; and GS784 variety Granny Smith currently marketed as Arctic Granny (petition number 10-161-01p), on February 18, 2015.

APHIS regulations in 7 CFR part 340 regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the regulatory requirements of part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest. The NF872 event was produced by the *Agrobacterium tumefaciens* mediated transformation of apple leaf tissue (OSF, 2016), and some of the introduced regulatory sequences come from plant pest organisms listed in 7 CFR 340.2 (OSF, 2016). Therefore, the NF872 event is considered a regulated article under APHIS regulations at 7 CFR part 340.

Potential impacts in this Plant Pest Risk Similarity Assessment are those that pertain to plant pest risk associated with the NF872 apple and its progeny and their use in the absence of confinement relative to the antecedent apple events. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if the NF872 event is any more likely than the OSF antecedent apple events to pose a plant pest risk. APHIS specifies in 7 CFR 340.6(e) that an extension request for nonregulated status shall include information to establish the similarity of the antecedent organism to the regulated article in question.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology'(51 FR 23302, 1986; 57 FR 22984, 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection

Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

B. Development of the NF872 Apple.

Okanagan Specialty Fruits has developed Arctic™ Fuji apple event NF872 by genetically engineering the commercially grown apple cultivar Fuji, to exhibit a non-browning phenotype. Browning of apple flesh due to damage, cutting or bruising, is caused by an enzymatic reaction catalyzed by polyphenol oxidase (PPO). Phenolic substrates for this reaction and PPO are separately compartmentalized in the cell, with PPO in plastids and phenolic substrates in the vacuole. Loss of compartmentalization occurs when cells are damaged; if little to no PPO is present in the cells, cell disruption does not lead to browning. These non-browning apples are intended to benefit the fresh cut and dehydrated apple markets by reducing browning associated with bruising and cutting, eliminating the need for chemical treatments to reduce browning of fresh cut apple slices (OSF, 2012). Browning also impacts the market for fresh fruit and juice. If the presence of PPO in cells is reduced or eliminated cell disruption does not lead to browning (OSF, 2012).

Cultivated apple is the most important temperate fruit crop in the world, and most commercially grown apple cultivars are diploid (2n=34) and some are triploid (3x=51). Apple has a long reproductive cycle, and there is a long period from hybridization to commercial establishment of a new cultivar, therefore, multiple-trait improvement would be difficult to achieve through conventional breeding. (OSF, 2012).

Event NF872 was obtained using the same binary plasmid as events GD743 and GS784, pGEN-03 carrying the PPO suppression transgene and NPTII selection marker flanked by *A. tumefaciens* T-DNA borders. The transgene (pGAS) is designed to simultaneously reduce expression of four polyphenol oxidase genes PPO2, GPO3, APO5 and pSR7 to induce a non-browning phenotype in apple fruit. Apple was the donor organism for the transgene. (OSF, 2016).

APHIS BRS completed a plant pest risk assessment (PPRA) and an environmental assessment (EA) for the OSF antecedent apple events GD743 and GS784 (USDA APHIS, 2014):
(http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml).
The EA fully addressed all resource areas of potential concern. For the antecedent petition, 10-161-01p, APHIS concluded on the basis of the EA that the impacts would not be significant. The agency issued a Finding of No Significant Impacts (FONSI) and made determinations of nonregulated status for each event (USDA APHIS, 2014).

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform APHIS of the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products,

APHIS assessed data and information presented in the extension request related to the similarity of the NF782 event to the OSF antecedent apple events (GD743 and GS784) and the transformation process; the source of the inserted genetic material, its function in both the donor organism and the GE crop event, and the integrity and number of loci inserted. The stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction have been determined for the antecedent events.

APHIS also assessed data presented in the extension request on whether the genetic modification results in expression of new genes, proteins, or enzymes, or changes in plant metabolism or composition in the NF872 event. The assessment encompasses a consideration of the expression of the PPO suppression transgene and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, anti-nutrients, or nutrient in apples derived from the NF872 event compared to OSF antecedent apple events GD743 and GS784, the cultivar from which it was derived, NF, as well as published nutritional data for apple (NDB09003) provided by the USDA National Nutrient Database for Standard Reference – Release 22 (2009) as referenced in OSF (2010).

