United States
Department of
Agriculture

Agrivida, Inc. Petition (19-176-01p) for the Determination of Nonregulated Status for Maize Event PY203: *Zea mays* Expressing a Phytase Gene Derived from *Escherichia coli* Strain K12

OECD Unique Identifier: AGV-PY203-4

Final Environmental Assessment

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TABLE OF CONTENTS Page

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
CAA	Clean Air Act
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations (United States)
CO	carbon monoxide
CO_2	carbon dioxide
CWA	Clean Water Act
DNA	deoxyribonucleic acid
EA	Environmental Assessment
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
FQPA	Food Quality Protection Act
FWS	U.S. Fish and Wildlife Service
HR	herbicide resistant
IPCC	Intergovernmental Panel on Climate Change
IWM	Integrated Weed Management
lb	pound
µg	micrograms
N_2O	nitrous oxide
NAAQS	National Ambient Air Quality Standards
NEPA	National Environmental Policy Act of 1969, as amended
NHPA	National Historic Preservation Act
NMFS	National Marine Fisheries Service
NOP	National Organic Program
NPS	non-point source
NWQI	National Water Quality Initiative
OECD	Organization for Economic Cooperation and Development
P	phosphorus

ACRONYMS AND ABBREVIATIONS

PIP	plant-incorporated protectant
PMI	phosphomannose isomerase
PPRA	Plant Pest Risk Assessment
PPA	Plant Protection Act
ppm	parts per million
TES	Threatened and Endangered Species
TSCA	Toxic Substances Control Act
U.S.	United States
USDA	U.S. Department of Agriculture
USDA-AMS	U.S. Department of Agriculture- Agricultural Marketing Service
USDA-APHIS or APHIS	U.S. Department of Agriculture-Animal and Plant Health Inspection Service
USDA-ARMS	U.S. Department of Agriculture-Agricultural Resource Management Survey
USDA-ERS	U.S. Department of Agriculture-Economic Research Service
USDA-NASS	U.S. Department of Agriculture-National Agricultural Statistics Service
USC	U.S. Code
USFWS	U.S. Fish & Wildlife Service
WPS	Worker Protection Standard for Agricultural Pesticides

1 PURPOSE AND NEED FOR AGENCY ACTION

1.1 Background

In June 2019, Agrivida, Inc. (Agrivida) submitted a petition (19-176-01p) to the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), requesting that PY203 maize (corn),[1] which was developed using genetic engineering, no longer be considered regulated under Title 7 of the Code of Federal Regulations part 340 (7 CFR part 340) (Agrivida 2019). As described in more detail below under Section 1.4–Requirement to Issue a Regulatory Status Determination, APHIS regulations at 7 CFR part 340 provide that any person may submit a petition to APHIS requesting that a an organism should not be regulated, because it is unlikely to pose a plant pest risk.

As part of evaluation of Agrivida's petition APHIS developed this Environmental Assessment (EA) to consider the potential impacts of a determination of nonregulated status for PY203 corn on the human environment.[2] The primary purpose of a NEPA analysis is to ensure agencies consider the environmental impacts of their actions in decision making. This EA is to provide a full and fair discussion of the potential environmental impacts, beneficial and adverse, so as to inform decision makers and the public of the potential outcomes of deregulation of PY203 corn, and ways to avoid or minimize any potential adverse impacts.

This EA has been prepared in compliance with the National Environmental Policy Act (NEPA, 42 U.S.C. § 4321 et seq.), the Council of Environmental Quality's (CEQ) NEPA-implementing regulations (40 CFR parts 1500-1508), and USDA and APHIS NEPA-implementing regulations (7 CFR part 1b, and 7 CFR part 372).

1.2 Purpose of PY203 Corn

Corn is the primary livestock feed grain in the United States, accounting for around 70% to 90% of total feed grain use on an annual basis (USDA-ERS 2020a). Phosphorus (P), an essential nutrient for livestock, occurs in corn and other grains and legumes primarily in the form of phytate, a storage form of phosphate comprised of six phosphate groups, which is not easily digested by non-ruminant animals (Alkarawi and Zotz 2014; Gupta et al. 2015). In corn, specifically, phytate comprises from 60% to 82% of the total plant phosphorus (West 2014).

