United States
Department of
Agriculture

The State University of New York College of Environmental Science and Forestry Petition (19-309-01p) for Determination of Nonregulated Status for Blight-Tolerant Darling 58 American Chestnut (*Castanea dentata*)

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Draft Environmental Impact Statement

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Executive Summary

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) received a request (APHIS Petition 19-309-01p) from the State University of New York College of Environmental Science and Forestry (ESF) seeking a determination of nonregulated status for Darling 58 American chestnut (*Castanea dentata* (Marsh.) that has been engineered to be tolerant to the fungal pathogen, *Cryphonectria parasitica* (ESF 2019). When APHIS receives a petition for nonregulated status of an organism currently regulated under its PPA authority codified in 7 CFR part 340, the Agency is required to make a decision. As a Federal agency, APHIS must also comply with applicable U.S. environmental laws and regulations because a decision on a petition for nonregulated status, whether positive or negative, is a final Agency action that might cause environmental impacts.

The petition stated that APHIS should not regulate Darling 58 American chestnut because it does not present a plant pest risk. In the event of a determination of nonregulated status, the nonregulated status would include Darling 58 American chestnut and any progeny derived from crosses between Darling 58 American chestnut and sexually compatible species including American chestnut (*C. dentata*), Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*), European chestnut (*C. sativa*), Chinese chinquapin chestnut (*C. henryi*), Allegheny Chinquapin chestnut (*C. pumila*), and Ozark Chinquapin Chestnut (*C. ozarkensis*) (ESF 2019).

Regulatory Authority

The Plant Protection Act of 2000 (PPA), as amended (7 U.S.C. §§ 7701-7772), provides the legal authorization for the APHIS plant protection mission. It authorizes the Agency to regulate the introduction of potential plant pests into the territorial boundaries of the United States, and their interstate movement within U.S. boundaries by establishing quarantine, eradication and control programs. Implementing rules, regulations and guidelines for this enabling legislation (PPA) are codified in Title 7 of the U.S. Code of Federal Regulations (CFR). Rules that implement this authority specific to organisms developed using genetic engineering have been published in 7 CFR part 340.

The regulations also provide a process to petition APHIS to determine that an organism developed using genetic engineering is nonregulated. An organism is no longer subject to the requirements of 7 CFR part 340 if APHIS determines that it is unlikely to pose a plant pest risk. A determination of nonregulated status means that the regulated organism is no longer subject to the regulations in 7 CFR part 340 and, therefore, there is no longer any authority for APHIS to require a permit or notification for the importation, interstate movement, or environmental release of the organism pursuant to 7 CFR part 340.

Two other agencies, the Federal Drug Administration (FDA) and the Environmental Protection Agency (EPA), are involved in regulating organisms developed using genetic engineering. The regulatory roles of USDA-APHIS, the FDA, and the EPA are described by the “Coordinated Framework,” a 1986 policy statement from the Office of Science and Technology Policy that describes the comprehensive Federal policy for ensuring the safety of biotechnology research and products.

The FDA regulates organisms developed using genetic engineering under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et seq.*). The FDA implements a voluntary consultation process to ensure that human food and animal feed safety issues or other regulatory issues, such as labeling, are resolved before commercial distribution of food derived from products developed using genetic engineering.

The EPA is responsible for regulating the sale, distribution, and use of pesticides, including pesticides that are produced by an organism through techniques of biotechnology. The EPA regulates plant incorporated protectants (PIPs) and microorganisms used as pesticides, e.g. bacteria, fungi, viruses, bacteriophages; both naturally occurring and genetically engineered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and certain microorganisms under the Toxic Substances Control Act (15 U.S.C. 53 *et seq.*).

Under FIFRA (7 U.S.C. 136 *et seq.*), the EPA regulates the use of pesticides (requiring registration of a pesticide for a specific use prior to distribution or sale of the pesticide for a proposed use pattern). The EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, and timing of its use; and storage and disposal practices. Prior to registration of a new pesticide or a new use for a previously registered pesticide, the EPA must determine that the pesticide will not cause unreasonable adverse effects on the environment and a reasonable certainty of no harm to humans when used in accordance with label instructions. The EPA reevaluates all pesticides every fifteen years (or shorter) to ensure they meet current standards for continued safe use (7 U.S.C. 136a(g)(1)(A)(iv)).

The EPA also sets tolerances for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the FFDCA. The EPA is required, before establishing pesticide tolerance, to reach a safety determination based on a finding of reasonable certainty of no harm under the FFDCA, as amended by the Food Quality Protection Act (FQPA). The FDA enforces the pesticide tolerances set by the EPA.

Purpose and Need for Agency Action

Under the authority of the plant pest provisions of the PPA and 7 CFR part 340, APHIS regulates the safe development and use of organisms developed using genetic engineering. Any party can petition APHIS to seek a determination of nonregulated status for an organism developed using genetic engineering that is regulated under 7 CFR part 340. As required by 7 CFR part 340, APHIS must respond to petitioners that request a determination of the regulated status of plants or organisms developed using genetic engineering such as Darling 58 American chestnut. When a petition for nonregulated status is submitted, APHIS must make a determination if the organism is unlikely to pose a plant pest risk. The petitioner is required to provide information under 7 CFR part 340 related to plant pest risk that the agency may use to compare the plant pest risk of the regulated organism to that of the unmodified organism. An organism developed using genetic engineering is no longer subject to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. In November 2019, APHIS received a petition from ESF requesting a determination of the regulated status for Darling 58 American chestnut. The purpose of the petition was to request nonregulated status for Darling 58 American chestnut because it does not pose a plant pest risk. APHIS prepared a PPRA and this

EIS to respond to the request and avoid the inappropriate use of public resources regulating a product developed using genetic engineering if the agency determines that it has no authority to do so.

Consistent with the Council of Environmental Quality's National Environmental Policy Act (NEPA) regulations and the USDA and APHIS NEPA implementing regulations and procedures (40 CFR parts 1500-1508, 7 CFR part 1b, and 7 CFR part 372), APHIS has prepared this draft Environmental Impact Statement (EIS) to consider the potential environmental impacts of an agency determination of nonregulated status. Specifically, this draft EIS has been prepared in order to evaluate the impacts on the quality of the human environment[1] that may result from a determination of nonregulated status of Darling 58 American chestnut.

Public Involvement

APHIS sought comments for the petition that is the subject of this EIS in a *Federal Register* notice dated August 19, 2020. The docket received a total of 4,320 public comments. Of the 4,320 comments received 1,619 comments were in opposition to deregulation of Darling 58 American chestnut, 2,698 comments were in favor of deregulation, and 3 were out of scope. The issues that were raised in the opposing comments were in regard to environmental impacts of the unconfined release of a forest tree developed using genetic engineering, impacts to native communities, human health and safety impacts of using a wheat gene, the need for long term studies, the potential for chestnut to be more susceptible to chestnut blight as well as other diseases, the potential for impacts to organic producers, impacts to trade, and general anti-biotech sentiments. The in favor of the petition comments emphasized the positive environmental and socio-economic benefits of restoring American chestnut to its original range.

As part of its scoping process to identify issues to address in this EIS, APHIS also published a Notice of Intent (NOI) to prepare an EIS and sought public input during a 30-day comment period (August 6 to September 7, 2021). Comments were submitted by individuals, academic researchers, and non-government organizations. The majority of comments submitted were opposed to a determination of nonregulated status for darling 58 American chestnut.

Alternatives Analyzed

In this EIS, APHIS considered two alternatives for its response to the ESF petition for nonregulated status. The two alternatives are: 1) continue to regulate Darling 58 American chestnut (No Action Alternative) and 2) approve the petition for nonregulated status of Darling 58 American chestnut (Preferred Alternative). These alternatives are further described here and in Chapter 2.

Alternative 1: No Action Alternative—Continue as Regulated

Under the No Action Alternative, APHIS would deny the petition because it was found to pose a plant pest risk. Darling 58 American chestnut and progeny derived from Darling 58 American

[1] Under NEPA regulations, the "human environment" includes "the natural and physical environment and the relationship of people with that environment" (40 CFR §1508.14).

chestnut would continue to be regulated under the regulations at 7 CFR part 340. Any introduction of Darling 58 American chestnut would still require authorization by APHIS. In addition, measures to ensure physical and reproductive confinement of Darling 58 American chestnut would continue to be implemented for any existing or new authorization. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of Darling 58 American chestnut.

This alternative is not the Preferred Alternative because APHIS has concluded through a PPRA that Darling 58 American chestnut is unlikely to pose a plant pest risk (USDA-APHIS 2020). Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status.

Alternative 2: Preferred Alternative – Determination that Darling 58 American chestnut is No Longer Regulated

Under this alternative, Darling 58 American chestnut and progeny derived from it would no longer be regulated under the regulations at 7 CFR part 340. APHIS would no longer require authorizations for introductions of Darling 58 American chestnut and progeny derived from this event. This alternative best meets the purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the PPA. Because the agency has concluded that Darling 58 American chestnut is unlikely to pose a plant pest risk (USDA-APHIS 2020), a determination of nonregulated status of Darling 58 American chestnut is a response that is consistent with the plant pest provisions of the PPA, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework. A determination of nonregulated status and this EIS would not necessarily apply to other blight tolerant American chestnut events as APHIS' regulatory practice is to review requests on a case-by-case basis.

Affected Environment and Environmental Consequences

Although the Preferred Alternative would allow for new plantings of Darling 58 American chestnut to occur anywhere in the United States the petitioner has stated their intention is ecological restoration and that initial distribution will consist of long-term research plots and relatively small-scale public horticultural plantings and will focus on areas where there are surviving small remnant American chestnut populations. For this reason, APHIS considered the affected environment for this EIS to be those areas of the United States where American chestnut was once a dominant forest tree.

Environmental issues are assessed individually in Chapter 4. The scope of this EIS analyzes the potential for direct and indirect impacts that might result from a determination of nonregulated status of Darling 58 American chestnut.

APHIS determined that the potential planting of Darling 58 American chestnut for restoration purposes or for any other purpose is likely to have some degree of impact on the environment. If blight-tolerant American chestnut were to become established as an important canopy tree, it would begin to influence ecosystem structure and function in these areas, as it did prior to the blight. Impacts to air quality, surface water and groundwater, soils, biodiversity, and habitats are all possible from planting Darling 58 American chestnut. The degree of environmental impacts will depend on a variety of factors that include the geographic locale, local biota, weather, inherent soil characteristics, and prevalence and diversity of wildlife. Given the historically slow spread rates, low propagule pressure, and need for disturbance to provide sufficient light for fast growth, the rate of increase would likely be very slow. Without aggressive restoration efforts, requiring considerable effort and coordination at landscape scales, it may require centuries before American chestnut becomes a significant presence in the landscape (Gustafson et al. 2017; Gustafson et al. 2018).

While it is difficult to predict what impacts Darling 58 American chestnut will have on forest biodiversity, in the long term if American chestnut were to become a dominant species again, it is reasonable to believe there are likely to be positive impacts on the biodiversity of animal species as chestnut provides a more stable and more abundant source of mast than oaks, hickory, and beech species (Diamond et al. 2000) while decreasing the abundance of some tree species such as oaks. As American chestnut grows faster than other hardwood species, there may be positive impacts on climate change through greater carbon sequestration. Species that became more prevalent during the demise of chestnut, such as oak and maple, may become less prevalent.

Chestnuts are the most abundant treenut after coconut (Davison et al. 2021a). The industry is centered in China (85% of world production) and is largely absent in the United States (Davison et al. 2021a). The few acres devoted to chestnut production in the United States are largely planted to Chinese chestnut due to its blight resistance (Revord et al. 2021). Agroforestry systems have been touted as a means to mitigate the environmental impacts of row crops and chestnuts have been proposed as a key component of such systems. If agroforestry systems become more widely adopted, plantings of chestnut may expand dramatically, and Darling 58 American chestnut could be used in agroforestry systems. However, commercial use of Darling 58 American chestnut is not anticipated at this time for the following reasons:

- Most growers consider Chinese chestnuts to be the best option currently available for establishing profitable orchards in eastern United States (Davison et al. 2021a). Darling 58 American chestnut was developed as a non-profit project for forest restoration. Its attributes have yet to be established and it is susceptible to other diseases such as ink disease caused by *Phytophthora cinnamomi* (ESF 2019). In contrast, Chinese chestnut has resistance to ink disease in addition to blight (Metaxas 2013). Furthermore, Chinese chestnut has been cultivated for over 2000 years allowing for the selection of favorable quality traits. It has been cultivated and bred in the United States since 1930 (Metaxas 2013). From the standpoint of performance characteristics, the risk of using Darling 58 American chestnut in a commercial venture is much higher than Chinese chestnut.

- Varieties created through genetic engineering typically have less public acceptance than conventional varieties. Darling 58 American chestnut would pose a risk to commercial growers from the standpoint of potential reduced public acceptance.

- Chinese chestnuts are smaller trees and more suitable for orchard planting than American chestnut

- Market surveys suggest American consumers prefer larger chestnuts (Aguilar et al. 2009) and American chestnuts are smaller than Chinese and Japanese chestnuts. Chinese chestnuts are also easier to peel (Metaxas 2013; Davison et al. 2021b).

In the early 1900s American chestnut was a valued timber tree (Wang et al. 2013) making up more than 25 percent of all timber cut in the Southern Appalachians and almost half of the timber cut in Connecticut (Hawley and Hawes 1918b; Hepting 1974; Wang et al. 2013). Most hardwood timberland forests are regenerated through natural means (i.e, seedlings in the understory are given the opportunity to grow upon the selective removal or clear cutting of larger trees) (Mississippi State Extension NA). While Darling 58 American chestnut may be able to establish and colonize much of the eastern United States if it shows enhanced tolerance to the fungus *C. parasitica*, it may require centuries before becoming a significant enough presence in the landscape to allow for timber harvest. The rate of increase will depend on the degree of human assistance. Darling 58 American chestnut was developed as a non-profit project for forest restoration in American chestnut's native range. If landowners have a strong interest in American chestnut restoration or the perception that Darling 58 American chestnut will provide more value to their forest than their existing tree stand and Darling 58 American chestnut seedlings become available in large numbers, the increase could be accelerated. As the climate and ecology of the eastern forests have changed in the last hundred years it is unknown whether Darling 58 American chestnuts will ever regain the dominance it exhibited in the nineteenth century. Darling 58 American chestnut's attributes have yet to be established for timber production and so the risk of using Darling 58 American chestnut in a commercial venture is likely too high for adoption in the foreseeable future.

No socioeconomic impacts are expected from deregulation of Darling 58 American chestnut for several reasons. As noted above, Darling 58 American chestnut is not expected to be used in commercial plantings for timber or nut production for the foreseeable future. The draft EIS considered whether Darling 58 American chestnut could impact commercial plantings of chestnut via cross pollination, especially those chestnuts produced for a biotech sensitive market. Gene flow from Darling 58 American chestnut to commercial chestnut is considered unlikely for several reasons. Darling 58 American chestnut is most likely to be planted in the native American chestnut range while the majority of commercial chestnut production (58%) occurs in states outside the native range (USDA-NASS 2019). Darling 58 American chestnut is a different species than the chestnut used for commercial production and so, successful hybridization occurs at a lower frequency than within the species (ESF 2019). Chinese chestnut also usually flowers earlier than American chestnut which decreases effective cross-pollination (Pennsylvania Chapter The American Chestnut Foundation 2006). And finally, chestnut pollen does not travel long distances. Effective pollination does not occur beyond 400 m (Forest et al. 1977; Russell 1987; Rutter 1990) so there is likely to be adequate isolation distances between Darling 58 American chestnut and chestnut orchards catering to the biotech sensitive markets. Chestnut farms catering to the biotech sensitive markets are likely to be low input operations catering directly to the consumer ("uPick" operations) which would not be subject to commingling in a supply chain (Gullickson 2019). Organic chestnut production today only represents 0.33% of the chestnuts sold in the United States (Davison et al. 2021a) though it is unknown whether organic chestnut production would capture additional market share if the industry dramatically expanded.

More detailed descriptions and analyses of the potential environmental consequences can be found in Chapter 4.

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ACRONYMS AND ABBREVIATIONS

AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
CAA	Clean Air Act
CFR	Code of Federal Regulations (United States)
CH_4	methane
CO_2	carbon dioxide
DNA	deoxyribonucleic acid
EIS	environmental impact statement
EO	Executive Order
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act of 1973
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHG	greenhouse gas
IPPC	International Plant Protection Convention
N_2O	nitrous oxide
NAAQS	National Ambient Air Quality Standards
NEPA	National Environmental Policy Act of 1969 and subsequent amendments
NHPA	National Historic Preservation Act
NMFS	National Marine Fisheries Service
NPS	Agricultural non-point source
OECD	Organization for Economic Cooperation and Development
PIP	plant incorporated protectants
PPRA	Plant Pest Risk Assessment
PPA	Plant Protection Act
T&E	threatened and endangered
USDA	U.S. Department of Agriculture
USDA-NASS	U.S. Department of Agriculture-National Agricultural Statistics Service
USC	United States Code
USFWS	U.S. Fish & Wildlife Service
WPS	Worker Protection Standard for Agricultural Pesticides

1 PURPOSE AND NEED FOR AGENCY ACTION

This document is intended to ensure compliance with the National Environmental Policy Act (NEPA). NEPA requires agencies to prepare an environmental impact statement (EIS) to be included in "every recommendation or report on proposals for legislation and other major Federal actions significantly affecting the quality of the human environment" (42 U.S.C. §4332(2)(C)).

The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) is currently engaged in decisionmaking relevant to its statutory authority to regulate Darling 58 American chestnut under the plant pest provisions of the Plant Protection Act (PPA). The Agency has determined that there are possible environmental impacts, as described in chapter 4, associated with whatever regulatory decision it renders. Therefore, this document has been prepared as part of this APHIS decisionmaking process.

1.1 State University of New York College of Environmental Science and Forestry (ESF) Petition for Nonregulated Status

In November 2019, APHIS received a petition from ESF requesting a determination of the regulated status of Darling 58 American chestnut. The purpose of the petition was to request nonregulated status for Darling 58 American chestnut, and any progeny derived from them to no longer be considered regulated under Title 7 of the Code of Federal Regulations part 340 (7 CFR part 340). Darling 58 American chestnuts have been engineered to be tolerant to the fungal pathogen, *Cryphonectria parasitica* (ESF 2019). Darling 58 American chestnut is currently regulated by APHIS.

APHIS prepared a plant pest risk assessment (PPRA) and this EIS to respond to the request and avoid the inappropriate use of public resources regulating a product developed using genetic engineering if the agency determines that it has no authority to do so; the need for this action.

1.2 Purpose of Darling 58 American Chestnut

The American chestnut, once one of the most abundant trees within its range in the eastern United States, was a fast-growing and long-lived canopy tree that produced a consistent crop of healthful nuts, was harvested for valuable lumber, and was considered a keystone species for wildlife. That ended when an invasive fungal pathogen, *C. parasitica*, was introduced from Asia and killed over 3 billion American chestnuts throughout their natural range.

Darling 58 American chestnut was developed using genetic engineering to be tolerant to the fungal pathogen, *C. parasitica,* chestnut blight (ESF 2019). Tolerance to chestnut blight in Darling 58 American chestnut was developed by the insertion of an oxalate oxidase (*OxO*) gene from wheat into an American chestnut line known as Ellis (ESF 2019). Tolerance to chestnut blight is achieved by detoxifying the oxalic acid produced by the fungus, preventing the acid from killing the chestnut's tissues. In the presence of OxO, the damage caused by the oxalic acid is restricted to superficial cankers (ESF 2019).

1.3 Coordinated Framework Review and Regulatory Review

Since 1986, the U.S. government has regulated organisms developed using genetic engineering pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (referred to as the Coordinated Framework). The Coordinated Framework, published by the White House Office of Science and Technology Policy, describes the regulatory roles and authorities for the three major agencies involved in regulating organisms developed using genetic engineering: the USDA APHIS, the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA). On January 4, 2017, the USDA, EPA, and FDA released a 2017 update to the Coordinated Framework (USDA-APHIS 2018), and the accompanying National Strategy for Modernizing the Regulatory System for Biotechnology Products (ETIPCC 2017). A more detailed description can be found in the original 1986 policy statement (51 FR 23302) and in the 2017 Coordinated Framework update (US-EPA 2017).

1.3.1 USDA-APHIS

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to the Plant Protection Act (PPA), as amended (7 U.S. Code (U.S.C.) 7701–7772), govern the introduction (importation, interstate movement, and environmental release) of organisms developed using genetic engineering that may pose a plant pest risk. An organism developed using genetic engineering is regulated under 7 CFR part 340 when APHIS has reason to believe that the organism may be a plant pest or APHIS does not have sufficient information to determine if the organism is unlikely to pose a plant pest risk. An organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that the organism is unlikely to pose a plant pest risk.

1.3.2 Environmental Protection Agency

The EPA is responsible for regulating the sale, distribution, and use of pesticides, including pesticides that are produced by organisms developed using genetic engineering, termed plant incorporated protectants. The EPA regulates pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 et seq.) and certain microorganisms under the Toxic Substances Control Act (TSCA) (15 U.S.C. 53 et seq.). Before a pesticide is legally used in the United States, the EPA must evaluate the pesticide to ensure that it will not cause unreasonable adverse effects on the environment and a reasonable certainty of no harm to humans when used in accordance with label instructions. Pesticides that complete this evaluation are issued a "registration" that permits their sale and use according to requirements set by the EPA. The EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Once registered, a pesticide may not legally be used unless the use is consistent with the approved directions for use on the pesticide's label. The overall intent of the label is to provide clear directions for effective product performance while minimizing risks to human health and the environment. Under FIFRA the EPA has a standard of reviewing pesticide registrations every 15 years (US-EPA 2011). The Food Quality Protection Act (FQPA) of 1996 amended FIFRA, and set a standard to reassess, over a 10-year period, all pesticide tolerances that were in place when the FQPA was signed, make a safety finding when setting tolerances that the pesticide can be used with "a reasonable certainty of no harm," take into consideration aggregate and cumulative effects/risks in assessing human health, and emphasize risks to special sub-populations such as infants and children (US-EPA 2015).

The EPA also sets tolerances (maximum limits) for pesticide residues that may remain on or in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the Federal Food, Drug, and Cosmetic Act (FFDCA; 21 U.S.C. 301 *et seq.*). In establishing a pesticide tolerance, the EPA conducts dietary risk assessments to ensure that all tolerances established for each pesticide and food product reach a safety determination based on a finding of reasonable certainty of no harm. The FDA enforce pesticide tolerances set by the EPA to ensure the safety of the nation's food supply.