Description of the genetic modification and inheritance of inserted DNA

Transformation of the NF872 event (*Malus domestica,* cv Fuji*)* was accomplished through *Agrobacterium tumefaciens* mediated transformation of apple leaf tissue using the binary transformation vector GEN-03 as described in the extension request (OSF, 2016). This same vector was used in the transformation of the antecedent apple events (GD743 and GS784), and contains 6,287 bps of DNA (T-DNA) consisting of the PGAS construct and the *nptII* selection marker gene flanked by *Agrobacterium tumefaciens* left and right T-DNA borders.

The construct PGAS is a chimeric PPO suppression transgene comprising fragments of four genes derived from apple (*Malus domestica*) in the sense orientation that belong to the apple PPO gene family. The genes code for four PPO proteins: PPO2, GPO3, APO5 and pSR7. Transcription of pGAS is directed by the duplicated enhancer CaMV 35S promoter from *Cauliflower Mosaic Virus* with the untranslated leader sequence from *Alfalfa mosaic virus* RNA4 (P70). A 3'untranslated region from the nopaline synthase gene (T_{NOS}) involved in transcription termination and polyadenylation, is used to terminate transcription of the transgene. The neomycin phosphotransferase type II gene (*nptII*) from *Escherichia coli* transposon Tn5 was used as a selectable marker for resistance to kanamycin. The *nptII* gene is under the control of the nopaline synthase promoter (P_{NOS}) and the terminator (T_{NOS}), both derived from *A. tumefaciens*.

Low coverage (20X) Illumina sequencing was used for whole genome sequencing of genomic DNA from NF872 and the parental NF control, combined with bioinformatics analysis for mapping the T-DNA insertions sites to generate insertion maps submitted to APHIS as part of the safety assessment.

Six T-DNA/apple junctions were identified indicating that there are three complex T-DNA insertions with multiple GEN-03 fragments in three chromosomes, CHR3, CHR13

and CHR17. The insertion in CHR3 includes approximately 1,400 bp of vector backbone sequence with no functional sequences. The other insertions in the other two chromosomes do not include vector backbone sequences. The insertion in CHR17 contains a partial CaMV 35S promoter, however, most of the functional elements of the promoter have been deleted. There is no potential for this partial CaMV 35S insertion to drive expression of unknown endogenous sequences and therefore it is not a safety issue (OSF, 2016).

The structure of the transgenes in the insertions in chromosomes CH3 and CHR 13 is such that there is no potential for expression of unknown endogenous sequences by orphaned promoter sequences. The CHR13 insertion does not include any binary vector backbone sequence.

Expression of inserted DNA and changes in gene expression, new proteins or metabolism

NF872 has been genetically engineered with a PPO suppression construct designed to reduce the expression of four apple genes coding for PPO proteins: PPO2, GPO3, APO5, and pSR7 (OSF, 2016). Therefore, the gene product is a chimeric, sense-silencing RNA rather than a functional protein or new enzyme. This is the same construct used in the antecedent apple events GD743 AND GS784 (OSF, 2012).

The structure of the transgene in the insertions in chromosomes CH3 and CHR13 is such that there is no potential for expression of unknown endogenous sequences by orphaned promoter sequences. The insertion in CHR17 contains a partial CaMV 35S promoter, but there is no potential for this partial CaMV35 S insertion to drive expression of unknown endogenous sequences (OSF, 2016).

The *nptII* gene was used for expression of nopaline synthase as a marker in the transformed apple. The FDA has previously identified the safe use of the *nptll* gene for the development of genetically modified cotton, oilseed rape and tomatoes for food and feed purposes (21 CFR 173.170 and 21 CFR 573.130, respectively) (OSF, 2016).