In ruminant animals (e.g., cattle, sheep, goats) the enzyme phytase naturally occurs in the gastrointestinal tract, produced by bacteria in the rumen of the stomach. Phytase degrades phytate into simpler bioavailable forms of phosphorus, which facilitates dietary phosphorus assimilation (Figure 1-1), essential for animal development and health (Konietzny and Greiner 2002; Li et al. 2016). Conversely, non-ruminant livestock (e.g., hogs, poultry) lack sufficient production of gastrointestinal phytase, and phosphorus in phytate form is poorly assimilated by these animals. Because phytate is poorly digested by

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[1] Maize is the botanical term used globally for the cereal plant *Zea mays*. In the United States maize is commonly referred to as corn. For consistency with the common plant name and petition APHIS uses the term maize, but also refers to corn in certain instances, such as in reference to food products. Both terms are used interchangeably in this document.

[2] 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).

non-ruminant animals, and phosphorus in phytate form is unavailable as a nutrient, this can pose challenges in utilizing corn grain for non-ruminant animal feed.

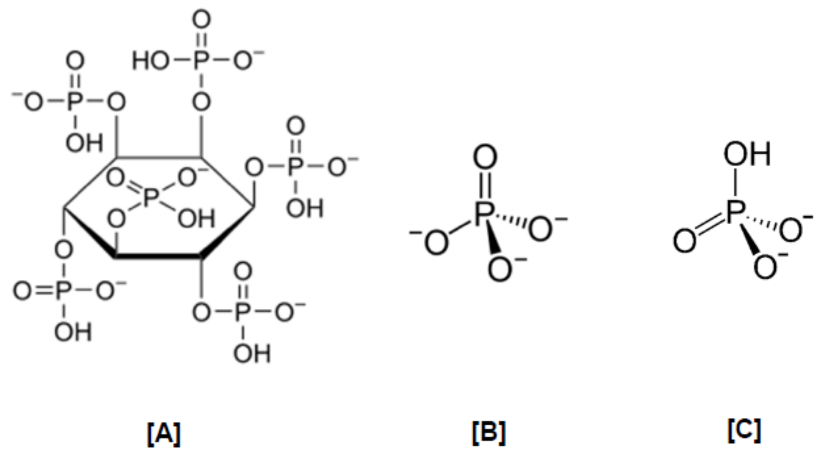

Figure 1-1. Phytate and Inorganic Phosphorus
Phosphorus is an essential nutrient that is required by all plant and animal cells for normal function. It is involved in a wide range of physiological processes (e.g., energy metabolism) and occurs in various forms in organisms, such as phosphate (a component of DNA and RNA), phospholipids (components of cell membranes), and phytate (a form of phosphorus storage). Phytate generally comprises around 50% to 85% of the total phosphorous in grains/legumes. The removal of phosphate groups from the phosphate inositol ring [A] is required for the digestion and assimilation of the majority of phosphate stored in plants. Digestible forms of inorganic phosphorus, that derive from dephosphorylation of the inositol ring, include phosphate (B], and hydrogen phosphate (C).

One method used to improve dietary phosphorus assimilation in non-ruminant animals is to supplement their feed with phytase, which helps breakdown phytate during digestion (Dersjant-Li et al. 2015; Ingelmann et al. 2018). Various phytase products are currently marketed for this purpose, such as Natuphos™ (BASF), Ronozyme™ (DSM), and Quantum Blue (AB Vista). The phytase in these products is derived from sources such as fungi and bacteria. Another strategy for ensuring the dietary phosphorus needs of non-ruminant animals are met is to add inorganic phosphorus to the feed (Li et al. 2016).

Using a genetic engineering process termed *Agrobacterium*-mediated transformation, Agrivida modified PY203 corn to increase expression of the enzyme phytase in PY203 corn, which will improve dietary phosphorus assimilation in non-ruminant livestock (Agrivida 2019). Phytase naturally occurs in corn, although expressed at low levels with commensurate low enzymatic activity (Rodehutscord et al. 2016; Ingelmann et al. 2018). Because PY203 corn has increased expression of phytase, the phytate in feeds utilizing PY203 corn can be more readily degraded to bioavailable forms of phosphorus during digestion. PY203 corn will be ground into a coarse meal (GRAINZYME® phytase) that will be used as a feed additive. Use of PY203 corn meal as feed additive would preclude or reduce the need for addition of inorganic phosphorus or microbial phytase to monogastric livestock feed. PY203 corn is intended to provide a cost effective means for providing phytase to monogastric animal feed, and facilitate dietary phosphorus assimilation via improved phytate digestion. PY203 corn may also be used for silage.