1.3.3 Food and Drug Administration

The FDA regulates organisms developed using genetic engineering under the authority of the FFDCA (21 U.S.C. 301 *et seq.*). The FDA published its policy statement concerning regulation of products derived from new plant varieties, including those derived from genetic engineering, in the *Federal Register* on May 29, 1992 (57 FR 22984). Under this policy, the FDA implements a voluntary consultation process to ensure that human food and animal feed safety issues or other regulatory issues, such as labeling, are resolved before commercial distribution of food developed using genetic engineering. This voluntary consultation process provides a way for developers to receive assistance from the FDA in complying with their obligations under Federal food safety laws prior to marketing.

More recently, in June 2006, the FDA published recommendations in "Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (US-FDA 2006) for establishing voluntary food safety evaluations for new non-pesticidal proteins produced by new plant varieties intended to be used as food, including plants developed using genetic engineering. Early food safety evaluations help make sure that potential food safety issues related to a new protein in a new plant variety are addressed early in development. These evaluations are not intended as a replacement for a biotechnology consultation with the FDA, but the information may be used later in the biotechnology consultation.

1.4 Purpose and Need for APHIS Action

Under the authority of the plant pest provisions of the Plant Protection Act (7 U.S.C. 7701 *et seq*.), the regulations in 7 CFR part 340, "Movement of Organisms Modified or Produced Through Genetic Engineering," regulate, among other things, the importation, interstate movement, or release into the environment of organisms modified or produced through genetic engineering that are plant pests or pose a plausible plant pest risk. APHIS recently revised 7 CFR part 340 and issued a final rule, published in the *Federal Register* on May 18, 2020 (85 FR 29790-29838, Docket No. APHIS-2018-0034).[2] The new Regulatory Status Review (RSR) process, which replaces the petition for determination of nonregulated status process, became effective for all crops as of October 1, 2021. The petition for a determination of nonregulated status subject of this EIS is being evaluated in accordance with the regulations at 7 CFR 340.6 (2020) as it was received by APHIS, in November 2019, prior to the implementation of the

[2]To view the final rule, go to www.regulations.gov and enter APHIS-2018-0034 in the Search field.

revised regulation. Pursuant to the terms set forth in 7 CFR 340.6 (2020), any person may submit a petition to APHIS seeking a determination that an organism should not be regulated under 7 CFR part 340. APHIS must respond to petitioners with a decision to approve or deny the petition. An organism produced using genetic engineering is no longer subject to the requirements of 7 CFR part 340 or the plant pest provisions of the PPA if APHIS determines, through conduct of a Plant Pest Risk Assessment (PPRA), that it is unlikely to pose a plant pest risk.

Consistent with the Council of Environmental Quality's (CEQ) NEPA regulations (40 CFR parts 1500-1508) and USDA and APHIS NEPA implementing regulations and procedures (7 CFR part 1b, and 7 CFR part 372), APHIS has prepared this EIS to consider the potential impacts of a determination of nonregulated status for Darling 58 American chestnut on the human environment.[3]

[3] The human environment includes the natural and physical environment and the relationship of people with that environment. When economic or social and natural or physical environmental effects are interrelated, the NEPA analysis may addresses these potential impacts as well (40 CFR §1508.14).

2 Scoping and Public Involvement

APHIS seeks public comment on petitions it receives that request a decision of nonregulated status for organisms developed using genetic engineering. APHIS does this through a notice published in the *Federal Register*. When the Agency decides to prepare an EIS as part of its decisionmaking process for a petition, prior to preparation, it also seeks public comments as part of its advance scoping process. Details about the public involvement process for the petition that is the subject of this document follows.

2.1.1 Public Comments for Petition 19-309-01p

On August 19, 2020, APHIS announced in the *Federal Register* that it was making ESF's petition available for public review and comment to help identify potential environmental and interrelated economic impacts that APHIS should consider in evaluation of the petition (85 FR 51008-51009). APHIS accepted written comments on the petition for a period of 60 days, until midnight, October 19, 2020. At the end of the comment period APHIS received a total of 4,320 public comments. Of the 4,320 comments received 1,619 comments were in opposition to deregulation of Darling 58 American chestnut, 2,698 comments were in favor of deregulation, and 3 were out of scope. The issues that were raised in the opposing comments were in regard to environmental impacts of the unconfined release of a forest tree developed using genetic engineering, impacts to native communities, human health and safety impacts of using a wheat gene, the need for long term studies, the potential for chestnut to be more susceptible to chestnut blight as well as other diseases, the potential for impacts to organic producers, impacts to trade, and general anti-biotech sentiments. The in favor of the petition comments emphasized the positive environmental and socio-economic benefits of restoring American chestnut to its original range. APHIS evaluated the comments and integrated the concerns raised into this draft EIS.

2.1.2 Public Scoping for this draft EIS

As part of its scoping process to identify issues to address in this EIS, APHIS also published a Notice of Intent (NOI) to prepare the EIS and sought public input during a 30-day comment period (August 6, 2021 to September 7, 2021). The docket received a total of 3,964 public comments from 3,967 submitters. Of the 3,964 comments received 3,807 comments were in opposition to deregulation of Darling 58 American chestnut, 156 comments were in favor of deregulation, and 1 was out of scope. Issues most frequently cited in public comments on the NOI included:

- the potential for gene flow from Darling 58 American chestnut to wild relatives
- the potential for Darling 58 American Chestnut to spread and become invasive
- impacts to wildlife, including pollinators, and threatened and endangered species
- the economic impacts, including impacts on organic and non-GMO producers
- non-target impacts specifically to beneficial fungus, the microbiome, and mycorrhizal networks
- impacts on the forest ecosystem
- the potential for Darling 58 American chestnut to be more susceptible to insects or other pathogens

- human health impacts from consuming nuts as well as potential allergies from pollen and the need for FDA clearance
- the potential for Darling 58 American chestnut to not be resistant to blight, act as a reservoir for blight, or the evolution of pathogen resistance
- the potential for unintended or off-target mutations and impacts from epigenetic changes
- impacts to tribal communities
- traditional cross breeding should be used to develop blight resistance

The issues discussed in this EIS were developed by considering the public input, including public comment received from the *Federal Register* notice announcing the availability of the petition (85 FR 51008-51009), the NOI, as well as issues raised in public comments submitted for other NEPA documents for organisms developed using genetic engineering, issues raised in lawsuits, and other issues raised by various stakeholders. These issues, including those regarding the potential reintroduction of American chestnut and the environmental and food/feed safety of plants developed using genetic engineering, were addressed to analyze the potential environmental impacts of Darling 58 American chestnut.

2.2 Issues Considered in this EIS

The list of resource areas considered in this draft EIS was developed by APHIS through experience in considering issues raised by the public, with specific attention to the issues raised in public comments submitted for this petition and EAs and EISs of other organisms developed using genetic engineering. The resource areas considered also address issues raised in previous and unrelated lawsuits, and issues that have been raised by various stakeholders for this and prior petitions. The resource areas considered in this draft EIS can be categorized as follows:

Action Area:

- Historic, Present, and Potential Future Range of American chestnut

Environmental Considerations:

- Soil Quality
- Water Resources
- Air Quality and Climate Change
- Animal Communities
- Plant Communities
- Gene Flow and Weediness
- Microorganisms
- Biodiversity

Human Health Considerations:

- Consumer Health

Livestock Health Considerations:

- Animal Feed/Livestock Health

Socioeconomic Considerations:

- Domestic Economic Environment
- Trade Economic Environment

3 ALTERNATIVES

This document analyzes the potential environmental consequences of a determination of nonregulated status of Darling 58 American chestnut. To respond favorably to a petition for nonregulated status, APHIS must determine that Darling 58 American chestnut is unlikely to pose a plant pest risk. Based on its PPRA (USDA-APHIS 2020), APHIS has concluded that Darling 58 American chestnut is unlikely to pose a plant pest risk. Therefore, in the absence of any new information, APHIS must determine that Darling 58 American chestnut is no longer subject to 7 CFR part 340.

Two alternatives are evaluated in this draft EIS: (1) No Action: Continuation as Regulated and (2) Preferred Alternative: Determination of Nonregulated Status of Darling 58 American chestnut. APHIS has assessed the potential for environmental impacts for each alternative in Chapter 4 Affected Environment and Environmental Consequences.

3.1 No Action Alternative: Continue as Regulated

Under the No Action Alternative, APHIS would deny the petition. Darling 58 American chestnut and progeny derived from Darling 58 American chestnut would continue to be regulated under the regulations at 7 CFR part 340. Any introduction of Darling 58 American chestnut would still require authorization by APHIS. In addition, measures to ensure physical and reproductive confinement of Darling 58 American chestnut would continue to be implemented. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of Darling 58 American chestnut.

This alternative is not the Preferred Alternative because APHIS has concluded through a PPRA that Darling 58 American chestnut is unlikely to pose a plant pest risk (USDA-APHIS 2020). Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status.

3.2 Preferred Alternative: Determination that Darling 58 American Chestnut is No Longer Regulated

Under this alternative, Darling 58 American chestnut and progeny derived from it would no longer be regulated under the regulations at 7 CFR part 340. APHIS would no longer require authorizations for introductions of Darling 58 American chestnut and progeny derived from this event. This alternative best meets the purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the PPA. Because the agency has concluded that Darling 58 American chestnut is unlikely to pose a plant pest risk (USDA-APHIS 2020), a determination of nonregulated status of Darling 58 American chestnut is a response that is consistent with the plant pest provisions of the PPA, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework.

3.3 Alternatives Considered but Dismissed from Detailed Analysis

APHIS has evaluated several additional alternatives for consideration in previous EAs for petitions for nonregulated status. For example, APHIS has considered alternatives that would entail approving a petition request in part, mandatory isolation or geographic restriction of plants developed using genetic engineering and those developed not using genetic engineering, and requirements for testing for the presence of plant material from plants developed using genetic engineering in plants that were not developed using genetic engineering.

Based on the PPRA for Darling 58 American chestnut (USDA-APHIS 2020), experience regulating organisms developed using genetic engineering, and broad general experience with plant varieties, APHIS determined that Darling 58 American chestnut trees are unlikely to pose a plant pest risk. Thus, the imposition of testing, release/planting, and/or isolation requirements on Darling 58 American chestnut would be inconsistent with the Agency's statutory authority under the plant pest provisions of the PPA, implementing regulations at 7 CFR part 340, and federal regulatory policies embodied in the Coordinated Framework. Because it would be unreasonable to evaluate alternatives absent any jurisdiction to implement them, these additional alternatives stated above were dismissed from detailed analysis in this EA.

3.4 Comparison of Alternatives

Table 1 presents a summary of the potential impacts associated with selection of either of the alternatives evaluated in this EIS. The impact assessment is presented in Chapter 4 of this EIS.

Table 1. Summary of Issues of Potential Impacts and Consequences of Alternatives.

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Meets Purpose and Need and Objectives	No	Yes
Management Practices		
Action Area for Darling 58 American chestnut	The American chestnut was once one of the most abundant trees within its range in the eastern United States. American chestnut was found at every elevation from sea level to over 5000 ft. from as far north as Maine and Ontario, Canada to as far south as Georgia and Mississippi, covering an approximate 200 million acres of land (Saucier 1973). As a consequence of blight, the abundance of chestnut has drastically declined; the number of live stems today is estimated at 431 ± 30.2 million, approximately	If Darling 58 American chestnut is granted nonregulated status and shows enhanced tolerance to the fungus *C. parasitica*, they may be able to establish and colonize much of the eastern United States where populations persist as stunted trees, becoming a self-sustaining forest tree species in its native range. Given the historically slow spread rates, low propagule pressure, and need for disturbance to provide sufficient light for fast growth, the rate of increase would likely be very slow. Without aggressive

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	10% of the pre-blight population (Dalgleish et al. 2015a). The American chestnut has been reduced from a dominant overstory tree to a small understory shrub (Elliott and Swank 2008; Dalgleish et al. 2015b).	restoration efforts, requiring considerable effort and coordination at landscape scales, it may require centuries before American chestnut becomes a significant presence in the landscape (Gustafson et al. 2017; Gustafson et al. 2018).
Physical Environment		
Soil Quality	American chestnut commonly grows on sandy loams in association with other hardwoods (Saucier 1973). American chestnut trees prefer well-drained, sandy, and slightly acidic (i.e. pH of 5 to 6) soils, often on slopes and ridges (Russell 1987; Wang et al. 2013). Forest ecosystem processes, including decomposition, nutrient cycling, and productivity, likely changed following chestnut's replacement by other species.	No significant differences were found in colonization by ectomycorrhizal fungi in Darling 58 American chestnut roots compared to non-transgenic controls, suggesting that the presence or expression of OxO in Darling 58 American chestnut does not pose risks to native soil fungi that are ecologically important for American chestnuts and other trees (ESF 2019). Introducing Darling 58 American chestnut back into eastern forests is unlikely to have negative impacts on soil quality.
Water Resources	All water in forested lands contain organic matter, inorganic matter, and dissolved gasses derived from the environment, organisms, and anthropogenic activities. The concentrations of all these substances, in addition to their biological, physical, and chemical effects, are the basic criteria of water quality (Chang 2013). Surface and groundwater resources are key outputs of forests. These water resources are essential to ecosystem processes and functions across the action area.	Introduction of Darling 58 American chestnut to eastern forests should not impact overall water resources in the area. American chestnut does not have different water requirements than those tree species currently found in eastern forests. Considering the relatively slow re-introduction rate and spread of Darling 58 American chestnuts, it is anticipated that impacts to water resources will remain relatively minimal across the landscape.

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Air Quality and Climate Change	Forests play a role in improving local and regional air quality. Rates of airborne pollution removal vary based on the pollutant type, leaf season length, and precipitation levels. Trees remove air pollution by the interception of particulate matter on plant surfaces and the absorption of gaseous pollutants through the leaf stomata (Nowak et al. 2014). Forests play an important role in global climate regulation. In 2007, U.S. forests absorbed an estimated 910 million metric tons of carbon dioxide equivalent, an amount equal to approximately 13 percent of the country's gross greenhouse gas emissions from industrial and other sources (Hanson et al. 2010). Carbon sequestration is an important ecosystem service provided by forests globally and represents a driving motivation for reforestation and conservation efforts worldwide.	Considering that it may require centuries before American chestnut becomes a significant presence in the landscape again, in the short term, Darling 58 American chestnut is unlikely to change the role forests play in regulating air quality. Because American chestnut is fast-growing, long-lived, and resistant to decay (Ellison et al. 2005; de Bruijn et al. 2014), its reintroduction could result in increased carbon sequestration and storage in the form of living and dead trees and durable wood products, however, the magnitude of this effect may be minor (Gustafson et al. 2017).
Biological Resources		
Animal Communities	Pre-blight, American chestnut was described as "the most important wildlife plant in the eastern United States" (Davis 2005). American chestnut produced a heavy mast crop of calorie packed seeds. American chestnut disappeared from forests before many systematic studies of wildlife food habits were undertaken (Hill 1992), but we know that native mammals such as white-tailed deer (*Odocoileus virginianus*), cottontail rabbit	American chestnut was considered a keystone species meaning it played a critical role in the function of the overall ecosystem. If Darling 58 American chestnuts were to become established, it would influence ecosystem structure and function in areas where they were introduced, as American chestnuts did prior to the blight (Paillet 2002).

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	(*Sylvilagus floridanus*), and black bears (*Ursus americanus*) all consumed chestnuts (Diamond et al. 2000; Wang et al. 2013), as well as many other vertebrates including rodents (Lichti et al. 2014) and birds including wild turkeys (*Meleagris gallopavo*), American crow (*Corvus brachyrhynchos*), blue jay (*Cyanocitta cristata*), ruffed grouse (*Bonasa umbellus*), and the extinct passenger pigeon (*Ectopistes migratorius*) (Webb 1986; Russell 1987) and heath hen (Hill 1992). Additionally, American chestnut provided a food source to numerous insect species, especially during the flowering period.	Since American chestnut provided a more stable and more abundant source of mast than oaks, hickory, and beech species (Diamond et al. 2000), if Darling 58 American chestnuts were to become established it could result in population increases of the species that feed on chestnut (Hill 1992). An increase in American chestnut would also result in a gradual decline in some co-occurring tree species. American chestnut will likely replace other tree species in proportion to their abundance, rather than replacing a single species or genus (Gustafson et al. 2017) and therefore those species will still be available to animal populations that use those trees. Oxalate oxidase is a common enzyme found in all grains, several other crops and food products, and many wild plants and microbes. Animal communities within Eastern forests are likely already exposed to the OxO gene in Darling 58 American chestnut. Additionally, nutrition analyses have confirmed that transgenic chestnuts are not nutritionally different than their wild-type relatives (ESF 2019). No impacts are expected to animal communities from exposure to the OxO gene in Darling 58 American chestnut.
Plant Communities	American chestnut can be found in naturalized populations throughout the eastern United States. The plant communities for	With an increase in American chestnut, some co-occurring tree species would gradually decline, the most likely being the same

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	American chestnut include all of the plants in a particular area, including native, introduced, desirable, and undesirable plants. The plant species in the action area may be generally characterized as forbes, vines, succulents, ferns, grasses, shrubs, and trees (BONAP 2020). Chestnuts were replaced mainly by oak and maple, and to a lesser extent, hickory, birch, black cherry, and others as dominant canopy tree species (Keever 1953; Stephenson 1986; Stephenson et al. 1991; Brewer 1995). No single species has emerged in a dominant role across a broad geographic range, comparable to the pre-blight status of American chestnut. Plant communities in the action area are likely to remain largely unchanged under the No Action Alternative.	trees (oak, maple, hickory, birch, and black cherry) that replaced American chestnut after chestnut blight was introduced. American chestnut will likely replace other tree species in proportion to their abundance, rather than replacing a single species or genus (Gustafson et al. 2017). Given the relatively close overlap between the niches of chestnut and oak (Keever 1953) competition from chestnut would likely affect oaks more than other species (Gustafson et al. 2017).
Gene Flow and Weediness	American chestnut is primarily wind-pollinated (Clapper 1954; Johnson 1988) and can outcross to other chestnut species, including Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*), European chestnut (*C. sativa*), and chinquapin (*C. pumila*) (Jaynes 1964) to form hybrids as well as other *Castanea* species where their distributions overlap. American chestnut can regenerate vegetatively by sprouting new shoots from the root collar, but cannot sprout from roots (Paillet	Under the Preferred Alternative, pollen mediated gene flow from Darling 58 American chestnut is possible. Darling 58 American chestnut is intended to be planted in proximity to native American chestnut trees with the hope that wild trees will flower and cross-pollinate to yield blight resistant seeds with the intention to increase the genetic diversity of the blight tolerant chestnuts. The transgenes from Darling 58 could also spread to related species through successful pollination with at least one transgenic parent

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	1984, 1993), so new shoots will be in the immediate location of the former tree; natural dispersal to a new location can take place only by seed. Chestnut dispersal is relatively slow without human intervention.	to produce viable offspring (ESF 2019). Gene flow to different species is not as likely because flowering times are not as synchronized, and trees may not be in proximity to Darling 58 American chestnut. Chestnut pollen is prone to desiccation and loses viability within as little as a few hours when air dried (Maynard 1991), so effective pollination becomes increasingly unlikely as pollen spreads farther from its source. Researchers have noted that trees need to be within 100 meters for successful pollination (Rutter 1990; Jacobs et al. 2011), and trees 300 m apart are essentially reproductively isolated from one another (Rutter 1990) American chestnut trees spread at an average rate of "no more than a few kilometers per century" (Paillet and Rutter 1989). It may take a century or more for blight tolerant chestnut trees to become dominant after the first pioneer trees become established in a given area. Slow natural colonization rates and frequent animal and pest pressure on seeds and seedlings (Clark et al. 2014), in addition with the limitations on pollen spread, suggest that chestnuts, regardless of type or transgene status, will not rapidly invade new areas (Cook and Forest 1978). Areas that are not intentionally planted with blight-

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
		tolerant chestnuts will likely remain without chestnuts for decades or longer (ESF 2019).
Biodiversity	In a forest ecosystem, biodiversity can be affected by several factors including climatic and soil conditions, evolution, changes in species' geographical ranges, population and community processes, and natural disturbances or those caused by human activities (Carnus et al. 2006).	Impacts to biodiversity are possible with the planting of Darling 58 American chestnut. Pre-blight, American chestnut was described as "the most important wildlife plant in the eastern United States" (Davis 2005), a keystone species that played a critical role in the function of the overall ecosystem. While it is difficult to predict what impacts Darling 58 American chestnut will have on forest biodiversity, especially since the overall ecosystem has changed since American chestnut disappeared from the landscape, it is reasonable to believe that if Darling 58 American chestnut shows enhanced tolerance to chestnut blight and the trees are able to establish and spread, in the long term it will have positive impacts on increasing the biodiversity of animals and micro-organisms while decreasing the abundance of some tree species such as oaks (Paillet 2002).
Human and Animal Health		
Risk to Human Health	Pre-blight American chestnut was regularly consumed by people living within its historic range. It is the responsibility of food and feed manufacturers to ensure that the products they introduce into commerce are safe and in	The transformation process in Darling 58 American chestnut do not cause nutritional differences beyond those already present in traditionally-bred chestnuts (ESF 2019) and is not expected to result in adverse human health

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	compliance with applicable laws and regulations. Organisms developed using genetic engineering for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market (US-FDA 2006).	effects from direct or indirect human contact. The Darling 58 American chestnut tree does not represent a source of new, potentially allergenic or anti-nutrient proteins; the oxalate oxidase gene and protein are commonly found in a variety of non-allergenic foods (ESF 2019). An indirect consequence that may result from the introduction of Darling 58 American chestnut is the potential effects from higher rodent populations including increased Lyme disease risk to humans (Dalgleish and Swihart 2012). While increased Lyme disease risk is possible, it is unlikely that this risk would be significant.
Risk to Animal Feed	Historically American chestnut was used as feed for hogs (Davis 2005). Chestnut has low calories with rich minerals, vitamins, and monounsaturated fatty acid (Joo et al. 2018). Currently, other varieties of chestnut are sometimes used as animal feed supplement for pigs (All About Feed 2011; Joo et al. 2018), young poultry and rabbits (Joo et al. 2018), and cattle (All About Feed 2011).	Nutritional analyses have confirmed that transgenic chestnuts are not nutritionally different than their wild-type relatives (ESF 2019). No impacts are expected to animals from exposure to the OxO gene in Darling 58 American chestnut if used in animal feed.
Socioeconomic		
Domestic Economic Environment	American chestnut was a valued timber tree (Wang et al. 2013) used for construction lumber, shingles, fence posts and rails, telephone and telegraph poles,	Darling 58 American chestnut would have the same qualities that made American chestnut a valued timber and nut tree pre-blight. The U.S. chestnut industry