Polyphenol oxidase (PPO) studies were conducted to determine the level of enzymatic activity in NF872 relative to the NF parental control. Samples of tissue culture leaves, mature leaves of field-grown plants, and skin layers of immature and mature fruit were used for these studies (OSF, 2016). For all tissues tested there was reduced PPO activity in NF872 compared to the NF control. In tissue culture leaves, the PPO activity was reduced by 76% (Table 4, OSF, 2016), but no statistical analysis was performed. However, in tests of PPO Specific Activity in leaves, immature fruit and mature fruit of NF872, there was a statistically significant reduction in PPO activity (76% reduction in leaves, 96% in immature fruit and 98% in mature fruit) compared to the NF control (Tables 5-7, OSF 2016).

Controlled experiments using an impact device were used to further verify reduction of PPO activity and enzymatic browning in response to mechanical bruising of mature fruit of NF872 relative to the NF control (OSF, 2016). The level of browning was reported

qualitatively by visual inspection and quantitatively with a Chroma Meter and reported as "Total Change in Color" following bruising of mature fruit using an impact device. A reduced browning response was observed on NF872 apples relative to those from the untransformed parent cultivar NF, further demonstrating success of the genetic transformation and resulting non browning phenotype (Table 8, OSF, 2016).

Compositional and nutritional evaluations were performed to determine whether NF872 apples are equivalent to apples of the parental cultivar NF, and to the published nutritional data for raw apple with skin (NDB09003 reference standard) provided by the USDA (2009). The NDB09003 reference standard is based on analytical data for 'Red Delicious', 'Golden Delicious', 'Gala', 'Granny Smith', and 'Fuji' raw apples with the skin. Apples from event NF872 and the control NF were subjected to nutritional and proximate analysis and measured for total phenolic content.

The composition of NF872 and of the NF control falls within the range of, or closely approximates the published data provided by the USDA (Table 27, OSF, 2016).
In terms of proximates (fat, protein, moisture, ash, carbohydrates, calories and sugar profile) and dietary fiber, NF872 apples are nutritionally equivalent to apple of the control NF (Table 28, OSF, 2016). Although NF872 apples were found to have significantly higher levels of potassium and vitamin C that NF apples. The potassium levels in both approximate the USDA norms. According to the Extension Request the higher levels of vitamin C in NF872 indicate a delay between processing the apples and measuring during testing (Table 28, OSF, 2016). NF872 apples appear to have higher levels of phenolics than NF, but the difference was not statistically significant. (Table 28, OSF, 2016).

APHIS reviewed the information provided by OSF in the extension request and determined the following:

- There are three complex T-DNA insertions with multiple GEN-03 fragments in three chromosomes, CHR3, CHR13 and CHR17 in NF872. (OSF, 2016).
- The insertion in chromosome CHR3 includes approximately 1,400 bp of vector backbone with no functional sequences. No backbone sequences were reported in the antecedent events.
- There is no potential for expression of the transgene in the insertions in chromosomes CH3 and CHR13. The insertion in CHR17 contains a partial CaMV 35S promoter with no potential to drive expression of unknown endogenous sequences.
- A statistically significant reduction in PPO activity was observed in NF872 over the control NF, in leaves and fruit, similarly to the antecedent events.
- The composition of NF872 and of the NF control falls within the range of, or closely approximates the published data provided by the USDA (OSF, 2016).
- In terms of proximates (fat, protein, moisture, ash, carbohydrates, calories and sugar profile) and dietary fiber, NF872 apples are nutritionally equivalent to apple of the control NF (OSF, 2016).

- No other changes in gene expression, metabolism or additional proteins between the NF872 event and OSF antecedent apple events GD743 and GS784, were observed.