For matters of clarity it should be noted that phytate is also referred to in the lay and peer reviewed literature as phytic acid, inositol polyphosphate, and inositol hexakisphosphate (IP6). In this EA the term phytate is used.

1.3 Coordinated Framework for the Regulation of Biotechnology

On June 26, 1986, the White House Office of Science and Technology Policy issued the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework), which outlined Federal regulatory policy for ensuring the safety of biotechnology products. The primary federal agencies responsible for oversight of biotechnology products are the USDA, the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA).

In 2015, the Executive Office of the President (EOP) issued a memorandum directing the USDA, EPA, and FDA to update the Coordinated Framework to clarify current roles and responsibilities in the regulation of biotechnology products; develop a long-term strategy to ensure that the Federal biotechnology regulatory system is prepared for the future products of biotechnology; and commission an independent, expert analysis of the future landscape of biotechnology products. On January 4, 2017, the USDA, EPA, and FDA released a 2017 update to the Coordinated Framework (USDA-APHIS 2020a), and accompanying National Strategy for Modernizing the Regulatory System for Biotechnology Products (ETIPCC 2017).

USDA-APHIS is responsible for protecting animal and plant health. USDA-APHIS regulates products of biotechnology that may pose a risk to agricultural plants and agriculturally important natural resources under the authorities provided by the plant pest provisions of the Plant Protection Act (PPA), as amended (7 U.S. Code (U.S.C.) 7701–7772), and implementing regulations at 7 CFR part 340.

The purpose of EPA oversight is to protect human and environmental health. The EPA regulates pesticides, including plant incorporated protectants (PIPs) that have been introduced into plants using genetic engineering, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*). In addition, the EPA regulates certain microorganisms produced using genetic engineering (agricultural uses other than pesticides) under the Toxic Substances Control Act (15 U.S.C. 53 *et seq.)*. 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.). The USDA and EPA monitor tolerances, and the FDA enforces tolerances—except for meat, poultry, catfish, and certain egg products that are regulated by the USDA—to ensure the safety of the nation's food supply (USDA-AMS 2019a; US-EPA 2020p).

The purpose of FDA oversight is to ensure human and animal foods and drugs are safe and sanitary. The FDA regulates a wide variety of products, including human and animal foods, cosmetics, human and veterinary drugs, and human biological products under the authority of the FFDCA and Food Safety Modernization Act.

A more detailed description of the roles and responsibilities of USDA, the EPA, and FDA under the Coordinated Framework can be found on USDA's website (USDA-APHIS 2020a).

1.4 Requirement to Issue a Regulatory Status Determination

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.[3] 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);[4] however, the final rule is being implemented in phases. APHIS' new Regulatory Status Review (RSR) process, which replaces the petition for determination of nonregulated status process, became effective on April 5, 2021 for corn, soybean, cotton, potato, tomato, and alfalfa. The RSR process is effective for all crops as of October 1, 2021. However, "Until RSR is available for a particular crop…APHIS will continue to receive petitions for determination of nonregulated status for the crop in accordance with the [legacy] regulations at 7 CFR 340.6." (85 FR 29815).

The petition for a determination of nonregulated status subject of this EA is being evaluated in accordance with the regulations at 7 CFR 340.6 (2020) as it was received by APHIS, in June, 2019. Pursuant to the terms set forth in the final rule, 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.

[3] Genetic engineering in the context of 7 CFR part 340 refers to biotechnology-based techniques that use recombinant, synthesized, or amplified nucleic acids to modify or create a genome. Various terms are used in the lay and peer review literature in reference to new plant varieties that have been developed using modern molecular biology tools, these include "agricultural biotechnology", "genetically engineered", and "genetically modified". In this EA, the terms "genetic engineering" and "biotechnology" may be used interchangeably. The term "transgenic" may also be used when discussing or referring to a transgene introduced into the genome of a plant. The USDA does not regulate plants that could have been developed through traditional breeding techniques—to include chemical and radiation based mutagenesis—as long as they are not plant pests or developed using plant pests.

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

2 SCOPING AND PUBLIC INVOLVEMENT

APHIS seeks public comment on EAs through notices published in the *Federal Register*. On March 6, 2012, APHIS announced in the *Federal Register* updated procedures for the way it solicits public comment on petitions for determinations of nonregulated status. Details on policy and procedures for public participation in the petition review and NEPA process are available in the *Federal Register* notice [5] and on the APHIS website (USDA-APHIS 2020d).