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	paneling, trim, furniture, coffins, interior decoration and firewood (Detwiler 1915; Buttrick 1925). In the early 1900s, American chestnut made up more than 25 percent of all timber cut in the Southern Appalachians, was the most valuable tree in southern New England, and constituted almost half of the timber cut in Connecticut (Hawley and Hawes 1918b; Hepting 1974; Wang et al. 2013). Seven years after discovery of the blight in 1904 it was estimated that the blight had done $25 million in damages to the chestnut industry (Buttrick 1915). The chestnut industry is in its infancy in the United States but is poised to grow due to increased consumer demand for chestnuts, increased demand for hardwood, and the potential for agroforestry to replace row crops as a means to mitigate climate change and the environmental impacts of conventional farming. U.S. domestic consumption is low at 0.1lb per capita but demand still greatly exceeds supply such that 90% of chestnuts consumed in the United States are imported. Currently, the preferred species for commercial planting is Chinese chestnut because of its disease resistance, shorter stature, shorter time to nut production, and superior nut qualities.	is expected to grow with other chestnut species regardless of the Darling 58 American chestnut regulatory status. Darling 58 American chestnut is not expected to be used in commercial plantings for timber or nut production for the foreseeable future. Chinese chestnut is preferred for nut production due to consumer preference for larger nut size and being easier to peel (Aguilar et al. 2009). Additionally, Darling 58 American chestnut was developed as a non-profit project for forest restoration. Its attributes have yet to be established for timber or nut production and so the risk of using Darling 58 American chestnut in a commercial venture is likely too high for adoption in the foreseeable future.
Trade Economic Environment	Chestnut production in 2018 was 2.4 million MT, more than any	Darling 58 American chestnut is not expected to alter the trade

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
	other tree nut except coconut (Davison et al. 2021a). Most of this production is in China. The United States does not have a significant chestnut industry, less than one percent of total world production (AgMRC 2021). The United States has 1,587 farms producing chestnuts on 4,228 acres (USDA-NASS 2019). It should be noted that due to chestnut blight these figures are primarily for Chinese chestnut and not for American chestnut. A few cultivars in production in the US are hybrids between American chestnut and Chinese chestnut or three-way crosses between American Chestnut, Chinese Chestnut and Japanese Chestnut (Revord et al. 2021).	economic environment for chestnut as it is not expected to be used in commercial plantings. Most growers consider Chinese chestnuts to be the best option currently available for establishing profitable orchards in eastern United States (Davison et al. 2021a). Darling 58 American chestnut was developed as a non-profit project for forest restoration. Its attributes have yet to be established and it is susceptible to other diseases such as ink disease caused by *Phytophthora cinnamomi* (ESF 2019). From the standpoint of performance characteristics, the risk of using Darling 58 in a commercial venture is much higher than Chinese chestnut. It also lacks some of the quality attributes that consumers prefer (large size and easy to peel) (Aguilar et al. 2009) and because it is created using genetic engineering may have less public acceptance. Darling 58 American chestnut is not expected to adversely impact the biotech sensitive market for chestnut because it is unlikely to cross pollinate or commingle with commercial chestnuts. Darling 58 American chestnut is not expected to be grown in proximity to chestnut species used for commercial purposes. The majority of commercial chestnut operations are outside the native range of American chestnut (USDA-NASS 2019)

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
		where Darling 58 American chestnut is expected to be planted. Commercial chestnut is expected to be predominantly a different species that is unlikely to overlap in flowering time. Effective pollination of chestnut occurs over relatively short distances (within 300 m), so isolation distances are manageable. Growers who cater to a biotech sensitive market are likely to sell direct to customers without going through a supply chain where commingling could happen.
Other Regulatory Approvals		
U.S.	In 2021, ESF initiated a consultation with the FDA on the safety of Darling 58 American chestnut pursuant to the voluntary consultation process for crop plants developed using genetic engineering (21 C.F.R. Parts 192 and 592). A food and feed safety and nutritional assessment of Darling 58 was submitted to the FDA for review (ESF 2021).	In 2021, ESF initiated a consultation with the FDA on the safety of Darling 58 American chestnut pursuant to the voluntary consultation process for crop plants developed using genetic engineering (21 C.F.R. Parts 192 and 592). A food and feed safety and nutritional assessment of Darling 58 was submitted to the FDA for review (ESF 2021).
Compliance with Other Laws		
CWA, CAA, EOs	Fully compliant	Fully compliant

4 AFFECTED ENVIRONMENT AND ENVIRONMENTAL CONSEQUENCES

4.1 Scope of Analysis

Evaluation of the Potential Impacts of Agency Action

An impact would be any change, beneficial or adverse, from existing (baseline) conditions described for the affected environment. Thus, impacts or effects means changes to the human environment that could result from approval of the petition, release of Darling 58 American chestnut and subsequent recolonization of eastern forests by American chestnut.

Pursuant to CEQ regulations (40 CFR § 1508.1(g), impacts/effects considered are those that are reasonably foreseeable and have a reasonably close causal relationship to the petition decision. Impacts/effects may occur soon after the Agency decision or occur later in time. Potential impacts/effects include ecological (such as the effects on natural resources and on the components and functioning of affected ecosystems), historic, cultural, social, or effects on public health. Economic effects, such as those on employment or markets, may also be considered. Impacts/effects include those resulting from actions that may have both beneficial and detrimental effects, even if on balance the agency believes that the effect will be beneficial (40 CFR § 1508.1(g)).

In considering whether the effects of the proposed action are significant, agencies are to analyze the potentially affected environment, and degree of the effects of the action in relation to the affected environment (40 CFR § 1501.3). Agencies should also consider connected actions consistent with 40 CFR§ 1501.9(e)(1). The potentially affected environment (summarized below) is defined by the area(s) potentially impacted by the proposed action (e.g., national, regional, or local), and associated resources (e.g., natural, cultural). In considering the degree of the effects, agencies are to consider the following, as appropriate to the proposed action:

- Short- and long-term effects.
- Both beneficial and adverse effects.
- Effects on public health and safety.
- Effects that would violate federal, state, tribal, or local laws protecting the environment.

Potentially Affected Environment

The potential environmental impacts of a forest tree developed using genetic engineering occur within the context of a forest's contribution to environmental change. Potential effects on the environment will depend on the success of Darling 58 chestnut to survive and spread over time. The former range of American chestnut covered approximately 200 million acres of land (Saucier 1973) along the Appalachian range, thus, the scale of potential impacts, should take into account the current state of eastern forests as well as the potential future forest systems should Darling 58 American chestnut be a successful restoration tree.

USDA-Forest Service defines a forest as a land cover type that is at least 10 percent stocked by single stemmed forest trees of any size that will be at least 4 meters tall at maturity (Wear 2013). Another characteristic of forests is that when viewed vertically, there is typically a 25 percent or greater canopy cover (Wear 2013). Forests provide valuable services in maintaining optimal air, water, and soil quality. Additionally, forests provide resources necessary to maintain animal and plant communities. Forest biodiversity is dependent on the intensity of management within the forest and is generally greater where forests are more structurally diverse.

Darling 58 American chestnut is intended to be used as a restoration tree to establish and colonize much of the eastern United States where stunted trees persist, once again becoming a self-sustaining forest tree species within its native range. It is within this context that APHIS evaluates the potential impacts of Darling 58 American chestnut on the human environment if released for restoration purposes.

4.2 Action Area

4.2.1 Previous Distribution

American chestnuts are a member of the genus *Castanea* in the plant family *Fagaceae*. *Fagaceae* contains approximately 900 species worldwide in 8 to 10 genera (Kremer et al. 2012), consisting of evergreen and deciduous trees and shrubs. The American chestnut was once one of the most abundant trees within its range in the eastern United States. American chestnut was found at every elevation from sea level to over 5000 ft. from as far north as Maine and Ontario, Canada to as far south as Georgia and Mississippi, covering an approximate 200 million acres of land (Saucier 1973).

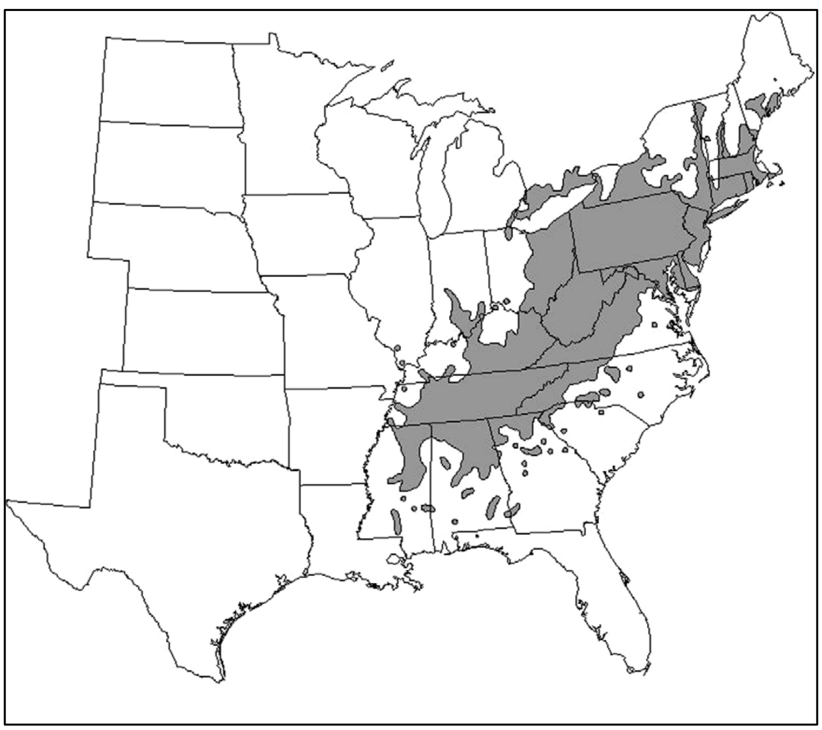

Figure 1. Natural range of American chestnut. (Jacobs 2007)

American chestnut was the predominant forest tree species in terms of both stand density and stature (Buttrick 1915; Braun 1950). The American chestnut was a fast-growing and long-lived canopy tree that produced a consistent crop of nuts, could be harvested for lumber, and was considered a keystone species for wildlife. In many areas, especially on mountain slopes in the Appalachian range, American chestnut was the dominant forest tree. Reports estimated that American chestnut lived several hundred years, up to an age of 400 to 600 years, in the southern Appalachians, though trees over 100 years of age, grow hollow in the center while continuing to grow in diameter (Zon 1904; Buttrick 1925). American chestnut trees were commonly recorded at heights of 70 to 100 feet, with diameters of 3 to 5 feet or more (Detwiler 1915; Buttrick 1925; Smith 2000).

4.2.2 Current Distribution and Blight

The range of American chestnut was naturally expanding northwestward at the time of blight introduction (Brewer 1995). The American chestnut is now found in the lower peninsula of Michigan (Brewer 1995), southwestern Wisconsin (Paillet and Rutter 1989), Illinois (Russell 1987), Iowa (Russell 1987; Farrar 2001), Louisiana, Missouri, and Florida (Dalgleish et al. 2015a). The observed expansion is presumably due to naturalized populations from historic plantings. Some trees planted in the 1800s in the Pacific Northwest remain alive and blight-free today (Gillis 2017).

Commercial chestnut production in the United States extends outside the native range of American chestnut (Michigan, California, Iowa, Ohio, and Florida are the top 5 producers (USDA-NASS 2019) and largely utilizes a different chestnut species, Chinese chestnut (*C. mollissima*), due to its superior resistance to chestnut blight. Domestic chestnut production also includes hybrids between Chinese chestnut and American chestnut (*C. dentata*) and triple hybrids between Chinese, American, and Japanese chestnut (*C. crenata*) (Revord et al. 2021). The hybrids have increased resistance to blight compared to American chestnut.

In the first decade of the twentieth century, a fungal canker disease was discovered in the Bronx Zoological Park, New York, which was disfiguring and quickly killing American chestnut trees (Merkel 1905). The fungus was described by mycologist William Murrill (Murrill 1906)) as *Diaporthe parasitica*, which was soon reclassified *Endothia parasitica* (Anderson and Anderson 1912) and later *Cryphonectria parasitica* (Murrill) Barr. (herein referred to as *C. parasitica*). In the decades following its discovery, the blight spread throughout the American chestnut range and killed over 3 billion trees. By 1940 virtually all American chestnut was considered functional extinction due to blight (Saucier 1973).

The blight fungus infects the stem through wounds or cracks in the bark and forms cankers on the aboveground portions of the plant. The cankers girdle and kill living stems. The pathogen kills living tissue primarily by secreting a toxin called oxalic acid (McCarroll and Thor 1978). Although the aboveground portion of the plant is killed by the fungus, the plants have the ability to sprout from the root collar and lower portion of the stem.

As a consequence of blight, the abundance of chestnut has drastically declined; the number of live stems today is estimated at 431 ± 30.2 million, approximately 10% of the pre-blight population (Dalgleish et al. 2015a). While data on the size class distribution before the blight is limited, the current population is highly skewed toward small trees with the vast majority of stems (estimated 84%) less than 2.5cm diameter at breast height (dbh) (Dalgleish et al. 2015a). The American chestnut has been reduced from a dominant overstory tree to a small understory shrub (Elliott and Swank 2008; Dalgleish et al. 2015b). Chestnuts were replaced mainly by oak and maple, and to a lesser extent, hickory, birch, black cherry, and others as dominant canopy tree species (Keever 1953; Stephenson 1986; Stephenson et al. 1991; Brewer 1995). No single species has emerged in a dominant role across a broad geographic range, comparable to the pre-blight status of American chestnut.

Under the No Action Alternative, Darling 58 American chestnut would remain regulated and would not be able to be planted without APHIS authorizations. American chestnut would remain stumps and small understory shrubs (Elliott and Swank 2008; Dalgleish et al. 2015b) in the forests of the eastern United States where populations persist.

4.2.3 Potential Future Distribution

American chestnuts are still found wherever American chestnut was a canopy tree before the blight. They persist as stumps and small trees, often multi-stemmed, as a result of the blight, and no longer occupy a dominant canopy position (Keever 1953; Woods and Shanks 1959; Good 1968; Mackey and Sivec 1973; Stephenson 1986; Elliott and Swank 2008; Ireland et al. 2011). If Darling 58 American chestnut shows enhanced tolerance to the fungus *C. parasitica*, they may be able to establish and colonize much of the eastern United States where populations persist as stunted trees, becoming a self-sustaining forest tree species in its native range. Given the historically slow spread rates, low propagule pressure, and need for disturbance to provide sufficient light for fast growth, the rate of increase would be very slow. Without aggressive restoration efforts, requiring considerable effort and coordination at landscape scales, it may require, centuries before American chestnut becomes a significant presence in the landscape (Gustafson et al. 2017; Gustafson et al. 2018). Despite the slow spread, the action area for this EIS are those areas of the United States where American chestnut was once a dominant forest tree.

<u>Forest Restoration with American Chestnut</u>

American chestnut is a fast-growing species with the ability to persist in shaded conditions and it responds favorably to forest management techniques that limit competition and increase available sunlight (Wang et al. 2013). One model found that an aggressive planting effort would be required to restore chestnut to its former prominence and that without this aggressive restoration effort, requiring considerable effort and coordination at landscape scales, it could take a millennium or more for chestnut to fully occupy its former landscapes (Gustafson et al. 2017).

American chestnut is a prolific seed producer able to produce fruit as early as age 4 when open-grown, or at about 8 to 20 years old when competing with other trees in the forest (Paillet and Rutter 1989). Reproduction starts earlier and yields more nuts in trees reproduced by coppice than in trees regenerated from seed (Wang et al. 2013). Chestnuts were historically an important food for wildlife, to the point that seed predation combined with insect damage likely made natural regeneration from seeds rare pre-blight (Detwiler 1915). As a result of the high demand for chestnuts by wildlife and humans, Paillet and Rutter (1989) reported that only 1 to 5 viable seeds per tree germinated into seedlings that survived for more than 1 year. Additionally, small seedlings are easily killed by fire or frosts (Wang et al. 2013). As a result, artificial regeneration of nursery-grown seedlings will be a crucial component of restoration.

Seeds may be sown directly in the soil or started in containers after cold stratification. Seedlings typically grow rapidly and with good form, supported by the seed's supply of carbohydrates and nutrients (ESF 2019). Once sufficient lines of blight-resistant seedlings are developed and tested, they can be planted, cross-pollinate with one another, and sexually reproduce. However, restoration of this species will probably always involve planting, due to the loss of seedlings from nut predation and high seedling mortality (Wang et al. 2013).

Chestnut is self-infertile, so trees will only produce seed when a sexually compatible, flowering tree is close enough to pollinate female flowers. Awareness of effective pollination distance is crucial in planning restoration plantings and seed production orchards and will be used by managers to predict and control the hybridization of various chestnut species and varieties (ESF 2019). If Darling 58 American chestnut is granted nonregulated status and shows enhanced tolerance to the fungus *C. parasitica*, the trees may be able to establish and colonize much of the eastern United States where stunted trees persist, once again becoming a self-sustaining forest tree species within its native range in the long term (centuries). Given the historically slow spread rates, low propagule pressure, and need for disturbance to provide enough light for fast growth, chestnut is not likely to spread significantly in the short term (decades).

4.3 Physical Environment

4.3.1 Soil Quality

Soil consists of solids (minerals and organic matter), liquids, and gases. This body of inorganic and organic matter is home to a wide variety of fungi, bacteria, and arthropods, as well as the growth medium for terrestrial plant life (USDA-NRCS 2004). Soil is characterized by its layers that can be distinguished from the initial parent material due to additions, losses, transfers, and transformations of energy and matter (USDA-NRCS 2010). It is further distinguished by its ability to support rooted plants in a natural environment. Soil plays a key role in determining the capacity of a site for biomass vigor and production in terms of physical support, air, water, temperature moderation, protection from toxins, and nutrient availability. Soils also determine a site's susceptibility to erosion by wind and water, and a site's flood attenuation capacity. Soil health may be monitored as an indicator of overall environmental health. Natural conditions and anthropogenic actions, such as soil preparation, planting, cultivating and irrigation, continuously affect and determine soil health, which in turn can alter the global environment (Lal 2008).

Soil properties including temperature, pH, soluble salts, the amount of organic matter, the carbon nitrogen ratio, the numbers of microorganisms, and soil fauna all vary seasonally, as well as over extended periods of time (USDA-NRCS 1999). Soil texture and organic matter levels directly influence its shear strength, nutrient holding capacity, and permeability. Soil taxonomy was established to classify soils according to the relationship between soils and the factors responsible for their character (USDA-NRCS 1999). Soils are organized into four levels of classification, the highest being the soil order. Soils are differentiated based on characteristics such as particle size, texture, and color, and classified taxonomically into soil orders based on observable properties such as organic matter content and degree of soil profile development (USDA-NRCS 2010). The Natural Resources Conservation Service (NRCS) maintains soil maps on a county level for the entire United States and its territories (Palm et al. 2007; USDA-NRCS 2010).

Although adapted to a variety of site conditions, American chestnut commonly grows on sandy loams in association with other hardwoods (Saucier 1973). American chestnut trees prefer well-drained, sandy, and slightly acidic (i.e. pH of 5 to 6) soils, often on slopes and ridges (Russell 1987; Wang et al. 2013). Alkaline or limestone-derived soils, or very wet or dry soils, do not support chestnut colonization (Paillet 2002).

American chestnut may have affected nutrient cycling and soil chemistry (Ellison et al. 2005). Forest ecosystem processes, including decomposition, nutrient cycling, and productivity, likely changed following chestnut's replacement by other species because American chestnut had a rapid growth rate and sprouting ability, wood with an extremely high tannin content, and leaves with a relatively low carbon to nitrogen ratio which influenced these ecosystem processes (Ellison et al. 2005). Decomposition of chestnut wood is much slower than other co-occurring hardwoods and its high tannin concentrations could restrict the mobilization of nutrients in soils. Additionally, chestnut's fast growth rate might have resulted in rapid sequestration of carbon and nutrients (Ellison et al. 2005).

Chestnut leaf litter decomposes more rapidly in the first year than oak or cherry leaf litter, and soils with chestnut leaf litter were shown to have lower nitrogen leaching rates and greater dissolved organic carbon than soils with cherry or oak leaf litter (Schwaner and Kelly 2019). These differences represent a potential for increased storage of carbon in surface soil of forests with successful introduction of Darling 58 American chestnut as the microbial community accumulates biomass in a nitrogen-limiting environment. Studies conducted by the petitioner showed there were no significant differences in colonization by ectomycorrhizal fungi in roots compared to non-transgenic controls, suggesting that the presence or expression of OxO in Darling 58 American chestnut does not pose risks to native soil fungi that are ecologically important for American chestnuts and other trees (ESF 2019). Introducing Darling 58 American chestnut back into eastern forests is unlikely to have negative impacts on soil quality.

4.3.2 Water Resources

The principal law governing the nation's water resources is the Federal Water Pollution Control Act of 1972, better known as the Clean Water Act. The Clean Water Act establishes water quality standards, permitting requirements, and monitoring to protect water quality. The EPA sets the standards for water pollution abatement for all waters of the United States under the programs contained in the Clean Water Act, but, in most cases, gives qualified states the authority to issue and enforce permits. Drinking water is protected under the Safe Drinking Water Act of 1974 (Public Law 93-523, 42 U.S.C. 300 *et seq.*) (US-EPA 2012).

Surface water in rivers, streams, creeks, lakes, and reservoirs support everyday life through the provision of water for drinking and other public uses, irrigation, and industry (USGS 2015). In 2010, about 75 percent of the freshwater used in the United States came from surface water sources, whereas the other 25 percent originated from groundwater (USGS 2015). Groundwater is water that flows underground and is stored in natural geologic formations called aquifers (USGS 2015). In the United States, approximately 40 percent of the population depends on groundwater for its drinking water supply (USGS 2018). Currently, the largest use of groundwater in the United States is irrigation, representing approximately 70 percent of all the groundwater pumped each day (USGS 2018).