D. Potential Plant Pest and Disease Impacts

APHIS assessed data and information presented in the extension request related to the similarity of the NF872 event to OSF antecedent apple events GD743 and GS784 to determine whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in the NF872 event that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses. APHIS also assessed whether NF872 event is more likely to have significantly increased disease and pest susceptibility as compared to the antecedent OSF apple events. Impacts or changes in similarity to the two antecedents were assessed to determine if they would (1) affect and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States; and supports trade and exports of U.S. agricultural products. PPQ responds to new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest, and there are a variety of insect, plant disease, mollusk, nematode or weed programs in PPQ (USDA APHIS, 2016). However, none specifically target pests of the NF872 event.

Because the NF872 event was obtained using the same transformation vector pGEN-03 used for transformation of the antecedent de-regulated events GD743 and GS784 and no significant changes in composition were detected from the expression of the pGEN-03 vector carrying the PPO suppression transgene, no significant changes in composition are expected from the expression of pGEN-03 in the NF872.

Similarly, the NF872 event is not expected to differ from the antecedents in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Non-target Organisms Beneficial to Agriculture

APHIS has previously evaluated the potential impacts on non-target organisms beneficial to agriculture that could result from the deregulation of OSF antecedent apple events. The OSF antecedent apple events were determined by APHIS to be unlikely to have an

adverse effect on non-target organisms in the environment (USDA APHIS, 2014). The genetic construct pGEN-03 used to transform the deregulated varieties contains the transgene (PGAS) designed to simultaneously reduce expression of four polyphenol oxidase genes for PPO2, GPO3, APO5 and pSR7 to induce a non-browning phenotype in apple fruit. Apple was the donor organism for the transgene.

Therefore, based on the high similarity of the NF872 event to the OSF antecedent apple events, the compositional similarity of the NF872 event to its parent variety, the unlikely impacts of non-target effects due to RNAi, and on the finding that the OSF antecedent apple events were unlikely to harm non-target organisms, APHIS concludes that it is unlikely that NF872 event will have an adverse effect on non-target organisms, including those beneficial to agriculture.

F. Potential for Enhanced Weediness of NF872 Apple

As documented in the PPRA prepared for the OSF antecedent apple events GD743 and GS784, they are no more likely to be a weed when compared to a conventional apple. Cultivated apple is not regarded as a weedy species although seedlings can be persistent and the species has escaped cultivation and naturalized in the U.S. (CFIA, 2014). The apple is a highly domesticated fruit tree species and cultivated varieties in the U.S are not listed as weeds or as Federal noxious weeds (Muenscher, 1980; 7 CFR part 360; USDA NRCS, 2016). Apple possesses few of the characteristics of those plants that are notably successful as weeds (Baker, 1974).

In addition to considerations of the known biology of apple, APHIS analyzed information submitted in the petition for the antecedent organisms, on a suite of agronomic characteristics and plant-disease and plant-insect interactions. This agronomic data from the field showed that the antecedents were not different from their non-transgenic comparator. The assessments concluded that the antecedents GD743 and GS784 were unlikely to become weeds. Based on the high similarity of the NF872 event to the OSF antecedent apple events modified with the same construct and expressing similar proteins, and on the finding that the antecedent organisms were unlikely to become weeds, APHIS concludes that it is unlikely that NF872 event will become a weed.

G. Potential Impacts on the Weediness of Any Other Plants with which the NF872 Apple Can Interbreed

APHIS evaluated the potential for gene introgression to occur from the NF872 antecedent apple events to sexually compatible wild relatives and considered whether such introgression would result in increased weediness.

The number of species in the genus Malus has been reported to include 25 species (Little, 1979) or 36 species and 44 accepted taxa (USDA-NRCS, 2016), including the cultivated and crab apples. The only species native to the Unites States are crab apples: *Malus angustifolia* (southern crab apple), *M. coronaria* (sweet crab apple), *M. diversifolia* (*syn= M. fusca*) (Oregon crab apple), *M. ioensis* (prairie crab apple) and *M. glabrata* (Biltmore crab