2.1 Public Involvement for Petition 19-176-01p

On April 16, 2020, APHIS announced in the *Federal Register* that it was making Agrivida's petition available for public review and comment to help identify potential environmental and interrelated economic issues and impacts that APHIS should consider in evaluation of the petition.[6] APHIS accepted written comments on the petition for a period of 60 days, until midnight June 15, 2020. At the end of the comment period, APHIS had received 13 comments on the petition. Two comments were in opposition to deregulation of Agrivida's phytase corn, 11 comments—received from academia, the agricultural industry, and farmers—were in favor of approval of the petition. A full record of each comment received is available online at www.regulations.gov [Docket No. APHIS–2019–0084].

On June 24, 2021, APHIS announced in the *Federal Register* that it was making Agrivida's draft EA, preliminary Finding of No Significant Impact (FONSI), draft plant pest risk assessment (PPRA), and preliminary regulatory status determination available for public review.[7] APHIS accepted written comments on these documents for a period of 30 days, until midnight July 26, 2021. At the end of the comment period, APHIS had received 4 comments on the draft EA. Two comments were in opposition to deregulation of Agrivida's phytase corn, 2 separate comments—received from the same individual, were in favor of approval of the petition. A full record of each comment received is available online at www.regulations.gov [Docket No. APHIS–2019–0084].

As discussed in the FR notice described above, if APHIS determines that no substantive information has been received that would warrant APHIS altering its preliminary regulatory determination or FONSI, or substantially change the analysis of impacts in the EA, the preliminary regulatory determination will become final and effective upon notification of the public through an announcement on APHIS' website.

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[5] Federal Register, Vol. 77, No. 44, Tuesday, March 6, 2012, p.13258 – Biotechnology Regulatory Services; Changes Regarding the Solicitation of Public Comment for Petitions for Determinations of Nonregulated Status for Genetically Engineered Organisms [http://www.gpo.gov/fdsys/pkg/FR-2012-03-06/pdf/2012-5364.pdf]

[6] Federal Register, Vol. 85, No. 74 / Thursday, April 16, 2020, p. 21170 – Agrivida, Inc.; Availability of a Petition for Determination of Nonregulated Status for Maize Genetically Engineered for the Production of Phytase Enzyme [https://www.govinfo.gov/content/pkg/FR-2020-04-16/pdf/2020-08065.pdf]

[7] Federal Register, Vol. 86, No. 119 / Thursday, June 24, 2021, p. 33209 – Agrivida, Inc.; Availability of a Draft Plant Pest Risk Assessment, Draft Environmental Assessment, Preliminary Determination, and Preliminary Finding of No Significant Impact for Determination of Nonregulated Status of Maize Developed Using Genetic Engineering for the Production of Phytase Enzyme: [https://www.govinfo.gov/content/pkg/FR-2021-06-24/pdf/2021-13341.pdf]

No further *Federal Register* notice will be published announcing the final regulatory status determination. There were no comments received that altered the analyses presented in this EA, nor the PPRA.

2.2 Issues Considered in this EA

APHIS developed a list of topics for consideration in this EA based on issues identified in public comments on the petition, public comments submitted for other EAs and Environmental Impact Statements (EISs) evaluating petitions for nonregulated status, prior EAs/EISs for biotechnology-derived corn varieties, the scientific literature on agricultural biotechnology, and issues identified by APHIS specific to wild and cultivated *Zea* and *Tripsacum* species. The following topics were identified as relevant to the scope of impacts analysis in this EA (40 CFR § 1501.2, and § 1501.3):

- Agricultural Production: Acreage and areas of corn production, agronomic practices and inputs
- Physical Environment: Soils, water resources, air quality
- Biological Resources: Soil biota, animal communities, plant communities, gene flow and weediness, biodiversity
- Public health and worker safety
- Food animal health and welfare
- Domestic economy and international trade
- Potential impacts on threatened and endangered species
- Compliance of the Agency's regulatory status decision with Executive Orders, and environmental laws and regulations to which the action is subject.

Because the introduced genes confer phytase activity to PY203 corn, which can improve phosphorus assimilation in non-ruminant animals that consume PY203 corn, the primary focus of this EA is on: (1) potential impacts on human and animal (livestock) health, (2) effects on wildlife that may consume PY203 corn or PY203 corn hybrids, and (3) gene flow and potential weediness.