All water in forested lands contain organic matter, inorganic matter, and dissolved gasses derived from the environment, organisms, and anthropogenic activities. The concentrations of all these substances, in addition to their biological, physical, and chemical effects, are the basic criteria of water quality (Chang 2013). Surface and groundwater resources are key outputs of forests. These water resources are essential to ecosystem processes and functions across the action area.

American chestnut is a relatively drought tolerant species, as suggested by its historical dominance on upland sites with well-drained, sandy soils (Wang et al. 2013). During an early-season drought in a hardwood forest in Pennsylvania, American chestnut saplings maintained higher leaf water potential than several associated oak (Quercus) species that are known for their drought tolerance (Abrams et al. 1990). When compared to co-occurring hardwood species American chestnut seedlings reported high water use efficiency during exposure to drought (Bauerle et al. 2006).

Drought stress or overwatering was occasionally unintentionally applied during growth chamber, greenhouse, and outdoor care of chestnuts by the petitioner, but Darling 58 offspring were not observed to respond any differently than non-transgenic relatives to these stresses (ESF 2019). Intentional experiments were not conducted by the petitioner on Darling 58 regarding abiotic stress tolerance, but numerous anecdotal observations on combined batches of transgenic and non-transgenic chestnuts have not shown obvious differences (ESF 2019).

If blight-tolerant American chestnut were to become established as an important canopy tree, it would begin to influence ecosystem structure and function in these areas, as it did prior to the blight (Paillet 2002). Chestnut leaf litter may alter aquatic ecology. Chestnut leaf litter decomposes more rapidly in the first year than oak or cherry leaf litter, and soils with chestnut leaf litter were shown to have lower N leaching rates and greater dissolved organic carbon than soils with cherry or oak leaf litter (Schwaner and Kelly 2019), potentially impacting water quality through a change in nutrient levels.

Introduction of Darling 58 American chestnut to eastern forests should not impact overall water resources in the area. As noted above, American chestnut does not have different water requirements than those tree species currently found in eastern forests. If American chestnut were to become established throughout its previous range, it is anticipated that the impacts to water quality would be similar to what it was prior to the blight (Paillet 2002), altering aquatic ecology and changing nutrient levels. These long-term impacts would be dependent on the location and density of American chestnut trees.

4.3.3 Air Quality and Climate Change

The Clean Air Act (CAA) requires the maintenance of National Ambient Air Quality Standards (NAAQS). The NAAQS, developed by the EPA to protect public health, establishes limits for six criteria pollutants: ozone, nitrogen dioxide, carbon monoxide, sulfur dioxide, lead, and Particulate Matter (US-EPA 2020a). The CAA requires states to achieve and maintain the NAAQS within their borders. Each state may adopt requirements stricter than those of the national standard and each is required by the EPA to develop a State Implementation Plan that contains strategies to achieve and maintain the national standard of air quality within the state. Areas that violate air quality standards are designated as non-attainment areas for the relevant pollutants, whereas areas that comply with air quality standards are designated as attainment areas (US-EPA 2020b).

Forests play a role in improving local and regional air quality. Trees can absorb or trap nitrogen dioxide, sulfur dioxide, and particulate matter 10 microns or less in size (Hanson et al. 2010). Rates of airborne pollution removal vary based on the pollutant type, leaf season length, and precipitation levels. Trees remove air pollution by the interception of particulate matter on plant surfaces and the absorption of gaseous pollutants (ozone, nitrogen dioxide, and sulfur dioxide) through the leaf stomata (Nowak et al. 2014). In 2010, trees and forests in the conterminous United States are estimated to have removed 17.4 million tonnes (t) of air pollution, with the ozone and nitrogen dioxide accounting for the greatest amount of pollution removal (Nowak et al. 2014). The impacts from introducing Darling 58 American chestnut into eastern forests is unlikely to change the role forests play in regulating air quality.

Climate change represents a statistical change in global climate conditions, including shifts in the frequency of extreme weather (Rosenzweig et al. 2001). The EPA has identified carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) as the key greenhouse gases (GHG) contributing to climate change. Greenhouse gases, including CO_2, CH_4, and N_2O, function as retainers of solar radiation (Aneja et al. 2009). While each of these gases occurs naturally in the atmosphere, human activity has significantly increased the concentrations of these gases since the beginning of the industrial revolution. The primary sources of GHG emissions in the United States are: Transportation (29%), Electricity production (25%), Industry (23%), Commercial and Residential (13%), and Agriculture (10%). In 2019, U.S. greenhouse gas emissions totaled 6,558 million metric tons of CO_2 equivalent (CO_2–eq), or 5,746 million metric tons of CO_2-eq after accounting for sequestration from the land sector (US-EPA 2021).

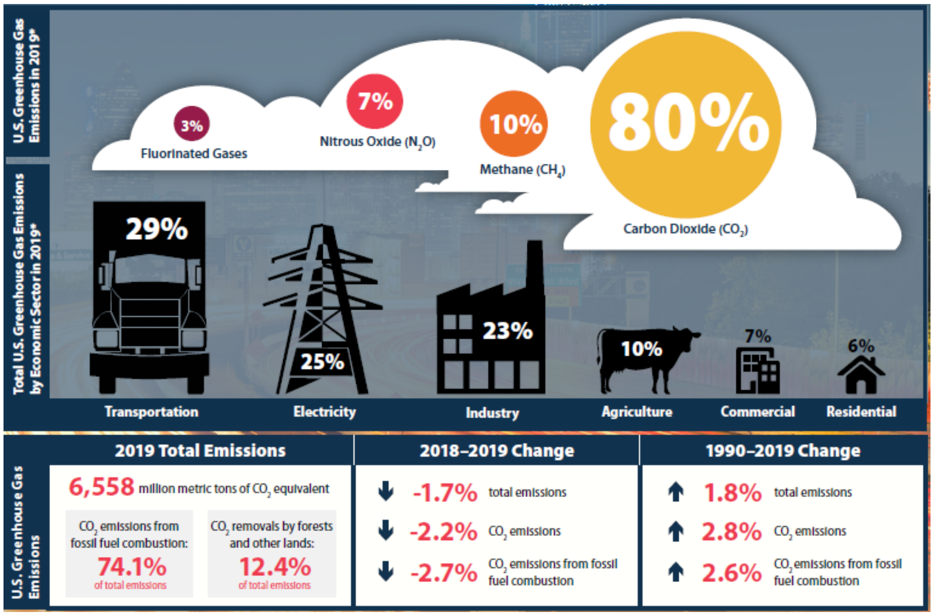

Figure 2. Sources of U.S. Greenhouse Gas Emissions
Source: (US-EPA 2021)

Forests play an important role in global climate regulation. In 2007, U.S. forests absorbed an estimated 910 million metric tons of carbon dioxide equivalent, an amount equal to approximately 13 percent of the country's gross greenhouse gas emissions from industrial and other sources (Hanson et al. 2010). Carbon sequestration is an important ecosystem service provided by forests globally and represents a driving motivation for reforestation and conservation efforts worldwide.

Because American chestnut is fast-growing, long-lived, and resistant to decay (Ellison et al. 2005; de Bruijn et al. 2014), its reintroduction could result in increased carbon sequestration and storage in the form of living and dead trees and durable wood products (Gustafson et al. 2017). Given relatively low decay rate of chestnut wood (de Bruijn et al. 2014), this carbon also would be expected to remain in storage for a longer period (Gustafson et al. 2017). Darling 58 American chestnut may provide rapid sequestration of carbon and nutrients, increasing long term carbon storage, especially when used in afforestation (Ellison et al. 2005; Gustafson et al. 2017).

4.4 Biological Resources

This section provides a summary of the biological environment and includes an overview of animals, plants, gene transfer, weeds and weediness, and biodiversity, as well as an assessment of the potential impacts to plant and animal communities and the potential for gene movement from a determination of nonregulated status of Darling 58 American chestnut.

4.4.1 Animal Communities

Animal communities include wildlife species and their habitats. Wildlife refers to both native and introduced species of mammals, birds, amphibians, reptiles, invertebrates, fish and shellfish. Animals that might be exposed to American chestnut would be individuals of species that typically inhabit eastern forests or feed on chestnuts.

Pre-blight, American chestnut was described as "the most important wildlife plant in the eastern United States" (Davis 2005). American chestnut produced a heavy mast crop of calorie packed seeds. Chestnut mast production in pre-blight forests was not directly measured, but estimates range from 270 kg/ha to 2500 kg/ha (Diamond et al. 2000; Gilland et al. 2012). Annual hard mast output is highly variable for most species and usually less than that produced by American chestnut, fluctuating between 9.5 kg/ha and 924.5 kg/ha annually for hickory, black oak, northern red oak, chestnut oak, white oak and scarlet oak combined (Diamond et al. 2000). American chestnut disappeared from forests before many systematic studies of wildlife food habits were undertaken (Hill 1992), but we know that native mammals such as white-tailed deer (*Odocoileus virginianus*), cottontail rabbit (*Sylvilagus floridanus*), and black bears (*Ursus americanus*) all consumed chestnuts (Diamond et al. 2000; Wang et al. 2013), as well as many other vertebrates including rodents (Lichti et al. 2014) and birds including wild turkeys (*Meleagris gallopavo*), American crow (*Corvus brachyrhynchos*), blue jay (*Cyanocitta cristata*), ruffed grouse (*Bonasa umbellus*), and the extinct passenger pigeon (*Ectopistes migratorius*) (Webb 1986; Russell 1987) and heath hen (Hill 1992).

Like many other large-seeded trees, American chestnut seeds were dispersed by wildlife species that included birds and squirrels (*Sciurus* spp.). The American crow, blue jay, wild turkey, ruffed grouse and passenger pigeon were major dispersers of large fruit and probably important for chestnut dispersal (Webb 1986; Russell 1987). Large mammals such as white-tailed deer or black bear may have also played an important role in the dispersal of American chestnut, as the bur is thought to be an evolutionary adaptation for hitchhiking on mammalian fur (Wang et al. 2013).

American chestnut provided a food source to numerous insect species, especially during the flowering period. Pollen feeders in the insect orders coleoptera (beetles), lepidoptera (moths) and hymenoptera (bees) have been observed visiting chestnut catkins (Clapper 1954; Opler 1978; Hasegawa et al. 2015; Tumminello 2016; Zirkle 2017). In addition to these observed interactions, chestnut pollen has been shown to be especially nutritious to bumble bees (*Bombus terrestris*) (Tasei and Aupinel 2008). Chestnut trees and seedlings are also known to be a food source and subject to damage by native insects including chestnut sawfly (*Craesus castaneae*) and periodical cicadas (*Magicicada spp.*), as well as non-native insects including Asiatic oak weevil (*Cyrtepistomus castaneus*) and chestnut gall wasp (*Dryocosmus kuriphilus*) (Cook et al. 2013; Clark et al. 2014; Clark et al. 2015). There is evidence suggesting some native insect species followed the tree to functional extinction (Opler 1978).

Forest leaf litter can be an important food source for invertebrate communities. A study by Smock and MacGregor (1988) on leaf litter nutritional quality showed that American chestnut leaf litter is of similar quality to that of pignut hickory (*Carya glabra*), both of which are of better nutritional quality than northern red oak leaf litter. In a laboratory experiment, leaf shredding stream invertebrates preferred chestnut and hickory leaf litter to oak, and growth rates were faster on chestnut and hickory than on oak. Oaks are the most important tree species to replace chestnut, with hickory and other species locally important in some areas (Smock and MacGregor 1988). In areas where oaks replaced chestnut, the change in leaf litter quality would have directly impacted leaf shredding insect populations, as well as downstream impacts such as reduction in fine particulate organic matter released as a byproduct of feeding (Smock and MacGregor 1988).

Preliminary laboratory tests of aquatic insect herbivory on various types of deciduous leaves indicated that mayfly (*Frenesia difficilis*) larvae preferred American chestnut leaves to most other leaves commonly found in eastern forests. Nine leaf types were studied: American beech (*Fagus grandifolia*), American chestnut, American sycamore (*Platanus occidentalis*), chestnut oak *(Quercus montana*), combined red and sugar maple (*Acer rubrum* and *Acer saccharum*), shagbark hickory (*Carya ovata*), tulip-tree (*Liriodendron tulipifera*), and white oak (*Quercus alba*). Only maple and shagbark hickory were preferred over American chestnut by mayfly larvae (ESF 2019).

Since American chestnut was considered a keystone species, it played a critical role in the function of the overall ecosystem. The loss of American chestnut and changes in food availability had far reaching effects, and may have contributed to more unstable, less resilient community dynamics (Kelly et al. 2008; Dalgleish and Swihart 2012). If Darling 58 American chestnuts were to become established, it would influence ecosystem structure and function in areas where they were introduced (Paillet 2002). Since American chestnut provided a more stable and more abundant source of mast than oaks, hickory, and beech species (Diamond et al. 2000), if Darling 58 American chestnuts were to become established it could result in population increases of the species that feed on chestnut (Hill 1992). The increase would be most pronounced during years of low seed production by other masting species, resulting in less fluctuation in those species that consume seeds (Dalgleish and Swihart 2012).

Higher populations could increase the pest potential of some species, and human-wildlife interactions could increase. Other indirect and complex consequences may result from changes in animal populations such as rodents. Higher rodent populations could potentially lead to increased pressure on songbirds as generalist predator populations increase, reduced gypsy moth (*Lymantria dispar*) outbreaks (moth pupae are eaten by mice), and increased Lyme disease risk to humans (Dalgleish and Swihart 2012).

The replacement of oak by Darling 58 American chestnut may increase macroinvertebrate activity, with potential consequences on population, community, and ecosystem levels since chestnut leaf litter is of higher nutritional value for aquatic macroinvertebrates than oak (Smock and MacGregor 1988). Additionally, slow decaying chestnut wood may increase stream channel complexity as it replaces faster decaying species, providing additional habitat for fish and invertebrates (Ellison et al. 2005).

Animal communities within Eastern forests are likely already exposed to the Oxalate oxidase gene in Darling 58 American chestnut. Oxalate oxidase is a common enzyme found in all grains, several other crops and food products, and many wild plants and microbes. A list of cultivated and wild plants containing the OxO gene can be found in Tables 4.2a and 4.2b in the petition (ESF 2019). Additionally, nutrition analyses have confirmed that transgenic chestnuts are not nutritionally different than their wild-type relatives (ESF 2019). No impacts are expected to animal communities from exposure to the OxO gene in Darling 58 American chestnut.

4.4.2 Plant Communities

As noted in Section 4.2 – Action Area, American chestnut can be found in naturalized populations throughout the eastern United States. The plant communities for American chestnut include all of the plants in a particular area, including native, introduced, desirable, and undesirable plants. The plant species in the action area represents a diverse variety of plant species, ranging from understory grasses to overstory trees.

Plant species in a particular area may be generally characterized as forbes, vines, succulents, ferns, grasses, shrubs, and trees (BONAP 2020). The plant classification descriptions below are derived from the Biota of North America Project[4] (BONAP 2020):

- Forbes - Herbaceous plants most commonly with relatively broad, usually pinnately veined leaves (contrasted with parallel-veined in "grass or grass-like" plants), with all perennating or overwintering organs at or below ground level. The forb category, which was originally established in an agricultural context to contrast with grass and grass-like plants, includes a wide range of herbaceous growth habits, especially if aquatic plants are added. Vining, creeping, and trailing herbs also are included within this broad category (in the BONAP system). Plants with annual stems becoming woody at the base are included as forbs. Primarily herbaceous plants bearing terminal buds at the tips of woody caudex branches at or near ground level are referred to the forb category; "cushion plants" belong with these.

- Vines - Perennial plants with woody, above-ground stems that bear overwintering buds and do not die back to a basal stem or rhizome in winter. Trees usually have a single main stem, are at least 4 meters tall, and have a more or less distinct and elevated crown. A few species produce normally short-lived but rapidly growing plants that occasionally attain tree-like proportions (e.g., *Ricinus communis*).

- Succulents – Plants with stems and leaves that are very soft, fleshy, and often filled with juice or sap.

- Ferns - Spore-producing but flowerless and seedless vascular plants that are usually differentiated into roots, stems, and leaf-like fronds.

- Grasses - Herbaceous plants with long, narrow, entire, parallel-veined leaves, often produced in a basal cluster, with all perennating or overwintering organs below the ground. The flowers of these plants usually are reduced in complexity and thus inconspicuous. Grasses and grass-like plants include all members of the monocot families Cyperaceae, Juncaceae, Juncaginaceae, and Poaceae, some members of the Liliaceae, and all members of the pteridophyte family Isoetaceae, but similar leaved-species occurring in numerous dicot families were not scored.

- Shrubs - Perennial plants with woody, above-ground stems that bear overwintering buds relatively evenly positioned on the stems and do not die back to a basal stem or rhizome in winter. Shrubs are multi-stemmed from the ground, generally attaining a low stature (variable in size but usually under 5 meters tall), and producing a poorly-defined crown. Some shrubs may be creeping (e.g., Juniperus horizontalis, Gaultheria hispidula); others

[4] Additional information on plant classes can be found on the BONAP website. http://www.bonap.org/ Last accessed September, 2020.

may be "mat-like" or "mound-like" (e.g., Arctostaphylos nevadensis). Various exceptional species are also placed here (e.g., Coreopsis gigantea, a "fleshy-stemmed shrub"; Coreopsismaritima, a "hollow-stemmed shrub"; Leucanthemum nipponicum, a "soft shrub"), and some primarily shrubby species that occasionally reach tree size are also characterized as trees

- Trees - Perennial plants with woody, above-ground stems that bear overwintering buds and do not die back to a basal stem or rhizome in winter. Trees usually have a single main stem, are at least 4 meters tall, and have a more or less distinct and elevated crown. A few species produce normally short-lived but rapidly growing plants that occasionally attain tree-like proportions (e.g., *Ricinus communis*).

As noted above in Section 4.2.3, if Darling 58 American chestnut is granted nonregulated status and shows enhanced tolerance to the fungus *C. parasitica*, the trees may be able to establish and colonize much of the eastern United States where stunted trees persist, once again becoming a self-sustaining forest tree species within its native range. Models have shown that to restore American chestnut to its former prominence it would require an aggressive planting effort and without that aggressive planting effort it could take a millennium or more for American chestnut to fully occupy its former landscapes (Gustafson et al. 2017).

With an increase in American chestnut, some co-occurring tree species would gradually decline, the most likely being the same trees that replaced American chestnut after chestnut blight was introduced. American chestnut will likely replace other tree species in proportion to their abundance, rather than replacing a single species or genus (Gustafson et al. 2017). Given the relatively close overlap between the niches of chestnut and oak (Keever 1953) competition from chestnut would likely affect oaks more than other species (Gustafson et al. 2017). There is no reason to believe that the reintroduction of American chestnut would result in reduction of any competing tree species to threatened or endangered levels.

4.4.3 Gene Flow and Weediness

Gene flow is a biological process that facilitates the production of hybrid plants, introgression of novel alleles, and evolution of new plant genotypes. Gene flow to and from an ecosystem can occur on both spatial and temporal scales. In general, plant pollen tends to represent the major reproductive method for moving across areas, while both seed and vegetative propagation tend to promote the movement of genes across time and space.

American chestnut is primarily wind-pollinated (Clapper 1954; Johnson 1988), though, insects, especially bees, likely play a role (Clapper 1954). Multiple bee species have been observed visiting catkins on American chestnuts and other chestnut species (Hasegawa et al. 2015; Tumminello 2016; Zirkle 2017). Chestnut species are considered self-incompatible (Clapper 1954; Russell 1987) although there is at least one report that self-fertilization may occur rarely in American chestnut, with < 1% to perhaps < 5% of the tree’s flowers (Rutter 1990).

To have high pollination rates, chestnut trees need to be within 30 to 100 m apart and trees further than 300 to 400 m apart will generally not pollinate each other (Forest et al. 1977; Russell 1987; Rutter 1990). Chestnut pollen can travel up to 100 km but effective pollination distances are much shorter due to rapid desiccation as pollen viability decreases with time spent in the air (Fernandez-Lopez and Alia 2003).

American chestnut can outcross to other chestnut species, including Chinese chestnut (*C. mollissima*), Japanese chestnut (*C. crenata*), European chestnut (*C. sativa*), and chinquapin (*C. pumila*) (Jaynes 1964) to form hybrids. Darling 58 chestnuts have produced offspring through controlled pollinations with C. dentata x C. mollissima F1 hybrids, Allegheny chinquapin, and European chestnut (ESF 2019). Additionally, hybridization has been shown to occur between American chestnut and other *Castanea* species where their distributions overlap. Although chestnut species are sexually compatible, crossing efficiency in the wild between species is less efficient than within the species because flowering times often are not coincident (Pennsylvania Chapter The American Chestnut Foundation 2006).

In the central and southern Appalachians, a hybrid population, *C. x neglecta,* has been described as plants with intermediate morphology between American chestnut and Allegheny chinquapin; (Hardin and Johnson 1985; Johnson 1988). Others have described a taxon called *C. x alabamensis* (Elias 1971), which may be another hybrid, though it has also been considered an isolated population of *C. ozarkensis* (Johnson 1988), or an entirely separate species (Ashe 1923; Graves 1950). More recent analyses of *C. x alabamensis* confirm it is morphologically and phylogenetically unique from *C. dentata* and not likely a hybrid, but leave its species status unresolved (Perkins 2016; Perkins et al. 2019). The evolutionary history of North American *Castanea* species is complicated by recent and past hybridization and incomplete lineage sorting, and is still not fully understood (Shaw et al. 2012).

Under the Preferred Alternative, pollen mediated gene flow from Darling 58 American chestnut to wild American chestnut populations is intended. To restore American chestnut to the landscape, ESF intends to intentionally cross-pollinate Darling 58 American chestnuts with surviving remnant American chestnut populations (ESF 2019). The transgenes from Darling 58 could spread to related species through successful pollination with at least one transgenic parent to produce viable offspring (ESF 2019). Chestnut growers have been managing unwanted pollination in chestnut orchards since pollen from certain hybrid or interspecific crosses can be detrimental to harvests. These same methods for controlling pollination by transgenic chestnuts can be applied to potential restoration programs if needed (ESF 2019).

American chestnut would also likely disperse through the spread of seeds. Chestnut seeds do not survive multiple years in natural conditions, so there is no seed bank (Davelos and JAROSZ 2004). However, rodents, including squirrels, eastern chipmunks (*Tamias striatus*) and mice (*Peromyscus* spp.), cache seeds for future consumption, including American chestnuts (Toumey and Korstain 1947; Lichti et al. 2014) and these seeds may germinate and become established seedlings. Blue jays (Darley-Hill and Johnson 1981; Johnson and Webb III 1989), crows (Zon 1904), and, historically, the now-extinct passenger pigeon (Webb 1986) also likely played a role in dispersing American chestnuts. Whole burs are possibly transported by large mammals such as black bear and white-tailed deer by hitchhiking on the animals fur (Wang et al. 2013).