apple) (Little, 1979). Many species and hybrids have been introduced and used as ornamentals, and some have escaped and become naturalized. The following are present in the United States: *M. baccata*, *M. floribunda*, *M. halliana*, *M. hupehensis*, *M. prunifolia*, *M. pumila*, *M. sargentii*, M. *spectabilis*, *M. sylvestris*, *M. toringo*, *M. zumi*, *Malus* x *arnoldiana* [*baccata* x *floribunda*], *Malus* x *dawsoniana* [*fusca* x *pumila*], *Malus* x *platycarpa* [*coronaria* x *pumila*], *Malus x purpurea* [*atrosanguinea* x *niedzwetzkyana*], *Malus* x *robusta*, *Malus* x *soulardii* [*ioensis* x *pumila*], *M. zumi, M.* x *magdeburgensis*, *M. mandshurica*, (USDA NRCS, 2016; CFIA, 2014). Although *Malus* x *zumi* and *Malus floribunda* have been described to be weedy under trees where starlings are known to roost in Ohio (Vincent and Cusick, 1998), none of the species and hybrids above is listed as a noxious weed on Federal or State weed lists (7 CFR part 360; USDA NRCS, 2016).

There is the potential for gene flow, hybridization and/or introgression of the introduced genetic material from cultivated apples to native *Malus* species with synchronous flowering in North America. Studies in Canada and in Europe have suggested that although hybridization potentially occurs, the gene pools between wild and feral cultivated apples remain distinct (CFIA, 2014). Although hybridization between cultivated apples and crab apples species can potentially occur when they are grown together, APHIS concluded that even if such introgression were to occur, this species is not considered a weed.

APHIS concluded that the gene silencing cassette originating from the NF872 antecedent apple events were unlikely to impact the weediness of wild *Malus* species, since the NF872 antecedent apple events do not exhibit characteristics that cause them to be any weedier than other cultivated apples. Therefore, the NF872 event is not expected to increase the weed risk potential of other species with which they can interbreed in the U.S. and its territories based on their similarity to the OSF antecedent apple events.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether significant changes to agricultural or cultivation practices from the NF872 antecedent apple events is likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

APHIS did not identify any significant changes to agricultural or cultivation practices (e.g. pesticide applications, tillage, irrigation, harvesting, rotations, management of volunteers, etc.) from the NF872 antecedent apple events and concluded that no impact on plant diseases or pests or their management is likely to occur. Based on the similarity of the NF872 event to the OSF antecedent apple events, APHIS concludes that it is unlikely that any significant changes to agriculture or cultivation practices would be associated with the NF872 event and therefore no impact on plant diseases or pests of their management is likely to occur.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which the NF872 Apple Cannot Interbreed

APHIS has previously examined the potential for the genetic material inserted into OSF antecedent apple events (GD743 and GS784) to be horizontally transferred without sexual reproduction to other organisms and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. The horizontal gene transfer (HGT) between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al., 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese, 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. APHIS has previously reviewed the potential for horizontal gene transfer from GE apple to bacteria, fungi, invertebrates, viruses, and parasitic plants (USDA APHIS, 2014)

APHIS previously concluded that HGT of the inserted genetic material from the OSF antecedent apple events to other organisms is highly unlikely, and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants. Therefore, APHIS concludes based on the similarity of these events that HGT from the NF872 event to other organisms is also highly unlikely.

J. Conclusion

APHIS has reviewed the information submitted in the extension request, supporting documents, and other relevant information to assess the similarity of plant pest risk of the NF872 event compared to the OSF antecedent apple events. APHIS concludes that the NF872event is **no more likely** to pose a plant pest risk than the previously deregulated OSF antecedent apple events, GD748 and GS784.

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L. Similarity Table

Description		Extension Request NF872 Petition 16-004-01p	Antecedent GS784 and GD743 Petition 10-161-01p	Comments
Organism		**Apple**	**Apple**	
Phenotype		Reduced Browning	Reduced Browning	Same phenotype
Genotype		**Construct pGEN-03**	**Construct pGEN-03**	
	P*NOS*	Nopaline synthase promoter sequence from Agrobacterium tumefaciens	Nopaline synthase promoter sequence from Agrobacterium tumefaciens	
	nptII	Neomycin phosphotransferase II gene from E. coli Tn5.	Neomycin phosphotransferase II gene from E. coli Tn5.	Same genes, promoters and spacers
	T*NOS*	Nopaline synthase terminator sequence from A. tumefaciens.	Nopaline synthase terminator sequence from A. tumefaciens.	