<u>Weediness of American chestnut</u>

The speed and extent to which a blight-tolerant American chestnut would become established as a dominant canopy tree in today's eastern U.S. forests is difficult to predict. American chestnut has the ability to regenerate vegetatively by sprouting new shoots from the root collar, which allows individual chestnuts to remain alive even in the presence of blight, and allows regeneration after fire (Hawley and Hawes 1918b; Toumey and Korstain 1947; Paillet 2002) or logging (Buttrick 1915; Faison and Foster 2014). The ability of chestnut trees to generate sprouts declines with stem age, but trees over 100 years old may retain the ability to produce sprouts (Zon 1904; Russell 1987). Chestnut does not sprout from roots (Paillet 1984, 1993), so new shoots will be in the immediate location of the former tree; natural dispersal to a new location can take place only by seed.

Since chestnut dispersal is relatively slow, it is likely that at least the first several decades of chestnut restoration will depend on people intentionally planting trees and caring for them (Gustafson et al. 2017). Chestnut cannot be restored in a short time frame and may require considerable effort and coordination at landscape scales (Gustafson et al., 2017).

Areas that are not intentionally planted with blight-tolerant chestnuts will likely remain without chestnuts for decades or longer (ESF 2019). Slow natural colonization rates and frequent animal and pest pressure on seeds and seedlings (Clark et al. 2014) suggest that chestnuts, regardless of type or transgene status, will not rapidly invade new areas (Cook and Forest 1978). A study by Paillet and Rutter (1989) of American chestnut trees outside of their natural range showed that chestnut trees spread at an average rate of "no more than a few kilometers per century" though the rate of spread appeared to be increasing with increased seed production by the established trees. American chestnut has the ecological capacity to achieve canopy dominance on favorable sites, but that it may take a century or more for blight tolerant chestnut trees to become dominant after the first pioneer trees become established in a given area (Paillet and Rutter 1989).

4.4.4 Biodiversity

Biodiversity refers to all plants, animals, and microorganisms interacting in an ecosystem (Wilson 1988). Biodiversity provides valuable genetic resources for crop improvement and also provides other functions beyond food, fiber, fuel, and income (Harlan 1975). The primary function of biological diversity is to contribute to ecosystem services. These ecosystem services may include: pollination, genetic introgression, biological control, nutrient recycling, competition against natural enemies, soil structure, soil and water conservation, disease suppression, control of local microclimate, control of local hydrological processes, and detoxification of noxious chemicals (Altieri 1999). The loss of biodiversity results in a need for costly management practices in order to provide these functions (Altieri 1999).

In a forest ecosystem, biodiversity can be affected by several factors including climatic and soil conditions, evolution, changes in species' geographical ranges, population and community processes, and natural disturbances or those caused by human activities (Carnus et al. 2006).

Impacts to biodiversity are possible with the planting of Darling 58 American chestnut. Pre-blight, American chestnut was described as "the most important wildlife plant in the eastern United States" (Davis 2005), playing a critical role in the function of the overall ecosystem. American chestnut provided a food source to numerous species. If Darling 58 American chestnut is granted nonregulated status and shows enhanced tolerance to the fungus *C. parasitica*, the trees may be able to establish and colonize much of the eastern United States, influencing ecosystem structure and function in areas where they were introduced, as American chestnuts did prior to the blight (Paillet 2002). Since American chestnut provided a more stable and more abundant source of mast than oaks, hickory, and beech species (Diamond et al. 2000), if Darling 58 American chestnuts were to become established it could result in population increases of the species that feed on chestnut (Hill 1992). The increase would be most pronounced during years of low seed production by other masting species, resulting in less fluctuation in those species that consume seeds (Dalgleish and Swihart 2012).

As noted above as American chestnut populations increase, some co-occurring tree species would likely gradually decline. American chestnut will likely replace other tree species in proportion to their abundance, however, given the relatively close overlap between the niches of chestnut and oak (Keever 1953) competition from chestnut would likely affect oaks more than other species (Gustafson et al. 2017). The replacement of oak by Darling 58 American chestnut may increase macroinvertebrate activity, with potential impacts to population, community, and ecosystem levels since chestnut leaf litter is of higher nutritional value for aquatic macroinvertebrates than oak (Smock and MacGregor 1988) and slow decaying chestnut wood may increase stream channel complexity providing additional habitat for fish and invertebrates (Ellison et al. 2005).

While it is difficult to predict what impacts Darling 58 American chestnut will have on forest biodiversity, it is reasonable to believe it will have positive impacts on increasing the biodiversity of animals and micro-organisms while decreasing the abundance of some tree species such as oaks in the long term.

4.5 Human Health

Human health considerations associated with plants developed using genetic engineering are those related to (1) the safety and nutritional value of the plants and their products to consumers, and (2) the potential health effects of pesticides that may be used in association with plants developed using genetic engineering. As for food safety, consumer health concerns are in regard to the potential toxicity or allergenicity of the introduced genes/proteins, the potential for altered levels of existing allergens in plants, or the expression of new antigenic proteins. Consumers may also be concerned about the potential consumption of pesticides on foods derived from plants developed using genetic engineering. In the case of American chestnut; the chestnuts produced could be used for food and feed purposes.

In the United States, plants developed using genetic engineering and other organisms are regulated and evaluated for public health and environmental safety under the Coordinated Framework for the Regulation of Biotechnology, described in Section 1.3. The safety assessment of plants derived through biotechnology includes characterization of the DNA insert or other genetic material, characterization of the biochemical and functional properties of the expressed protein(s), and compositional analysis of the plants developed using genetic engineering.

<u>Food Safety</u>

Under the FFDCA and the Food Safety Modernization Act (FSMA), it is the responsibility of food and feed manufacturers to ensure that the products they introduce into commerce are safe and in compliance with applicable laws and regulations. Organisms developed using genetic engineering for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market (US-FDA 2006). Although a voluntary process, thus far all applicants who wish to commercialize a variety developed using genetic engineering that will be included in the food supply have completed a consultation with the FDA. APHIS considers the voluntary FDA assessment in evaluating the potential impacts of a determination on nonregulated status of a plant or other organism developed using genetic engineering.

Food safety reviews frequently compare the compositional characteristics of the plant developed using genetic engineering with plants not developed using genetic engineering, conventional varieties of that plant. Compositional characteristics evaluated in these comparative tests typically include plant components such as protein, fat, carbohydrates, ash, minerals, dietary fiber, essential and non-essential amino acids, fatty acids, vitamins, and anti-nutrients. Various developers have performed characterization analyses of trait genes and proteins, safety assessments of the genes and proteins, compositional analyses of food and feed, and safety and nutritional assessments of food and feed products derived from plants developed using genetic engineering containing these traits (i.e., those submissions listed at (US-FDA 2020; USDA-APHIS 2021). The FDA evaluates the submission and responds to the developer by letter with any concerns it may have or additional information it may require.

ESF plans to consult with the FDA on the safety of Darling 58 American chestnut pursuant to the voluntary consultation process for crop plants developed using genetic engineering (21 C.F.R. Parts 192 and 592). A food and feed safety and nutritional assessment of Darling 58 will be submitted to the FDA for review (ESF 2019).

In addition, foods derived from plants developed using genetic engineering typically undergo a safety evaluation among international agencies before entering foreign markets, including reviews under Codex Alimentarius guidelines, the European Food Safety Agency (EFSA), and Australia and New Zealand Food Standards Agency (ANZFS) (e.g., see (WHO 2005; FAO 2009; EFSA 2015)).

In general, based on over 15 years of peer reviewed research and regulatory review, rather broad agreement among the scientific and regulatory communities has emerged that food products derived from plants developed using genetic engineering currently on the market are as safe as and nutritionally equivalent to their counterparts not developed using genetic engineering, and pose no more risks than foods derived from conventional crop varieties (e.g., see (CAST 2005; WHO 2005; Batista and Oliveira 2009; Ronald 2011; AAAS 2012; AMA 2012; DeFrancesco 2013; Goldstein 2014; Nicolia et al. 2014), and reviews by FDA (US-FDA 2020), EFSA (EFSA 2015), and ANZFS (ANZFS 2015)).

While the safety of foods derived from current crops developed using genetic engineering has been established through peer reviewed research and regulatory agency reviews (e.g., (WHO 2005; Batista and Oliveira 2009; AAAS 2012; AMA 2012; DeFrancesco 2013; Goldstein 2014), and others), some consumers may worry about potential negative health effects from food derived from plants developed using genetic engineering; such as through the consumption of introduced DNA, or changes in nutritional quality or allergenicity. Consequently, consumer preferences can tend towards avoidance of food derived from plants developed using genetic engineering unless such food contains perceptible benefits (Lucht 2015).

The Darling 58 American chestnut tree does not represent a source of new, potentially allergenic or anti-nutrient proteins; the oxalate oxidase gene and protein are commonly found in a variety of non-allergenic foods (ESF 2019). The OxO enzyme (and its encoding gene) is eaten by over a billion people daily in wheat and other grains, and has not been identified as an allergen in any known reports (ESF 2019). Additionally, the fact that the OxO enzyme is present in corn, rice, sorghum, and many other foods that are not considered allergens (Hefle et al. 1996; FDA 2006) provides support for a lack of allergenicity in OxO.

Oxalate oxidase and similar enzymes are consumed and handled daily in cereal grains and many other foods without any reports of toxicity, and we have found no evidence that it should be considered a toxin (ESF 2019).

Under the Preferred Alternative, potential impacts to human health are not anticipated to be different from those under the No Action Alternative. The presence of the OxO transgene or the transformation process in Darling 58 American chestnut do not cause nutritional differences beyond those already present in traditionally-bred chestnuts (ESF 2019) and is not expected to result in adverse human health effects from direct or indirect human contact.

Other indirect consequences may result from introduction of Darling 58 American chestnut and successful re-establishment of American chestnut to eastern forests. A more stable and more abundant source of mast could result in population increases of the species that feed on chestnut (Hill 1992) including rodents. Potential effects of higher rodent populations could include increased Lyme disease risk to humans (Dalgleish and Swihart 2012). While increased Lyme disease risk is possible, it is unlikely that this risk would be significant given the historically slow spread rates and that the rate of increase would be very slow. The increased mast availability that leads to the increased rodent populations could require centuries.

4.6 Animal Feed

Historically American chestnut was used as feed for hogs (Davis 2005). Unlike other nuts and seeds, chestnut has low calories with rich minerals, vitamins, and monounsaturated fatty acid (Joo et al. 2018). Currently, other varieties of chestnut not meeting quality standards are used as an animal feed supplement for pigs (All About Feed 2011; Joo et al. 2018), have been added to the diet of young poultry and rabbit to enhance the growth performance by the improvement of gastrointestinal microflora stability (Joo et al. 2018), and have been added to the diets of cattle in the last few months of fattening for the market (All About Feed 2011). Additionally tannins derived from chestnuts have been used with calves, sheep, and other animals as an alternative to antibiotics (Liu et al. 2011a; Liu et al. 2011b; Buccioni et al. 2015; Bonelli et al. 2018).

It is unlikely that American chestnut would be used for animal feed in the near future as chestnut dispersal is relatively slow and it will take at least several decades for chestnut restoration to reach the point of having a surplus nut crop. However, nutritional analyses have confirmed that transgenic chestnuts are not nutritionally different than their wild-type relatives (ESF 2019). No impacts are expected to animals from exposure to the OxO gene in Darling 58 American chestnut if used in animal feed.

It is the responsibility of feed manufacturers to ensure that the products they market are safe and properly labeled. Feed derived from American chestnut developed using genetic engineering must comply with all applicable legal and regulatory requirements, which are designed to protect human health. To help ensure compliance, a voluntary consultation process with the FDA may be implemented before release of commodity products with origins from plants developed using genetic engineering as animal feed into the market.

4.7 Socioeconomics

4.7.1 Domestic Economic Environment

American chestnut was a valued eastern hardwood species due to its use as a timber tree, its abundant nut production, and its secondary wood products (Wang et al. 2013). Historically American chestnut was used for construction lumber, shingles, fence posts and rails, telephone and telegraph poles, paneling, trim, furniture, coffins, interior decoration and firewood (Detwiler 1915; Buttrick 1925). American chestnut cordwood supplied tanneries one-half of their raw source of tannin (Buttrick 1925) and was also used in the manufacture of wood pulp (Buttrick 1925).

In the early 1900s, American chestnut made up more than 25 percent of all timber cut in the Southern Appalachians, was the most valuable tree in southern New England, and constituted almost half of the timber cut in Connecticut (Hawley and Hawes 1918b; Hepting 1974; Wang et al. 2013). The timber yield of American chestnut varied greatly depending on the dominance of American chestnut in the stand. Buttrick (1925) estimated that pure stands of American chestnut could yield as high as 20,000 board feet per acre while mixed stands would yield an average of 4,000 board feet per acre. The yield on slopes was estimated at 2,000 to 3,000 board feet per acre and that on ridges was reported at 1,500 board feet per acre (Buttrick 1925). Seven years after discovery of the blight in 1904 it was estimated that the blight had done $25 million in damages to the chestnut industry (Buttrick 1915).

Darling 58 American chestnut would have the same qualities that made American chestnut a valued timber and nut tree pre-blight. The Chinese primarily cultivate chestnuts for nut production (Qin and Feng 2009) and have selected chestnut trees that grow well in orchards, though some varieties of Chinese chestnut were found to have high story canopies suitable for timber (Diller 1947; Commender 2017). European chestnut, which grows about the same size as American chestnut, is grown for nuts, timber, or both (Pereira-Lorenzo et al. 2009). American chestnut, with its vigorous upright growth and open canopy is valued for high quality lumber (Davison et al. 2021a) and the nuts, while sweet tasting, are less valuable for human consumption based on their size.

Chestnuts in Timber Production

In 2012, 76% of timberland forests in the eastern United States were privately owned (58% by non-corporate entities, 24% by corporate entities) and 24% of the timberland forests were on public lands (USDA-FS 2014). In the United States, very few hardwood trees are planted in plantations (Zhang and Polyakov 2010). Most hardwood timberland forests are regenerated through natural means (i.e, seedlings in the understory are given the opportunity to grow upon the selective removal or clear cutting of larger trees) (Mississippi State Extension NA). In cases where the seedlings in the understory are undesirable, landowners may incur the expense of planting seeds or seedlings to improve the quality of the forest (Mississippi State Extension NA). While this is not a common practice for hardwood trees it may become more widely practiced as hardwood trees with improved genetics are developed. Darling 58 American chestnut was developed as a non-profit project for forest restoration in American chestnut's native range. Darling 58 American chestnut seedlings are intended to be planted in eastern forests. As the climate and ecology of the eastern forests have changed in the last hundred years it is unknown whether Darling 58 American chestnuts will ever regain the dominance it exhibited in the nineteenth century. For example, ink disease, to which Darling 58 American chestnut is susceptible has been a problem in the Southern United States (Russell 1987; Gailing and Nelson 2017) and climate change might expand the problem northward. Nevertheless, the possibility remains that if Darling 58 American chestnut is granted nonregulated status and shows enhanced tolerance to the fungus *C. parasitica*, the trees may be able to establish and colonize much of the eastern United States where stunted trees persist, once again becoming a self-sustaining forest tree species within its native range and potentially providing a valuable source of lumber. Human assistance will be essential for the restoration to be successful. However, given the historically slow spread rates, low propagule pressure, and need for disturbance to provide enough light for fast growth, the rate of increase may require centuries before becoming a significant enough presence in the landscape to allow for timber harvest. The rate of increase will also depend on the degree of human assistance. If landowners have a strong interest in American chestnut restoration or the perception that Darling 58 American chestnut will provide more value to their forest than their existing tree stand and Darling 58 American chestnut seedlings become available in large numbers, the increase could be accelerated. Pre-blight it was recommended that American chestnut be maintained in a rotation of 80 years (Hawley and Hawes 1918a; Pinchot 2011) for timber production and it has been noted that "regular and plentiful" nut crops are produced after 20 years (Zon 1904; Paillet and Rutter 1989). Darling 58 American chestnut's attributes have yet to be established for timber production and so the risk of using Darling 58 American chestnut in a commercial venture is likely too high for adoption in the foreseeable future. Silviculture for timber production from American chestnut has not had the opportunity to advance for over one hundred years and would likely evolve if the restoration is successful.

Chestnuts in Agroforestry systems

Agroforestry has been touted as a solution to the negative environmental impacts caused by monoculture row crops (Davison et al. 2021a). In agroforestry, trees are integrated into the agricultural landscape providing direct economic benefits through food, fodder, and lumber, climate change mitigation and adaptation through carbon sequestration, and ecosystem services such as soil retention, increasing soil water infiltration and storage, reducing evaporation of soil moisture, protecting crops from wind stress, stabilizing air and soil temperatures, increasing biodiversity, and preventing nutrient runoff (Davison et al. 2021a). Chestnuts, specifically Chinese chestnuts, have been proposed as a key tree species in agroforestry systems due to their rapid growth, high yield, and consistent production. Agroforestry pilot projects utilizing chestnut have been started at both public institutions: University of MO Horticulture and Agroforestry

Research[5] ; SUNY Adirondack Warren County Conservation District[6]; North Carolina A and T University Four Farms, U NE Horning State Farm Demonstration Forest, and Penn State University Rodale Farm[7]; and private ventures in New York[8] and MO[9]. If these systems show promise, chestnut plantings, but not necessarily American chestnut, are likely to increase.

Chestnut Nut Production

There is renewed interest in chestnuts as a food source because the nuts are considered a nutritious food. Chestnuts are a source of dietary fiber, contain a significant amount of vitamin C, but contain no cholesterol or gluten (Warmund 2011). Unlike other nuts, they are low in fat and rich in carbohydrates (Warmund 2011). The nuts could be processed into a gluten free flour alternative (Warmund 2011), however chestnut processing is virtually non-existent in the United States because demand for fresh chestnuts exceeds the meager supply produced in the United States (Davison et al. 2021a).

Chinese chestnuts have been cultivated and selected for improved varieties for over 2000 years in Asia. The chestnut grown in Asia is a different species from American chestnut. As a result of the extensive cultivation and selection for nut production, Chinese chestnut has superior qualities for commercial nut production. In response to the blight harming American chestnuts, Chinese chestnuts, which are resistant to both blight and ink disease caused by *Phytophthora cinnamomi* (ESF 2019) have been explored for commercial uses in the United States. An active breeding program utilizing Chinese chestnut began in the 1930's to attempt to adapt varieties to climates within the United States as well as to test Asiatic chestnuts for the production of timber (Diller 1947). The nascent chestnut industry in the United States revolves principally around Chinese chestnut (*C. mollissima* Bl.) though there are some operations using European, Japanese, and hybrid chestnuts (Revord et al. 2021). The Agroforestry program at the University of MO Center for Agroforestry has been breeding chestnuts and supporting the chestnut industry for 20 years. They recently announced a Chestnut Improvement Network, a participatory breeding program led by UMCA and partner growers to expand and accelerate efforts in chestnut breeding (Revord et al. 2022). Chinese chestnut is the most prevalent species in the breeding program due to its superior nut qualities, climatic adaptation, resistance to chestnut blight and phytophthora root rot, and shorter stature amenable to orchard production. Most growers consider Chinese chestnuts to be the best option currently available for establishing profitable orchards in eastern United States (Davison et al. 2021a). In contrast, Darling 58 American chestnut's commercial attributes are untested and it remains susceptible to phytophthora root rot, has smaller nuts unfavored by U.S. consumers (Aguilar et al. 2009), and is taller than Chinese chestnut making it less desirable for nut production in an orchard setting though it may be more suitable for timber (Davison et al. 2021a). Furthermore, crops created through genetic engineering have less public acceptance than conventional crops and as a long-term investment, would also be less likely to be used on that basis for nut production. Therefore, despite the promise of an expanding chestnut industry,

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[5] FarmHorticulture and Agroforestry Research Farm // Missouri Agricultural Experiment Station
[6] Pilot Program To Develop Agroforestry Plan Will Be Implemented At SUNY Adirondack - Glens Falls Business Journal
[7] USDA/ 2011. Agroforestry USDA reports to America, Fiscal years 2011-2012-Comprehensive Version
[8] Propagate Case Study — Propagate (propagateag.com)),
[9] USDA/ 2011. Agroforestry USDA reports to America, Fiscal years 2011-2012-Comprehensive Version: Bringing chestnuts back to American landscapes and diets one graft at a time

Darling 58 American chestnut is not expected to be used in commercial nut operations for the foreseeable future.

American chestnut can cross-pollinate with other chestnut species where their distributions overlap (Jaynes 1964) potentially leading to impacts to chestnut producers catering to a biotech-sensitive market. Currently organic chestnut production only represents 0.33% of the chestnuts sold in the United States (Davison et al. 2021a); it is unknown whether organic chestnut production would capture additional market share if the industry dramatically expanded. American chestnut is primarily wind-pollinated (Clapper 1954; Johnson 1988). To have high pollination rates, chestnut trees need to be within 30 to 100 m apart and trees further than 300 to 400 m apart will generally not pollinate each other (Forest et al. 1977; Russell 1987; Rutter 1990). Chestnut pollen can travel up to 100 km but effective pollination distances are much shorter due to rapid desiccation as pollen viability decreases with time spent in the air (Fernandez-Lopez and Alia 2003). Managing unwanted pollination of chestnut orchards is already an issue that is addressed by chestnut growers, since pollen from certain hybrid or interspecific crosses can be detrimental to harvests. Small effective pollination distances for chestnut mean that such management is achievable. Controlling pollination by transgenic chestnuts after implementation of potential restoration programs would be similarly manageable for growers if needed (ESF 2019). There are unlikely to be any impacts to biotech-sensitive chestnut producers from a determination of non-regulated status for Darling 58 American chestnut for several reasons:

1. As noted above, Darling 58 American chestnut is not expected to be used in commercial nut plantings for the foreseeable future.

2. Darling 58 American chestnut is most likely to be planted in the native American chestnut range while the majority of commercial chestnut production (58%) occurs in states outside the native range (USDA-NASS 2019).

3. Darling 58 American chestnut is a different species than the chestnut used for commercial production. As such, successful hybridization occurs at a lower frequency than within the species (ESF 2019). Chinese chestnut also usually flowers earlier than American chestnut which decreases effective cross-pollination (Pennsylvania Chapter The American Chestnut Foundation 2006).

4. Chestnut pollen does not travel long distances. Effective pollination does not occur beyond 400 m so there is likely to be adequate isolation distances between Darling 58 American chestnut and chestnut orchards catering to the biotech-sensitive market.

5. Chestnut farms catering to the biotech-sensitive market are likely to be low input operations catering directly to the consumer (“uPick” operations) (Gullickson 2019), which would not be subject to commingling in a supply chain.

4.7.2 Trade Economic Environment

Worldwide demand for chestnuts exceeds all other nuts except for coconuts and peanuts (Davison et al. 2021a). China alone produces 1.9 million Metric tons (M MT) of chestnut (Modor Intelligence 2022) which is greater than worldwide production of almonds (1.6 M MT), walnuts (1.0 M MT), cashews (0.89 M MT), pistachios (0.80 M MT), or hazelnuts (0.55 M MT) (International Nut and Dried Fruit Council 2022). The value of the nut is related to its size. Generally, the value of chestnuts ranges from $0.75 to $2.50 per pound wholesale and from $2.00 to $5.00 per pound retail, depending on the market (AgMRC 2021). The United States does not have a significant chestnut industry, less than one percent of total world production, despite being one of the few nations in the world that can grow chestnuts (AgMRC 2021). The United States has 1,587 farms producing chestnuts on 4,228 acres (USDA-NASS 2019). The top five states with the most chestnut acreage were Michigan, California, Iowa, Ohio, and Florida (USDA-NASS 2019). These farms primarily produce chestnuts for fresh market domestic consumption. Because demand greatly exceeds supply, about 90% of chestnuts consumed in the United States are imported, 3,200 metric tons of chestnuts in 2017 (AgMRC 2021). The United States has a very low per capita consumption of chestnuts (0.1 lb/person/yr) compared to Europe at 1 lb per capita and Korea at 4 lbs per capita. Chestnuts are grown in 27 countries and worldwide production in 2018 was 2.4 million tons with a value of $5.4 billion (Davison et al. 2021a). Growth is projected to increase annually by 2.2% over the next five years (Davison et al. 2021a). Growth is being driven by an expanding middle class around the world and increased interest in healthy eating and gluten free alternatives to grain (Davison et al. 2021a). The current U.S. consumption of 0.1 pounds per capita would support 20,000 acres of chestnuts on U.S. farms. If the United States reached the European level, that would support 200,000 acres of chestnuts and result in a $1.2 billion chestnut industry. If U.S. consumers replaced a portion of the starch they consume in the form of grains and vegetables with chestnuts, this level of consumption would support 40,000,000 acres of chestnuts (Davison et al. 2021a). As noted above, expansion of chestnut planting for food and feed is not likely to use Darling 58 American chestnut. Therefore, Darling 58 American chestnuts are anticipated to have minimal impacts on trade.

4.8 Compliance with Federal and State Laws and Regulations, Executive Orders, Policies, and Treaties

4.8.1 Federal Laws and Regulations

The laws most relevant to APHIS determinations of regulatory status are the National Environmental Policy Act of 1969 (NEPA), the Clean Water Act of 1972 (CWA), the Safe Drinking Water Act of 1974 (SDWA), the Clean Air Act of 1970 (CAA), the Endangered Species Act of 1973 (ESA), and the National Historic Preservation Act of 1966 (NHPA). Compliance with the requirements of the ESA has been addressed in Chapter 6. Compliance with the requirements of NEPA, CWA, SDWA, CAA, and NHPA, are specifically addressed in the following subsections.

4.8.2 National Environmental Policy Act (NEPA)

NEPA (42 United States Code (U.S.C) 4321, *et seq*.) is designed to ensure transparency and communication of the possible environmental effects of federal actions prior to implementation. The Act and implementing regulations require federal agencies to document, in advance and in detail, the potential effects of their actions on the human environment, so as to ensure that there is a full understanding of the possible environmental outcomes of federal actions by both the decision-makers and the public. This EIS documents the potential environmental outcomes of the alternatives considered, approval or denial of ESF's petition, and is consistent with the requirements of NEPA and Council on Environmental Quality implementing regulations at 40 CFR parts 1500-1508.

4.8.3 Compliance with the Clean Air Act, Clean Water Act, and Safe Drinking Water Act

The CAA, CWA, and SDWA authorize the EPA to regulate air and water quality in the United States. Apart from the blight-tolerance, Darling 58 American chestnut are equivalent to native chestnut varieties. Therefore, the potential impacts on water resources and air quality are the same under both the No Action and Preferred Alternatives. Darling 58 American chestnut are expected to be used for forest restoration, which could contribute to potential positive impacts on air quality, and potentially water quality. The sources and degree of these impacts would be small or no different than that which occurs in current forest systems. As discussed in this EIS, the blight-tolerance in Darling 58 American chestnut presents no known risks to water or air quality. Considering these factors, a determination of nonregulated status for Darling 58 American chestnut would not lead to circumstances that resulted in non-compliance with the requirements of the CWA, CAA, and SDWA.

4.8.4 Executive Orders Related to Domestic Issues

The following executive orders require consideration of the potential impacts of the Federal action to various segments of the population.

- **EO 12898 – Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations**

  This EO requires federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

- EO 13045 – Protection of Children from Environmental Health Risks and Safety Risks

  Children may suffer disproportionately from environmental health and safety risks due to their developmental stage, higher metabolic rates, and behavior patterns, as compared to adults. This EO requires each federal agency to identify, assess, and address the potential environmental health and safety risks that may disproportionately affect children.

The No Action and Preferred Alternatives were analyzed with respect to EO 12898 and EO 13045. Neither alternative evaluated in this EIS is expected to have disproportionate adverse impacts on minorities, low-income populations, or children. As reviewed in the EIS, it is highly improbable the trait genes and gene products in Darling 58 American chestnut present any risks to human health, nor to animal health and welfare. Darling 58 American chestnut would primarily be used for forest restoration projects and not as a nursery or orchard crop and is expected to have overall beneficial impacts on the environment.

- **EO 13175 – Consultation and Coordination with Indian Tribal Governments**

> Executive departments and agencies are charged with engaging in consultation and collaboration with tribal governments; strengthening the government-to-government relationship between the United States and Indian tribes; and reducing the imposition of unfunded mandates upon Indian tribes. This EO emphasizes and pledges that federal agencies will communicate and collaborate with tribal officials when proposed federal actions have potential tribal implications.

Tribal entities are recognized as independent governments and agricultural activities on tribal lands would only be conducted if approved by the tribe. Tribes would have control over any potential conflict with cultural resources on tribal properties. Approval nor denial of the petition is not expected to have any effect on Indian tribal self-governance or sovereignty, tribal treaties, or other rights.

Consistent with EO 13175, APHIS sent a letter of notification and request for comment and consultation on the proposed action to federally recognized tribes on August 24, 2020. This letter contained information regarding the ESF petition request and Darling 58 American chestnut. Additionally, this notification asked tribal leaders to contact APHIS if they believed that there were potentially significant impacts to tribal lands or resources that should be considered. APHIS received two replies, one from the San Manuel Band of Mission Indians, stating they have elected to not consult on this announcement, and one from the Ysleta del Sur Pueblo tribe, stating they had no comments for APHIS on ESF's petition request. Additionally, APHIS sent a letter informing tribes of APHIS' intent to prepare an EIS on August 6, 2021. APHIS received two replies. One from the Sac & Fox Tribe of the Mississippi in Iowa (Meskwaki Nation) and one from the Klamath Tribes, both stating they had no comments for APHIS on ESF's petition request.

APHIS will continue to consult and collaborate with tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests, in accordance with EO 13175. A determination of nonregulated status for Darling 58 American chestnut will not adversely impact cultural resources on tribal properties.

The No Action and Preferred Alternatives were analyzed with respect to EO 12898, EO 13045, and EO 13175. Neither alternative is expected to have a disproportionate adverse impact on minorities, low-income populations, or children. Nor is any alternative expected to have potential Tribal implications.

- **EO 13751 – Safeguarding the Nation from the Impacts of Invasive Species**

  Invasive species are a significant issue in the United States, causing both adverse economic and environmental impacts. This EO directs federal agencies to take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause.

American chestnut is not listed in the United States as a noxious weed species by the Federal government (USDA-NRCS 2020), nor is it listed as an invasive species by major invasive plant data bases. Based on observations and data submitted by the applicant and reviewed by APHIS, Darling 58 American chestnut is not expected to become weedy or invasive. While Darling 58 American chestnut is expected to be resistant to chestnut blight and therefore able to survive unlike other chestnut varieties currently found in Eastern forests, it's slow spread without human intervention, slow natural colonization rates and frequent animal and pest pressure on seeds and seedlings (Clark et al. 2014) make it unlikely to be invasive. As part of its PPRA, APHIS evaluated the potential weediness and invasiveness of Darling 58 American chestnut and concluded that it is unlikely that Darling 58 American chestnut will become weedy or invasive in areas where it is grown (USDA-APHIS 2020).

- **EO 13186 – Responsibilities of Federal Agencies to Protect Migratory Birds**

  Federal agencies taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations are directed to develop and implement, within two years, a Memorandum of Understanding (MOU) with the Fish and Wildlife Service that shall promote the conservation of migratory bird populations.

Chestnuts can provide food sources for migratory birds, along migratory routes in North America. Migratory birds may visit chestnuts to feed on chestnuts or insects on chestnut trees, which provides a valuable source of nutrition to migratory birds. As reviewed in this EIS, it is highly unlikely the trait genes and their products present any risks to the health of migratory birds. Oxalate oxidase is a common enzyme found in all grains, several other crops and food products, and many wild plants, on which birds may forage and the *nptII* gene and associated NPTII enzyme are present in soil and aquatic bacteria and animal gastrointestinal flora (ESF 2019). The genes and gene products inserted into Darling 58 American chestnut are already found in the environment. Because migratory birds that forage on Darling 58 American chestnut are unlikely to be adversely affected by ingesting the chestnuts or other plant parts, it is unlikely that a determination of nonregulated status for Darling 58 American chestnut would have a negative impact on migratory bird populations.

4.9 Conclusions: Potential Impacts on the Human Environment

As discussed in the Scope of Analysis for this EIS (Section 4.1), in considering whether the effects of the proposed action could be significant, APHIS analyzed the affected environment and degree of the potential effects identified (40 CFR § 1501.3). As part of this analysis APHIS considered those requirements outlined in sections 102(2)(C)(ii),(iv), and (v) of NEPA, 40 CFR § 1502.16– Environmental consequences, 40 CFR § 1501.3–Determine the appropriate level of NEPA review, 40 CFR § 1502.24–Environmental review and consultation requirements, and 40 CFR § 1502.15–Affected environment, which are addressed below.

4.9.1 Adverse environmental effects that cannot be avoided should the proposal be implemented.

Planting long lived trees for restoration purposes or for any other purpose is likely to have some degree of impact on the environment, as discussed in this EIS. If blight-tolerant American chestnut were to become established as an important canopy tree, it would begin to influence ecosystem structure and function in these areas, as it did prior to the blight. Impacts to air quality, surface water and groundwater, soils, biodiversity, and habitats are all possible from planting Darling 58 American chestnut. The degree of environmental impacts will depend on a variety of factors that include the geographic locale, local biota, weather, inherent soil characteristics, and prevalence and diversity of insect pests. Given the historically slow spread rates, low propagule pressure, and need for disturbance to provide sufficient light for fast growth, the rate of increase would likely be very slow. Without aggressive restoration efforts, requiring considerable effort and coordination at landscape scales, it may require centuries before American chestnut becomes a significant presence in the landscape (Gustafson et al. 2017; Gustafson et al. 2018). The scale of potential impacts is anticipated to remain relatively minimal across the landscape.

4.9.2 The relationship between short-term uses of man's environment and the maintenance and enhancement of long-term productivity.

Planting of Darling 58 American chestnut would not be considered a short-term use of the environment since the trees can live hundreds of years. Long-term productivity depends on the sustainable use of natural resources. Restoration with Darling 58 American chestnut is expected to occur on long-term research plots and relatively small-scale public horticultural lands before being planted on a larger scale in eastern forests and utilize similar resources as other forest trees.

4.9.3 Irreversible or irretrievable commitments of resources that would be involved in the proposal should it be implemented.

An irreversible or irretrievable commitment of resources refers to impacts on or losses of resources that cannot be recovered or reversed. Irreversible commitments of resources are those that cause either direct or indirect use of natural resources such that the resources cannot be restored or returned to their original condition. Irreversible impacts entail the loss of future options, and applies primarily to the use of nonrenewable resources such as fossil fuels, and resources that are renewable only over long time spans. Irretrievable is a term that involves the loss of productive value or use of resources. For example, certain opportunities can be foregone during the conduct of a proposed action, during which a resource cannot be used. These actions may be reversible or temporary, but the utilization opportunities foregone during the action are irretrievable.

Production of Darling 58 American chestnut may involve the irreversible consumption of nonrenewable resources. The forests of today differ greatly from the forests of a hundred years ago in land use, species composition (including many nonnative species not formerly present), fire regimes, and climate change. If blight-tolerant American chestnut were to become established as an important canopy tree, it would begin to influence ecosystem structure and function in these areas, as it did prior to the blight. With an increase in American chestnut, the populations of some co-occurring tree species would gradually decline. Chestnut will likely replace other tree species in proportion to their abundance, rather than replacing a single species or genus. Darling 58 American chestnut spread entails the irretrievable removal of some natural habitat as it replaces other tree species from the landscape.

Renewable and nonrenewable resources utilized for Darling 58 American chestnut production would differ little from that of other forest trees. Any irreversible or irretrievable commitments of resources in Darling 58 American chestnut production would be the same as or very similar to that of other forest trees. Darling 58 American chestnut is expected to be produced on long-term research plots and relatively small-scale public horticultural lands before being planted on a larger scale in eastern forests. Restoration efforts will be primarily managed by The American Chestnut Foundation.

4.9.4 Whether the action would violate or conflict with a federal or state laws or local requirements governing protection of the environment.

As reviewed in Section 4.8.3, approval of the petition would not lead to circumstances that resulted in non-compliance with any federal, state, or local laws and regulations providing protections for environmental and human health. ESF plans to consult with the FDA on the safety of Darling 58 American chestnut pursuant to the voluntary consultation process for crop plants developed using genetic engineering (21 C.F.R. Parts 192 and 592). A food and feed safety and nutritional assessment of Darling 58 will be submitted to the FDA for review (ESF 2019).

4.9.5 Possible conflicts between the proposed action and the objectives of federal, regional, state, tribal, and local land use plans, policies, and controls for the area concerned.

There are no conflicts with approval of the petition, and subsequent planting of Darling 58 American chestnut, with federal, state, tribal, or local land use plans or policies.

Federal Lands

There are four major federal land management agencies that administer 606.5 million acres (as of September 30, 2018). These are the Bureau of Land Management, Fish and Wildlife Service (FWS), National Park Service in the Department of the Interior, and the Forest Service in the USDA. A fifth agency, the Department of Defense, administers 8.8 million acres in the United States (as of September 30, 2017). Together, the five agencies manage about 615.3 million acres, or 27% of the U.S. land base (CRS 2020). Many other agencies administer the remaining federal acreage. The lands administered by the four major agencies are managed primarily for purposes related to preservation, recreation, and development of natural resources (CRS 2020).

APHIS approval of the petition would have no effect on lands governed by federal land management agencies. Any planting of Darling 58 American chestnut on federal lands would require approval by a federal land management agency.

Tribal Nations

Executive Order 13175–Consultation and Coordination with Indian Tribal Governments, charges executive departments and agencies with engaging in consultation and collaboration with tribal governments; strengthening the government-to-government relationship between the United States and Indian tribes; and reducing the imposition of unfunded mandates upon Indian tribes. The EO emphasizes and pledges that federal agencies will communicate and collaborate with tribal officials when proposed federal actions have potential tribal implications. Tribal entities are recognized as independent governments and agricultural activities on tribal lands would only be conducted if approved by the tribe. Tribes would have control over any potential conflict with cultural resources on tribal properties. Approval nor denial of the petition would not have any effect on Indian tribal self-governance or sovereignty, tribal treaties, or other rights. APHIS conducted outreach to tribal nations informing tribes of ESF's petition by sending a letter on August 24, 2020. APHIS received two replies, one from the San Manuel Band of Mission

Indians, stating they have elected to not consult on this announcement, and one from the Ysleta del Sur Pueblo tribe, stating they had no comments for APHIS on ESF's petition request. Additionally, APHIS sent a letter informing tribes of APHIS' intent to prepare an EIS on August 6, 2021. APHIS received two replies. One from the Sac & Fox Tribe of the Mississippi in Iowa (Meskwaki Nation) and one from the Klamath Tribes, both stating they had no comments for APHIS on ESF's petition request.

State and Local Land Use Plans and Policies

The PPA contains a preemption clause (7 U.S.C. § 7756) that prohibits state regulation of any, "plant, biological control organism, plant pest, noxious weed, or plant product" to protect against plant pests or noxious weeds if the Secretary (USDA) has issued regulations to prevent the dissemination of biological control organisms, plant pests, or noxious weeds within the United States. The PPA preemption clause does however allow states to impose additional prohibitions or restrictions based on special needs supported by sound scientific data or risk assessment. Consequently, while the PPA limits states' issuance of laws and regulations governing organisms developed using genetic engineering and bars conflicting state regulation, it does allow state oversight when there is a special need for additional prohibitions or restrictions.

States use a variety of mechanisms to regulate the movement or release of organisms developed using genetic engineering within their jurisdiction. For example, South Dakota simply authorizes holders of a federal permit issued under 7 CFR part 340 to use it within the state (SD Stat § 38-12A-31 (2015)). Minnesota issues state permits for release of organisms developed using genetic engineering only after federal applications or permits are on file (MN Stat § 18F.07 (2015)). Nebraska may rely on APHIS or other experts before they issue their permit (NE Code § 2-10,113 (2015)). These illustrative examples show the range of state approaches to regulating the movement and release of organisms developed using genetic engineering within state boundaries.

Neither of the alternatives considered would affect APHIS partnerships with states in the oversight of organisms developed using genetic engineering, to include the planting of Darling 58 American chestnut. Under both alternatives, APHIS would continue working with states. The range of state legislation addressing agricultural biotechnology, namely in the way of permitting, crop protection, seed regulation, and economic development, would be unaffected by denial or approval of the petition.

4.9.6 Energy requirements and conservation potential of various alternatives and mitigation measures.

The energy requirements involved with the full life cycle of Darling 58 American chestnut production would differ little from that of other forest trees. However, the conservation potential of planting Darling 58 American chestnut could be substantial. American chestnut was considered a keystone species, described as "the most important wildlife plant in the eastern United States" (Davis 2005), and played a critical role in the function of the overall ecosystem. If Darling 58 American chestnuts were to become established, it would influence ecosystem structure and function in areas where they were introduced, as American chestnuts did prior to the blight (Paillet 2002). Additionally, because American chestnut is fast-growing, long-lived, and resistant to decay (Ellison et al. 2005; de Bruijn et al. 2014), its reintroduction could result in increased carbon sequestration and storage in the form of living and dead trees and durable wood products (Gustafson et al. 2017). However, given the historically slow spread rates, low propagule pressure, and need for disturbance to provide enough light for fast growth, the rate of increase would likely be very slow. Without aggressive restoration efforts, requiring considerable effort and coordination at landscape scales, it may require centuries before conservation impacts are seen (Gustafson et al. 2017; Gustafson et al. 2018).

4.9.7 Natural or depletable resource requirements and conservation potential of various alternatives and mitigation measures.

There are no depletable resource requirements unique to the production of Darling 58 American chestnut. Use of natural resources (e.g., irrigation water, soils, fertilizers) would be no different than that of other trees in eastern forests. Available mitigation measures to curtail potential environmental impacts, such as those summarized below in 4.9.9, would likewise not differ.

4.9.8 Urban quality, historic and cultural resources, and the design of the built environment, including the reuse and conservation potential of various alternatives and mitigation measures.

Darling 58 American chestnut may be grown in proximity to historic or cultural resources. The National Historic Preservation Act of 1966 and its implementing regulations (36 CFR part 800) requires federal agencies to: 1) determine whether activities they propose constitute "undertakings" that have the potential to cause effects on historic properties and 2) if so, to evaluate the effects of such undertakings on such historic resources and consult with the Advisory Council on Historic Preservation (i.e., State Historic Preservation Office, Tribal Historic Preservation Officers), as appropriate.

Approval of the petition is not a decision that would directly or indirectly result in alteration of the character or use of historic properties protected under the NHPA, nor would it result in any loss or destruction of cultural or historical resources. There are no proposed major ground disturbances; no new physical destruction or damage to property; no alterations of property, wildlife habitat, or landscapes; and no prescribed sale, lease, or transfer of ownership of any property. This action is limited to a determination of nonregulated status of Darling 58 American chestnut. This action would not convert land use to nonagricultural use and, therefore, would have no adverse impact on prime farmland.

Based on these findings, including the assumption that EPA label use instructions are in place to protect unique geographic areas and that those label use instructions are adhered to, a determination of nonregulated status of Darling 58 American chestnut is not expected to impact unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas.

4.9.9 Means to mitigate adverse environmental impacts.

Darling 58 American chestnut was developed with the intent of restoring a native tree to its former range. The American chestnut was once one of the most abundant trees within its range in the eastern United States. As a former keystone species, Darling 58 American chestnut has the potential to influence ecosystem structure and function in eastern forests, as it did prior to the blight, if it were to become established. As discussed throughout this EIS, the impacts of a determination of nonregulated status for Darling 58 American chestnut are unlikely to be adverse. Additionally, because of the historically slow spread rates, low propagule pressure, and need for disturbance to provide enough light for fast growth, the rate of increase would likely be very slow. Without aggressive restoration efforts, requiring considerable effort and coordination at landscape scales, it may require centuries before conservation impacts are seen (Gustafson et al. 2017; Gustafson et al. 2018).

4.9.10 Economic and technical considerations, including the economic benefits of the proposed action.

Economic considerations have been evaluated in Section 4.7 Socioeconomics. The economic impacts associated with the introduction of Darling 58 American chestnut for restoration would be potentially beneficial.

4.9.11 The degree to which the action may adversely affect the endangered or threatened species or its habitat that has been determined to be critical under the Endangered Species Act of 1973.