		Extension Request NF872 Petition 16-004-01p	Antecedent GS784 and GD743 Petition 10-161-01p	Comments
		Second cassette	Second cassette	
Genotype	**P70**	Duplicated-enhancer CaMV 35S promoter from *Cauliflower mosaic virus* with untranslated leader sequence from *Alfalfa mosaic virus* RNA4	Duplicated-enhancer 35S promoter from *Cauliflower mosaic virus* with untranslated leader sequence from *Alfalfa mosaic virus* RNA4	Same genes, promoters, and spacers.
	PGAS	A chimeric sense suppression sequence of four fragments of four PPO genes from *Malus* × *domestica.*	A chimeric sense suppression transgene from *Malus* × *domestica.*	PGAS consists of 394 to 457 bp regions of four apple PPO genes (PPO2, GPO3, APO5, and pSR7) in tandem that upon transcription is designed to suppress the expression of these four members of the apple PPO gene family
	T*NOS*	Nopaline synthase terminator sequence from *A. tumefaciens.*	Nopaline synthase terminator sequence from *A. tumefaciens.*	
Transformation Method		*Agrobacterium tumefaciens*–mediated	*Agrobacterium tumefaciens*–mediated	Same
Insert and Copy Number		Complex insertions in chromosomes CHR3, CHR 13 and CHR 17: CHR3 insert has three GEN-03 T-DNA fragments and vector backbone.	Unknown	Proposed insertional positions and placement speculated in original petition. Low coverage (20X) Illumina sequencing with bioinformatics analyses were used to map and describe the insertions in the apple genome, for the submission of the extension request.

	CHR13 insert has two GEN-03 T-DNA fragments. CHR 17 insertion has two GEN-03 T-DNA fragments.		
Description	**Extension Request NF872 Petition 16-004-01p**	**Antecedent GS784 and GD743 Petition 10-161-01p**	**Comments**
Compositional analysis	Compositionally equivalent to conventional apple	Compositionally equivalent to conventional apple	Same
Backbone Absent	No	Yes	Approximately 1400 bp of non-functional vector backbone was detected in CHR3 in event N872.
Mechanism of Action	*PPO2*: reduces enzymatic browning *GPO3*: reduces enzymatic browning *APO5*: reduces enzymatic browning *pSR7*: reduces enzymatic browning	*PPO2*: reduces enzymatic browning *GPO3*: reduces enzymatic browning *APO5*: reduces enzymatic browning *pSR7*: reduces enzymatic browning	Same The sequences and sources of the four PPO gene sequences in *PGAS* are described in the Patent application "Genetically modified reduced-browning fruit-producing plants and produced fruit thereof, and method of obtaining such" (Armstrong and Lane, 2009)

Date of antecedent EA/ EIS	N/A	February 2015	

Description	**Extension Request NF872 Petition 16-004-01p**	**Antecedent GS784 and GD743 Petition 10-161-01p**	**Comments**
Plant Pest Risk			
Disease and pest susceptibilities	Similar to antecedent	Unlikely to change disease and pest susceptibilities	
Impacts on beneficial non-targets	Similar to antecedent	Unlikely to impact beneficial non-target organisms	
Enhanced weediness	Similar to antecedent	Unlikely to enhance weediness	
Enhanced weediness of relatives	Similar to antecedent	Unlikely to enhance weediness of relatives	
Changes to agriculture or cultivation practices	Similar to antecedent	Unlikely to change agriculture or cultivation practices	

Horizontal Gene Transfer	Similar to antecedent	Unlikely to affect the probability of horizontal gene transfer	
Plant Pest Risk	Similar to antecedent	Unlikely to pose a plant pest risk	