The evaluation of impacts from a determination of nonregulated status of Darling 58 American chestnut, and subsequent introduction into the environment, can be found in Appendix 1 of this EIS. Darling 58 American chestnut has been found to have no adverse effect on listed species or species proposed for listing, and would not affect designated habitat or habitat proposed for designation.

4.9.12 The degree to which the proposed action affects public health or safety.

Approval of the petition and subsequent planting of Darling 58 American chestnut would not present any risks to public health or worker safety. As reviewed in Section 4.5, it is unlikely that humans would be negatively impacted by consuming chestnuts from Darling 58 American chestnut. Oxalate oxidase and similar enzymes are consumed and handled daily in cereal grains and many other foods without any reports of toxicity, and we have found no evidence that it should be considered a toxin (ESF 2019). As discussed previously in this EIS, there are no health hazards presented by consumption of Darling 58 American chestnuts. As reviewed in Section 4.9.4, ESF is consulting with the FDA as to the safety of food and feed derived from Darling 58 American chestnut (ESF 2019).

4.9.13 Whether the affected environment includes reasonably foreseeable environmental trends and planned actions in the affected areas.

Approval of the petition would provide for the release of Darling 58 American chestnut into the environment, subject to any FDA consultation, and EPA and state requirements. As of October, 2021, APHIS has not issued a determination of nonregulated status for any chestnut varieties developed using genetic engineering. However, APHIS has issues determinations of nonregulated status for three fruit trees; apple, papaya, and plum. APHIS maintains a publicly available list of petitions and determinations of nonregulated status on its website (USDA-APHIS 2021). Seeds for crops developed using genetic engineering were commercially introduced in the United States for major field crops in 1996, with adoption rates increasing rapidly in the years that followed.

Farmers generally adopt a crop developed using genetic engineering based on the benefits they can derive from it, such as effective insect pest or weed control, increased crop yields per acre, increased farm net returns, and time savings (Fernandez-Cornejo et al. 2014; Livingston et al. 2015). Potential net benefits are a function of the particular crop farmed and geographic location; agronomic input and market commodity prices; existing on-farm crop production systems; and farmer abilities and preferences (Fernandez-Cornejo et al. 2014; Livingston et al. 2015).

Unlike field crops, Darling 58 American chestnut was developed with the intent of restoring a native tree to its former range. The American chestnut was once one of the most abundant trees within its range in the eastern United States. Because of chestnut blight, the abundance of American chestnut drastically declined and was reduced from a dominant overstory tree to a small understory shrub (Elliott and Swank 2008; Dalgleish et al. 2015b). As a former keystone species, Darling 58 American chestnut has the potential to influence ecosystem structure and function in eastern forests, as it did prior to the blight, if it were to become established. As discussed throughout this EIS, the impacts of a determination of nonregulated status for Darling 58 American chestnut are likely to be minimal and require centuries before American chestnut becomes a significant enough presence in the landscape to see these impacts (Gustafson et al. 2017; Gustafson et al. 2018).

Appendix 1 Threatened and Endangered Species

The Endangered Species Act (ESA) of 1973, as amended, is one of the most far-reaching wildlife conservation laws ever enacted by any nation. Congress passed the ESA to prevent extinctions facing many species of fish, wildlife and plants. The purpose of the ESA is to conserve endangered and threatened species, and the ecosystems on which they depend, as key components of America's heritage. To implement the ESA, the U.S. Fish & Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS), other Federal, State, and local agencies, Tribes, non-governmental organizations, and private citizens. Before a plant or animal species can receive the protection provided by the ESA, one of the Services must first add it to the Federal list of threatened and endangered wildlife and plants. Threatened and endangered (T&E) species are plants and animals at risk of becoming extinct throughout all or part of their geographic range (endangered species) or species likely to become endangered in the foreseeable future throughout all or a significant portion of their ranges (threatened species).

The Services add a species to the list when they determine it is endangered or threatened because of any of the following factors or a combination thereof:

- The present or threatened destruction, modification, or curtailment of its habitat or range;
- Overutilization for commercial, recreational, scientific, or educational purposes;
- Disease or predation;
- The inadequacy of existing regulatory mechanisms; or
- The natural or manmade factors affecting its survival.

Once an animal or plant is added to the list, in accordance with the ESA, protective measures apply to the species and its habitat. These measures include protection from adverse effects of Federal activities.

Requirements for Federal Agencies

Section 7 (a)(2) of the ESA requires that federal agencies, in consultation with USFWS and/or the NMFS, ensure that any action they authorize, fund, or carry out is "not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat." It is the responsibility of the federal agency taking the action to assess the effects of their action and to consult with the USFWS and NMFS if it is determined that the action "may affect" listed species or designated critical habitat. To facilitate their ESA consultation requirements, APHIS met with the USFWS from 1999 to 2003 to discuss factors relevant to APHIS' regulatory authority and effects analysis for petitions for nonregulated status and developed a process for conducting an effects determination consistent with the PPA (Title IV of Public Law 106-224). APHIS uses this process to help fulfill its obligations and responsibilities under Section 7 of the ESA for biotechnology regulatory actions.

The APHIS regulatory authority over organisms developed using genetic engineering is limited to those organisms for which it has reason to believe might be a plant pest or those for which APHIS does not have sufficient information to determine that the organism is unlikely to pose a plant pest risk. In this case, ESF requests that the USDA APHIS consider that Darling 58

American chestnut does not pose a plant pest risk. After completing a Plant Pest Risk Assessment, if APHIS determines that Darling 58 American chestnut seeds, plants, or parts thereof do not pose a plant pest risk, then this organism would no longer be subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340, and therefore, APHIS must reach a determination that this organism is no longer regulated.

As part of its EIS analysis, APHIS analyzed the potential effects of Darling 58 American chestnut on the environment, including any potential effects to T&E species and critical habitat. As part of this process, APHIS thoroughly reviews product information and data related to the organism developed using genetic engineering to inform the ESA effects analysis and, if necessary, the biological assessment. For each transgene/transgenic plant the following information, data, and questions are considered by APHIS:

- A review of the biology, taxonomy, and weediness potential of the crop plant and its sexually compatible relatives;
- Characterization of each transgene with respect to its structure and function and the nature of the organism from which it was obtained;
- A determination of where the new transgene and its products (if any) are produced in the plant and their quantity;
- A review of the agronomic performance of the plant including disease and pest susceptibilities, weediness potential, and agronomic and environmental impact;
- Determination of the concentrations of known plant toxicants (if any are known in the plant);
- Analysis to determine if the transgenic plant is sexually compatible with any T&E species of plants or a host of any T&E species; and
- Any other information that may inform the potential for an organism to pose a plant pest risk.

APHIS met with USFWS officials on June 15, 2011, to discuss and clarify whether APHIS has any obligations under the ESA regarding analyzing the effects on T&E species that may occur from use of pesticides associated with plants developed using genetic engineering. As a result of these joint discussions, USFWS and APHIS have agreed that it is not necessary for APHIS to perform an ESA effects analysis on pesticide use associated with plants developed using genetic engineering because EPA has both regulatory authority over the labeling of pesticides under FIFRA, and the necessary technical expertise to assess pesticide effects on the environment. APHIS has no statutory authority to authorize or regulate the use of pesticides by growers. Under APHIS' Part 340 regulations, APHIS only has the authority to regulate Darling 58 American chestnut or any organism developed using genetic engineering as long as APHIS believes they may pose a plant pest risk. APHIS has no regulatory jurisdiction over any other risks associated with organisms developed using genetic engineering including risks resulting from the use of pesticides with those organisms.

In following this review process, APHIS, as described below, has evaluated the potential effects that a determination of nonregulated status of Darling 58 American chestnut may have, if any, on federally-listed T&E species (mammals, bird, amphibians, reptiles, invertebrates, fish and shellfish) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation. APHIS also considered potential effects of Darling 58 planted outside the historic range of the American chestnut. While planting outside the range of conventional American chestnut is not part of this petition, APHIS considered it reasonably certain to occur in the event Darling 58 American chestnut is made available to the public. However, American chestnut trees spread at an average rate of "no more than a few kilometers per century" (Paillet and Rutter 1989) and will likely take centuries for chestnut to have a significant presence on the landscape. Considering this, and since planting outside the historic range would likely occur for non-restoration purposes in anthropogenically modified environments such as landscaping, parks, and arboretums, APHIS does not expect Darling 58 American chestnut to have negative effects on T&E species outside its historic range (Figure 1).

Potential Effects of Darling 58 American Chestnut on T&E Species

While a determination of nonregulated status for Darling 58 American chestnut would allow for plantings anywhere within the United States, APHIS limited the action area to the 25 states where American chestnut historically occurred (Figure 1). Darling 58 American chestnut is intended to be used as a restoration tree to establish and colonize much of the eastern United States where stunted trees persist as stumps and small trees. APHIS obtained and reviewed the USFWS list of T&E species for these states from the USFWS Environmental Conservation Online System (USFWS 2022). The species list can be found at the end of this appendix. Our analysis focused on potential effects on terrestrial, avian, and aquatic species that could come in contact with and otherwise carry out life functions (nesting, feeding, rearing, etc) in the vicinity or on Darling 58 American chestnut tree itself within the action area, where American chestnut was once the dominant forest tree (Figure 1). Accordingly, the issues discussed herein focus on the potential environmental consequences of approval of the petition for nonregulated status of Darling 58 American chestnut on T&E species and critical habitat in the action area, where Darling 58 could be planted.

For its analysis on T&E plants and critical habitat, APHIS focused on the potential differences between Darling 58 American chestnut and conventional American chestnut; the potential for ecosystem effects; and the potential for gene movement to native plants, listed species, and species proposed for listing. According to information submitted by the petitioner and reviewed by APHIS, Darling 58 American chestnut is phenotypically and biochemically comparable to conventional American Chestnut (ESF 2019). No significant differences have been observed in terms of plant pest risk traits such as competitiveness, responses to other pests, interactions with other organisms in the environment, or survival (besides blight tolerance) (ESF 2019). Therefore, Darling 58 American chestnut should present no additional weediness traits or plant pest risks than wild-type American chestnuts or traditionally bred hybrids. The American chestnut is not considered an invasive, fast colonizing tree, and the OxO gene will not change these traits. Therefore, Darling 58 American chestnut is not expected to invade or alter critical habitat in ways that would be detrimental to T&E species. Areas that are not intentionally planted with blight-tolerant chestnuts will likely remain without chestnuts for decades or longer (ESF 2019). Darling 58 American chestnut could effectively be excluded from critical habitat if needed.

Independent nutrition analyses have confirmed that transgenic chestnuts are not nutritionally different than their wild-type relatives. Even with the ubiquity of OxO in the environment and agriculture, there are no reports of this enzyme being detrimental to human or animal health, having adverse effects on the environment, or being a plant pest risk.

For its analysis of effects on T&E animals, APHIS focused on the implications of exposure to the OxO gene expressed in Darling 58 American chestnut as well as the tree itself, and the ability of the Darling 58 American chestnut to serve as a host or forage. For plant species, APHIS focused on the implications of displacement and the potential for gene flow to other plant and tree species including its potential to hybridize for a T&E Species.

Threatened and Endangered Plant Species and Critical Habitat

Data provided by ESF (2019) were used in the APHIS analysis of the weediness potential for Darling 58 American chestnut, and further evaluated for the potential to impact T&E species and critical habitat.

No differences were detected between Darling 58 chestnut and conventional chestnut in growth, reproduction, or interactions with pests and diseases, other than the intended effect of resistance to chestnut blight (ESF 2019). American chestnut trees spread at an average rate of "no more than a few kilometers per century" (Paillet and Rutter 1989). Further, slow natural colonization rates and frequent animal and pest pressure on seeds and seedlings (Clark et al. 2014) suggest chestnuts, regardless of transgene status, will not rapidly invade new areas (Cook and Forest 1978). Accordingly, APHIS has concluded that the determination of nonregulated status of Darling 58 American chestnut does not present a plant pest risk, does not present an increased risk of weediness, and does not present an increased risk of gene flow.

Chestnuts were replaced mainly by oak and maple, and to a lesser extent, hickory, birch, black cherry, and others as dominant canopy tree species (Keever 1953; Stephenson 1986; Stephenson et al. 1991; Brewer 1995). No single species has emerged in a dominant role across a broad geographic range, comparable to the pre-blight status of American chestnut. With an increase in American chestnut (Darling 58), some co-occurring tree species would gradually decline, most likely the same trees that replaced American chestnut after the blight was introduced. As discussed above, American chestnut would likely replace other tree species in proportion to their abundance, rather than replacing a single species or genus (Gustafson et al. 2017). Given the relatively close overlap between the niches of chestnut and oak (Keever 1953) competition from chestnut would likely affect oaks more than other species (Gustafson et al. 2017). There is no reason to believe that the reintroduction of American chestnut would result in reduction of any competing tree species to threatened or endangered levels or reduce any tree species to such a level as to impact T&E species depending on those resources. Darling 58 American chestnut is not expected to invade or alter critical habitat in ways that would be detrimental to T&E species. Areas that are not intentionally planted with blight-tolerant chestnuts will likely remain without chestnuts for decades or longer (ESF 2019). Darling 58 American chestnut could effectively be excluded from critical habitat if needed.

Studies conducted by the petitioner showed there were no significant differences in colonization by ectomycorrhizal fungi in roots compared to non-transgenic controls, suggesting that the presence or expression of OxO in Darling 58 American chestnut does not pose risks to native soil fungi that are ecologically important for American chestnuts and other trees (ESF 2019). As such, Darling 58 American chestnut is not expected to have impacts on soil quality.

APHIS also evaluated the potential of Darling 58 American chestnut to cross with a listed species. None of the relatives of Darling 58 American chestnut are Federally listed (or proposed) as endangered or threatened species (http://www.fws.gov/endangered/). Therefore, the presence of Darling 58 chestnut in the environment will not result in movement of the inserted genetic material to any endangered or threatened species.

After reviewing the list of threatened and endangered plant species, and laboratory and field data that confirms no sexual compatibility of any T&E species with Darling 58 American chestnut, as well as studies that confirm no negative effects to soil quality, that Darling 58 American chestnut would not reduce competing trees to threatened or endangered levels, and that Darling 58 American chestnut could be effectively excluded from critical habitat if needed, APHIS determined that Darling 58 American chestnut will have no effect on threatened or endangered plant species or on their critical habitat.

Threatened and Endangered Animal Species and Critical Habitat

Threatened and endangered animal vertebrate and invertebrate species that may be exposed to Darling 58 American chestnut would be those T&E species that inhabit areas where Darling 58 American chestnut would be planted for restoration purposes.

APHIS considered the possibility that Darling 58 American chestnut could serve as an ecosystem component for a threatened or endangered species (i.e., listed invertebrates or other organisms that may use the Darling 58 American chestnut tree to complete its lifecycle). APHIS reviewed the complete T&E species database available on the FWS website (USFWS 2022) and found several animal groups that would use Darling 58 chestnut as an important component in the completion of its lifecycle.

MAMMALS

Pre-blight, American chestnut was described as "the most important wildlife plant in the eastern United States" (Davis 2005). Native mammals such as white-tailed deer (*Odocoileus virginianus*), cottontail rabbit (*Sylvilagus floridanus*), and black bears (*Ursus americanus*) all consumed chestnuts (Diamond et al. 2000; Wang et al. 2013), as well as many other vertebrates including rodents (Lichti et al. 2014) and birds including wild turkeys (*Meleagris gallopavo*), American crow (*Corvus brachyrhynchos*), blue jay (*Cyanocitta cristata*), ruffed grouse (*Bonasa umbellus*), and the extinct passenger pigeon (*Ectopistes migratorius*) (Webb 1986; Russell 1987) and heath hen (Hill 1992). Other mammalian wildlife benefit indirectly from the American chestnut. Bats, weasels, mountain lion, bobcat, and other predators prey on rodents and other organisms that consume American chestnuts. As discussed below, the decline of the American chestnut likely had far-reaching effects on food chains that disrupted the entire forest ecosystem.

Studies suggest the decline in the Allegheny wood rat (a Federal species of concern) may have coincided with the reduction in American chestnut, noting the chestnut is an important food source for the rodent (Wright and Kirkland 2000). Other wildlife such as squirrels may have declined greatly as a result of habitat destruction and loss of chestnut hard mast during fall and winter, and avian and mammalian predators also decreased as a result of decline of these prey populations (Hill 1992). An important feature of the American chestnut is its reliability to produce mast (flowers and nuts) compared to other nut-producing trees that have out-competed the chestnut since the blight. In addition to producing more mast, chestnut was the most reliable of all the nut-producing trees in the eastern forest. Because of its relatively late bloom (May through June), it could produce mast despite late-spring frost, unlike almost all other mast food sources. Many other trees, including oaks and hickories, avoid the effects of granivores (seed and grain consumers) eating all their nuts by surprising them with intermittent high-mast years (Burke 2013). This evidence suggests forest restoration with Darling 58 chestnut may reduce systematic impacts on ecosystem food chains as both predatory and prey mammals increase survival, benefiting the entire forest ecosystem, including threatened and endangered mammals

INVERTEBRATES

American chestnut provided a food source to numerous insect species, especially during the flowering period. The blight and subsequent reduction in American chestnut is thought to have caused at least five indigenous insect species to become extinct or extremely rare (Office of Technology Assessment 1993). Pollen feeders in the insect orders coleoptera (beetles), lepidoptera (moths), diptera (flies), hymenoptera (bees) and others have been observed visiting chestnut catkins (Clapper 1954; Opler 1978; Hasegawa et al. 2015; Tumminello 2016; Zirkle 2017). Darling 58 American chestnut is compositionally equivalent to and as safe and nutritious as the forage produced from conventional American chestnut (ESF 2019). Since American chestnut provided a more stable and more abundant source of mast than oaks, hickory, and beech species (Diamond et al. 2000), if Darling 58 American chestnuts were to become established it could result in population increases of the animal and insect species that feed on chestnut (Hill 1992). The increase would be most pronounced during years of low seed production by other masting species, resulting in less fluctuation in those species that consume seeds (Dalgleish and Swihart 2012).

AQUATIC SPECIES

The replacement of oak by Darling 58 American chestnut may also increase macroinvertebrate activity with potential consequences on population, community, and ecosystem levels since chestnut leaf litter is of higher nutritional value for aquatic invertebrates than oak (Smock and MacGregor 1988). Over the long term, this could help restore functional habitat for those T&E species that use aquatic invertebrates for forage, primarily fish species. Further, slow decaying chestnut wood may increase stream channel complexity as it replaces faster decaying species such as oak. Over time, this would provide additional conservation value for critical habitat for T&E fish and invertebrates (Ellison et al. 2005).

Introduction of Darling 58 American chestnut should not impact overall water quality resources in the action area used by listed animal and insect species. American chestnut does not have different water requirements than those tree species currently found in the action area (ESF 2019).

AVIANS

Migratory birds may visit chestnuts to feed on chestnuts or insects on chestnut trees, which provides a valuable source of nutrition to migratory birds. As reviewed in this EIS, it is highly unlikely the trait genes and their products present any risks to the health of migratory birds. Oxalate oxidase is a common enzyme found in all grains, several other crops and food products, and many wild plants, on which birds may forage and the *nptII* gene and associated NPTII enzyme are present in soil and aquatic bacteria and animal gastrointestinal flora (ESF 2019). The genes and gene products inserted into Darling 58 American chestnut are already found in the environment. Because migratory birds and other avian species that forage on Darling 58 American chestnut are unlikely to be adversely affected by ingesting the chestnuts or other plant parts, it is unlikely that a determination of nonregulated status for Darling 58 American chestnut would have any effect on threatened and endangered migratory bird populations.

ESF plans to consult with the FDA on the safety of Darling 58 American chestnut pursuant to the voluntary consultation process for crop plants developed using genetic engineering (21 C.F.R. Parts 192 and 592). A food and feed safety and nutritional assessment of Darling 58 American chestnut will be submitted to the FDA for review (ESF 2019).

Summary of Effects and Determination

As discussed above, several experiments have been performed on OxO-expressing American chestnuts, and results consistently confirm a lack of plant pest risks or non-target effects. Studies have been conducted on Darling 58 American chestnut, offspring of Darling 58 American chestnut, and on older legacy events that also express OxO. These experiments included observing mycorrhizal colonization of chestnut roots, aquatic and terrestrial insect herbivory on leaves, wood frog tadpoles feeding on leaf litter, leaf litter decomposition, interactions with nearby plants, and use by bumble bees of OxO-containing chestnut pollen. Nutritional composition and tannin concentrations of the OxO-containing nuts have been evaluated by commercial testing labs, and the OxO enzyme was queried against allergen, gluten, and toxin databases. In all cases, the blight-tolerant transgenic American chestnut trees were shown to be equivalent to wild-type American or traditionally bred hybrid chestnuts (ESF 2019). No effects on T&E species are expected from the OxO gene in Darling 58 American chestnut.

After reviewing the possible effects of determining nonregulated status of Darling 58 American chestnut, APHIS has not identified any stressor that could negatively affect the reproduction, numbers, or distribution of a listed T&E species or species proposed for listing. APHIS also considered the potential effect of a determination of nonregulated status of Darling 58 American chestnut on designated critical habitat or habitat proposed for designation and could identify no differences from effects that would occur from the current state of eastern forests. Consumption of Darling 58 American chestnut nuts, wood or leaf material by any listed species or species proposed for listing will not result in a toxic or allergic reaction. While APHIS determined that the planting of Darling 58 American chestnut will likely result in some degree of impacts to the environment, negative impacts to threatened and endangered species or critical habitat that occur where it would be planted is unlikely. Any long-term benefits wouldn’t be measurable for decades or longer. As such, no effects are expected to listed and proposed T&E species and critical habitat where Darling 58 American chestnut would be planted.

Based on these factors, APHIS has concluded that a determination of nonregulated status of Darling 58 American chestnut, will have no effect on listed species or species proposed for listing, and would not affect designated habitat or habitat proposed for designation. Because of this no-effect determination, consultation under Section 7(a)(2) of the Act or the concurrences of the USFWS or NMFS is not required.

Table 2. List of T&E Species within American Chestnut Historic Range

Scientific Name	Common name	Status	Group
Acipenser oxyrinchus (=oxyrhynchus) desotoi	Gulf sturgeon	Threatened	Fishes
Aconitum noveboracense	Northern wild monkshood	Threatened	Flowering Plants
Aeschynomene virginica	Sensitive joint-vetch	Threatened	Flowering Plants
Agalinis acuta	Sandplain gerardia	Endangered	Flowering Plants
Alasmidonta atropurpurea	Cumberland elktoe	Endangered	Clams
Alasmidonta heterodon	Dwarf wedgemussel	Endangered	Clams
Alasmidonta raveneliana	Appalachian elktoe	Endangered	Clams
Alligator mississippiensis	American alligator	Similarity of Appearance (Threatened)	Reptiles
Amaranthus pumilus	Seabeach amaranth	Threatened	Flowering Plants
Amblema neislerii	Fat threeridge (mussel)	Endangered	Clams
Ambystoma bishopi	Reticulated flatwoods salamander	Endangered	Amphibians
Ambystoma cingulatum	Frosted Flatwoods salamander	Threatened	Amphibians
Amphianthus pusillus	Little amphianthus	Threatened	Flowering Plants
Anguispira picta	Painted snake coiled forest snail	Threatened	Snails
Antrolana lira	Madison Cave isopod	Threatened	Crustaceans
Apios priceana	Prices potato-bean	Threatened	Flowering Plants
Arabis georgiana	Georgia rockcress	Threatened	Flowering Plants
Arabis perstellata	Braun's rock-cress	Endangered	Flowering Plants
Asclepias meadii	Mead's milkweed	Threatened	Flowering Plants
Asplenium scolopendrium var. americanum	American hart's-tongue fern	Threatened	Ferns and Allies
Astragalus bibullatus	Guthrie's (=Pyne's) ground-plum	Endangered	Flowering Plants
Astragalus robbinsii var. jesupii	Jesup''s milk-vetch	Endangered	Flowering Plants
Athearnia anthonyi	Anthony's riversnail	Endangered	Snails
Baptisia arachnifera	Hairy rattleweed	Endangered	Flowering Plants
Betula uber	Virginia round-leaf birch	Threatened	Flowering Plants
Boechera serotina	Shale barren rock cress	Endangered	Flowering Plants
Bombus affinis	Rusty patched bumble bee	Endangered	Insects
Brychius hungerfordi	Hungerford's crawling water Beetle	Endangered	Insects
Calidris canutus rufa	Red knot	Threatened	Birds
Cambarus callainus	Big Sandy crayfish	Threatened	Crustaceans

Scientific Name	Common name	Status	Group
Cambarus cracens	Slenderclaw crayfish	Endangered	Crustaceans
Cambarus veteranus	Guyandotte River crayfish	Endangered	Crustaceans
Campeloma decampi	Slender campeloma	Endangered	Snails
Canis lupus	Gray wolf	Endangered	Mammals
Cardamine micranthera	Small-anthered bittercress	Endangered	Flowering Plants
Caretta caretta	Loggerhead sea turtle	Threatened	Reptiles
Carex lutea	Golden sedge	Endangered	Flowering Plants
Charadrius melodus	Piping Plover	Endangered	Birds
Chelonia mydas	Green sea turtle	Threatened	Reptiles
Chrosomus saylori	Laurel dace	Endangered	Fishes
Cirsium pitcheri	Pitcher's thistle	Threatened	Flowering Plants
Clematis morefieldii	Morefields leather flower	Endangered	Flowering Plants
Clematis socialis	Alabama leather flower	Endangered	Flowering Plants
Conradina verticillata	Cumberland rosemary	Threatened	Flowering Plants
Corynorhinus (=Plecotus) townsendii virginianus	Virginia big-eared bat	Endangered	Mammals
Cottus paulus (=pygmaeus)	Pygmy Sculpin	Threatened	Fishes
Crystallaria cincotta	diamond Darter	Endangered	Fishes
Cumberlandia monodonta	Spectaclecase (mussel)	Endangered	Clams
Cyprinella caerulea	Blue shiner	Threatened	Fishes
Cyprogenia stegaria	Fanshell	Endangered	Clams
Dalea foliosa	Leafy prairie-clover	Endangered	Flowering Plants
Dermochelys coriacea	Leatherback sea turtle	Endangered	Reptiles
Dromus dromas	Dromedary pearlymussel	Endangered	Clams
Drymarchon couperi	Eastern indigo snake	Threatened	Reptiles
Echinacea laevigata	Smooth coneflower	Endangered	Flowering Plants
Elassoma alabamae	Spring pygmy sunfish	Threatened	Fishes
Elimia crenatella	Lacy elimia (snail)	Threatened	Snails
Ellipsoptera puritana	Puritan tiger beetle	Threatened	Insects
Elliptio chipolaensis	Chipola slabshell	Threatened	Clams
Elliptio lanceolata	Yellow lance	Threatened	Clams
Elliptio spinosa	Altamaha Spinymussel	Endangered	Clams
Elliptoideus sloatianus	Purple bankclimber (mussel)	Threatened	Clams
Epioblasma brevidens	Cumberlandian combshell	Endangered	Clams
Epioblasma capsaeformis	Oyster mussel	Endangered	Clams
Epioblasma florentina florentina	Yellow blossom (pearlymussel)	Endangered	Clams
Epioblasma florentina walkeri (=E. walkeri)	Tan riffleshell	Endangered	Clams
Epioblasma metastriata	Upland combshell	Endangered	Clams
Epioblasma obliquata obliquata	Purple Cat's paw (=Purple Cat's paw pearlymussel)	Endangered	Clams

Scientific Name	Common name	Status	Group
Epioblasma obliquata perobliqua	White catspaw (pearlymussel)	Endangered	Clams
Epioblasma othcaloogensis	Southern acornshell	Endangered	Clams
Epioblasma penita	Southern combshell	Endangered	Clams
Epioblasma rangiana	Northern riffleshell	Endangered	Clams
Epioblasma torulosa gubernaculum	Green blossom (pearlymussel)	Endangered	Clams
Epioblasma torulosa torulosa	Tubercled blossom (pearlymussel)	Endangered	Clams
Epioblasma triquetra	Snuffbox mussel	Endangered	Clams
Epioblasma turgidula	Turgid blossom (pearlymussel)	Endangered	Clams
Eretmochelys imbricata	Hawksbill sea turtle	Endangered	Reptiles
Erimonax monachus	Spotfin Chub	Threatened	Fishes
Erimystax cahni	Slender chub	Threatened	Fishes
Etheostoma akatulo	bluemask darter	Endangered	Fishes
Etheostoma boschungi	Slackwater darter	Threatened	Fishes
Etheostoma chermocki	Vermilion darter	Endangered	Fishes
Etheostoma chienense	Relict darter	Endangered	Fishes
Etheostoma etowahae	Etowah darter	Endangered	Fishes
Etheostoma nuchale	Watercress darter	Endangered	Fishes
Etheostoma osburni	Candy darter	Endangered	Fishes
Etheostoma percnurum	Duskytail darter	Endangered	Fishes
Etheostoma phytophilum	Rush Darter	Endangered	Fishes
Etheostoma rubrum	Bayou darter	Threatened	Fishes
Etheostoma scotti	Cherokee darter	Threatened	Fishes
Etheostoma sellare	Maryland darter	Endangered	Fishes
Etheostoma spilotum	Kentucky arrow darter	Threatened	Fishes
Etheostoma susanae	Cumberland darter	Endangered	Fishes
Etheostoma trisella	Trispot darter	Threatened	Fishes
Etheostoma wapiti	Boulder darter	Endangered	Fishes
Fundulus julisia	Barrens topminnow	Endangered	Fishes
Fusconaia burkei	Tapered pigtoe	Threatened	Clams
Fusconaia cor	Shiny pigtoe	Endangered	Clams
Fusconaia cuneolus	Finerayed pigtoe	Endangered	Clams
Fusconaia escambia	Narrow pigtoe	Threatened	Clams
Fusconaia masoni	Atlantic pigtoe	Threatened	Clams
Geum radiatum	Spreading avens	Endangered	Flowering Plants
Glaucomys sabrinus coloratus	Carolina northern flying squirrel	Endangered	Mammals
Glyptemys muhlenbergii	bog turtle	Similarity of Appearance (Threatened)	Reptiles
Gopherus polyphemus	Gopher tortoise	Threatened	Reptiles
Graptemys flavimaculata	Yellow-blotched map turtle	Threatened	Reptiles

Scientific Name	Common name	Status	Group
Graptemys oculifera	Ringed map turtle	Threatened	Reptiles
Grus canadensis pulla	Mississippi sandhill crane	Endangered	Birds
Gymnoderma lineare	Rock gnome lichen	Endangered	Lichens
Habroscelimorpha dorsalis dorsalis	Northeastern beach tiger beetle	Threatened	Insects
Hamiota altilis	Finelined pocketbook	Threatened	Clams
Hamiota australis	Southern Sandshell	Threatened	Clams
Hamiota perovalis	Orangenacre mucket	Threatened	Clams
Hamiota subangulata	Shinyrayed pocketbook	Endangered	Clams
Hedyotis purpurea var. montana	Roan Mountain bluet	Endangered	Flowering Plants
Helenium virginicum	Virginia sneezeweed	Threatened	Flowering Plants
Helianthus schweinitzii	Schweinitz's sunflower	Endangered	Flowering Plants
Helianthus verticillatus	Whorled Sunflower	Endangered	Flowering Plants
Helonias bullata	Swamp pink	Threatened	Flowering Plants
Hemistena lata	Cracking pearlymussel	Endangered	Clams
Hexastylis naniflora	Dwarf-flowered heartleaf	Threatened	Flowering Plants
Hudsonia montana	Mountain golden heather	Threatened	Flowering Plants
Hymenoxys herbacea	Lakeside daisy	Threatened	Flowering Plants
Iliamna corei	Peter's Mountain mallow	Endangered	Flowering Plants
Iris lacustris	Dwarf lake iris	Threatened	Flowering Plants
Isoetes louisianensis	Louisiana quillwort	Endangered	Ferns and Allies
Isoetes melanospora	Black spored quillwort	Endangered	Ferns and Allies
Isoetes tegetiformans	Mat-forming quillwort	Endangered	Ferns and Allies
Isotria medeoloides	Small whorled pogonia	Threatened	Flowering Plants
Lampsilis abrupta	Pink mucket (pearlymussel)	Endangered	Clams
Lampsilis virescens	Alabama lampmussel	Endangered	Clams
Lasmigona decorata	Carolina heelsplitter	Endangered	Clams
Laterallus jamaicensis ssp. jamaicensis	Eastern Black rail	Threatened	Birds
Leavenworthia crassa	Fleshy-fruit gladecress	Endangered	Flowering Plants
Leavenworthia exigua laciniata	Kentucky glade cress	Threatened	Flowering Plants
Lemiox rimosus	Birdwing pearlymussel	Endangered	Clams
Lepidochelys kempii	Kemp's ridley sea turtle	Endangered	Reptiles
Leptoxis ampla	Round rocksnail	Threatened	Snails
Leptoxis foremani	Interrupted (=Georgia) Rocksnail	Endangered	Snails
Leptoxis plicata	Plicate rocksnail	Endangered	Snails
Leptoxis taeniata	Painted rocksnail	Threatened	Snails
Lepyrium showalteri	Flat pebblesnail	Endangered	Snails
Lesquerella lyrata	Lyrate bladderpod	Threatened	Flowering Plants
Lesquerella perforata	Spring Creek bladderpod	Endangered	Flowering Plants

Scientific Name	Common name	Status	Group
Liatris helleri	Heller's blazingstar	Threatened	Flowering Plants
Lindera melissifolia	Pondberry	Endangered	Flowering Plants
Lioplax cyclostomaformis	Cylindrical lioplax (snail)	Endangered	Snails
Lirceus usdagalun	Lee County cave isopod	Endangered	Crustaceans
Lycaeides melissa samuelis	Karner blue butterfly	Endangered	Insects
Lynx canadensis	Canada Lynx	Threatened	Mammals
Lysimachia asperulaefolia	Rough-leaved loosestrife	Endangered	Flowering Plants
Margaritifera marrianae	Alabama pearlshell	Endangered	Clams
Marshallia mohrii	Mohr's Barbara's buttons	Threatened	Flowering Plants
Marstonia ogmorhaphe	Royal marstonia (snail)	Endangered	Snails
Marstonia pachyta	Armored snail	Endangered	Snails
Medionidus acutissimus	Alabama moccasinshell	Threatened	Clams
Medionidus parvulus	Coosa moccasinshell	Endangered	Clams
Medionidus penicillatus	Gulf moccasinshell	Endangered	Clams
Medionidus simpsonianus	Ochlockonee moccasinshell	Endangered	Clams
Medionidus walkeri	Suwannee moccasinshell	Threatened	Clams
Menidia extensa	Waccamaw silverside	Threatened	Fishes
Mesodon clarki nantahala	noonday snail	Threatened	Snails
Microhexura montivaga	Spruce-fir moss spider	Endangered	Arachnids
Mimulus michiganensis	Michigan monkey-flower	Endangered	Flowering Plants
Mycteria americana	Wood stork	Threatened	Birds
Myotis grisescens	Gray bat	Endangered	Mammals
Myotis septentrionalis	Northern Long-Eared Bat	Threatened	Mammals
Myotis sodalis	Indiana bat	Endangered	Mammals
Necturus alabamensis	Black warrior (=Sipsey Fork) Waterdog	Endangered	Amphibians
Necturus lewisi	Neuse River waterdog	Threatened	Amphibians
Neonympha mitchellii francisci	Saint Francis' satyr butterfly	Endangered	Insects
Neonympha mitchellii mitchellii	Mitchell's satyr Butterfly	Endangered	Insects
Nerodia erythrogaster neglecta	Copperbelly water snake	Threatened	Reptiles
Nicrophorus americanus	American burying beetle	Threatened	Insects
Notropis albizonatus	Palezone shiner	Endangered	Fishes
Notropis cahabae	Cahaba shiner	Endangered	Fishes
Notropis mekistocholas	Cape Fear shiner	Endangered	Fishes
Noturus baileyi	Smoky madtom	Endangered	Fishes
Noturus crypticus	Chucky Madtom	Endangered	Fishes
Noturus flavipinnis	Yellowfin madtom	Threatened	Fishes
Noturus furiosus	Carolina madtom	Endangered	Fishes
Noturus stanauli	Pygmy madtom	Endangered	Fishes
Noturus trautmani	Scioto madtom	Endangered	Fishes

Scientific Name	Common name	Status	Group
Novisuccinea chittenangoensis	Chittenango ovate amber snail	Threatened	Snails
Oarisma poweshiek	Poweshiek skipperling	Endangered	Insects
Obovaria choctawensis	Choctaw bean	Endangered	Clams
Obovaria retusa	Ring pink (mussel)	Endangered	Clams
Orconectes shoupi	Nashville crayfish	Endangered	Crustaceans
Oxypolis canbyi	Canby's dropwort	Endangered	Flowering Plants
Palaemonias alabamae	Alabama cave shrimp	Endangered	Crustaceans
Palaemonias ganteri	Kentucky cave shrimp	Endangered	Crustaceans
Parvaspina collina	James spinymussel	Endangered	Clams
Parvaspina steinstansana	Tar River spinymussel	Endangered	Clams
Pedicularis furbishiae	Furbish lousewort	Endangered	Flowering Plants
Pegias fabula	Littlewing pearlymussel	Endangered	Clams
Percina antesella	Amber darter	Endangered	Fishes
Percina aurolineata	Goldline darter	Threatened	Fishes
Percina aurora	Pearl darter	Threatened	Fishes
Percina jenkinsi	Conasauga logperch	Endangered	Fishes
Percina rex	Roanoke logperch	Endangered	Fishes
Percina tanasi	Snail darter	Threatened	Fishes
Peromyscus polionotus ammobates	Alabama beach mouse	Endangered	Mammals
Phaeognathus hubrichti	Red Hills salamander	Threatened	Amphibians
Phoxinus cumberlandensis	Blackside dace	Threatened	Fishes
Physaria globosa	Short's bladderpod	Endangered	Flowering Plants
Picoides borealis	Red-cockaded woodpecker	Endangered	Birds
Pituophis melanoleucus lodingi	Black pinesnake	Threatened	Reptiles
Pityopsis ruthii	Ruth's golden aster	Endangered	Flowering Plants
Platanthera integrilabia	White fringeless orchid	Threatened	Flowering Plants
Platanthera leucophaea	Eastern prairie fringed orchid	Threatened	Flowering Plants
Plethobasus cicatricosus	White wartyback (pearlymussel)	Endangered	Clams
Plethobasus cooperianus	Orangefoot pimpleback (pearlymussel)	Endangered	Clams
Plethobasus cyphyus	Sheepnose Mussel	Endangered	Clams
Plethodon nettingi	Cheat Mountain salamander	Threatened	Amphibians
Plethodon shenandoah	Shenandoah salamander	Endangered	Amphibians
Pleurobema clava	Clubshell	Endangered	Clams
Pleurobema curtum	Black clubshell	Endangered	Clams
Pleurobema decisum	Southern clubshell	Endangered	Clams
Pleurobema furvum	Dark pigtoe	Endangered	Clams
Pleurobema georgianum	Southern pigtoe	Endangered	Clams
Pleurobema hanleyianum	Georgia pigtoe	Endangered	Clams
Pleurobema marshalli	Flat pigtoe	Endangered	Clams

Scientific Name	Common name	Status	Group
Pleurobema perovatum	Ovate clubshell	Endangered	Clams
Pleurobema plenum	Rough pigtoe	Endangered	Clams
Pleurobema pyriforme	Oval pigtoe	Endangered	Clams
Pleurobema strodeanum	Fuzzy pigtoe	Threatened	Clams
Pleurobema taitianum	Heavy pigtoe	Endangered	Clams
Pleurocera foremani	Rough hornsnail	Endangered	Snails
Pleuronaia dolabelloides	Slabside Pearlymussel	Endangered	Clams
Pleuronaia gibber	Cumberland pigtoe	Endangered	Clams
Polygyriscus virginianus	Virginia fringed mountain snail	Endangered	Snails
Potamilus capax	Fat pocketbook	Endangered	Clams
Potamilus inflatus	Inflated heelsplitter	Threatened	Clams
Pseudemys alabamensis	Alabama red-bellied turtle	Endangered	Reptiles
Pseudemys rubriventris bangsi	Plymouth Redbelly Turtle	Endangered	Reptiles
Ptilimnium nodosum	Harperella	Endangered	Flowering Plants
Ptychobranchus greenii	Triangular Kidneyshell	Endangered	Clams
Ptychobranchus jonesi	Southern kidneyshell	Endangered	Clams
Ptychobranchus subtentus	Fluted kidneyshell	Endangered	Clams
Quadrula cylindrica cylindrica	Rabbitsfoot	Threatened	Clams
Quadrula cylindrica strigillata	Rough rabbitsfoot	Endangered	Clams
Quadrula fragosa	Winged Mapleleaf	Endangered	Clams
Quadrula stapes	Stirrupshell	Endangered	Clams
Rana sevosa	dusky gopher frog	Endangered	Amphibians
Reginaia rotulata	Round Ebonyshell	Endangered	Clams
Rhodiola integrifolia ssp. leedyi	Leedy's roseroot	Threatened	Flowering Plants
Rhus michauxii	Michaux's sumac	Endangered	Flowering Plants
Rhynchospora knieskernii	Knieskern's Beaked-rush	Threatened	Flowering Plants
Ribes echinellum	Miccosukee gooseberry	Threatened	Flowering Plants
Sagittaria fasciculata	Bunched arrowhead	Endangered	Flowering Plants
Sagittaria secundifolia	Kral's water-plantain	Threatened	Flowering Plants
Salmo salar	Atlantic salmon	Endangered	Fishes
Sarracenia oreophila	Green pitcher-plant	Endangered	Flowering Plants
Sarracenia rubra ssp. alabamensis	Alabama canebrake pitcher-plant	Endangered	Flowering Plants
Sarracenia rubra ssp. jonesii	Mountain sweet pitcher-plant	Endangered	Flowering Plants
Scaphirhynchus albus	Pallid sturgeon	Endangered	Fishes
Scaphirhynchus suttkusi	Alabama sturgeon	Endangered	Fishes
Schwalbea americana	American chaffseed	Endangered	Flowering Plants
Scirpus ancistrochaetus	Northeastern bulrush	Endangered	Flowering Plants
Scutellaria montana	Large-flowered skullcap	Threatened	Flowering Plants

Scientific Name	Common name	Status	Group
Silene polypetala	Fringed campion	Endangered	Flowering Plants
Sistrurus catenatus	Eastern Massasauga (=rattlesnake)	Threatened	Reptiles
Sisyrinchium dichotomum	White irisette	Endangered	Flowering Plants
Solidago houghtonii	Houghton's goldenrod	Threatened	Flowering Plants
Solidago shortii	Short's goldenrod	Endangered	Flowering Plants
Solidago spithamaea	Blue Ridge goldenrod	Threatened	Flowering Plants
Somatochlora hineana	Hine's emerald dragonfly	Endangered	Insects
Speoplatyrhinus poulsoni	Alabama cavefish	Endangered	Fishes
Spigelia gentianoides	Gentian pinkroot	Endangered	Flowering Plants
Spiraea virginiana	Virginia spiraea	Threatened	Flowering Plants
Sterna dougallii dougallii	Roseate tern	Endangered	Birds
Sternotherus depressus	Flattened musk turtle	Threatened	Reptiles
Stygobromus hayi	Hay's Spring amphipod	Endangered	Crustaceans
Thalictrum cooleyi	Cooley's meadowrue	Endangered	Flowering Plants
Theliderma intermedia	Cumberland monkeyface (pearlymussel)	Endangered	Clams
Theliderma sparsa	Appalachian monkeyface (pearlymussel)	Endangered	Clams
Thelypteris pilosa var. alabamensis	Alabama streak-sorus fern	Threatened	Ferns and Allies
Torreya taxifolia	Florida torreya	Endangered	Conifers and Cycads
Toxolasma cylindrellus	Pale lilliput (pearlymussel)	Endangered	Clams
Trichechus manatus	West Indian Manatee	Threatened	Mammals
Trillium persistens	Persistent trillium	Endangered	Flowering Plants
Trillium reliquum	Relict trillium	Endangered	Flowering Plants
Triodopsis platysayoides	Flat-spired three-toothed Snail	Threatened	Snails
Tulotoma magnifica	Tulotoma snail	Threatened	Snails
Vermivora bachmanii	Bachman's warbler (=wood)	Endangered	Birds
Villosa fabalis	Rayed Bean	Endangered	Clams
Villosa perpurpurea	Purple bean	Endangered	Clams
Villosa trabalis	Cumberland bean (pearlymussel)	Endangered	Clams

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No additional parties requested physical copies of the EIS. In addition to this distribution list APHIS notified all of its stakeholders of the availability of the EIS for review and comment.

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Appendix 5 INDEX