3 ALTERNATIVES

NEPA implementing regulations (40 C.F.R. 1500 – 1508) require agencies to evaluate alternatives to the proposed action that would avoid or minimize adverse impacts, or enhance the quality of the human environment, while meeting the purpose and need for the Agency's action (in this case, a regulatory decision). Two alternatives are evaluated in this EA: (1) No Action, denial of the petition, which would result in the continued regulation of PY203 corn, and (2) Preferred Alternative, approval of the petition, which would result in a determination of nonregulated status for PY203 corn.

3.1 No Action Alternative: Deny the Petition Request

One of the alternatives that APHIS considers is a "No Action Alternative", consistent with CEQ regulations at 40 CFR § 1502.14. Under the No Action Alternative APHIS would deny the petition request for nonregulated status and PY203 corn would remain regulated under 7 CFR part 340. Permits issued by APHIS would be required for the introduction of PY203 corn. Because APHIS concluded in its PPRA that PY203 corn is unlikely to pose a plant pest risk (USDA-APHIS 2020b), denial of the petition would not be a scientifically nor legally sound response as it would not meet the purpose and need in providing a science based regulatory status decision to the petitioner, pursuant to 7 CFR part 340.

3.2 Preferred Alternative: Approve the Petition–Determination of Nonregulated Status for PY203 Corn

Under this alternative APHIS would approve the petition request. PY203 corn and progeny derived from it would no longer be subject to APHIS regulation under 7 CFR part 340. Permits issued by APHIS would no longer be required for introductions of PY203 corn. Because it was determined that, based on the scientific evidence before the Agency, PY203 corn is unlikely to pose a plant pest risk (USDA-APHIS 2020b), this alternative satisfies the purpose and need to respond to the petition for nonregulated status with a science based regulatory status decision, pursuant to the requirements of 7 CFR part 340, and the Agency's statutory authority under the PPA.

3.3 Alternatives Considered but Dismissed from Detailed Analysis in the EA

APHIS has evaluated other alternatives for consideration in this EA. For example, APHIS has considered an alternative that would entail approving the petition request in part. APHIS has also received public comments on EAs and EISs stating the APHIS should require mandatory isolation or geographic restriction of a biotechnology-derived crop from other conventionally bred cropping systems, and/or require testing for the presence of crop plant material in conventionally bred crops and commodities. APHIS has considered these options as well.

Based on the PPRA for PY203 corn, APHIS concluded that PY203 corn is unlikely to pose a plant pest risk (USDA-APHIS 2020b). Thus, approval of the petition in part and/or the imposition of testing, release, and/or isolation requirements on PY203 corn would be outside 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 alternatives were dismissed from detailed analysis in this EA.

3.4 Summary of the No Action and Preferred Alternative Analyses

Table 3-1 presents a summary of the environmental impacts associated with the No Action Alternative and Preferred Alternative that are evaluated in this EA. Detailed analysis of the affected environment and environmental impacts are discussed in Chapter 4.

Table 3-1. Summary of Potential Impacts of the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate PY203 Corn**	**Preferred Alternative: Approve the Petition for Nonregulated Status for PY203 Corn**
Meets Purpose and Need and Objectives	No	Yes
Management Practices		
Acreage and Areas of Corn Production	Denial of the petition would have no effect on the areas or acreage utilized for corn production. Fluctuations in production areas and acreage would be relative to weed, insect pest, and disease pressures, and market demand for corn commodities. Regulated field trials would be conducted on lands allocated for this purpose.	Approval of the petition could result in minor increase in U.S. corn acreage. Agrivida states that the area required to produce sufficient PY203 corn to meet the demands of the poultry and swine production markets is about 10,000 acres, which is around 0.01% of U.S. corn acreage. Annual corn acreage normally fluctuates around several million acres. Thus, any increase in acreage utilized for PY203 corn production would be considered *de minimis*.
Agronomic Practices and Inputs	Agronomic practices and inputs used in corn crop production, to include regulated field trials, would be unaffected by denial of the petition.	Agronomic practices and inputs used for PY203 corn production would be the same as for other corn varieties.
Corn Produced Using Genetic Engineering	Denial of the petition would have no effect on grower choice in the planting of biotechnology-derived and conventionally bred feed corn.	Approval of the petition would provide for cultivation of a biotechnology-derived corn with increased phytase expression. This would be expected to be of benefit to the animal feed industry.
Physical Environment		
Soil Quality	Agronomic practices and inputs associated with corn production potentially impacting soils, to include regulated field trials, would continue along current trends.	The agronomic practices and inputs are the same for both PY203 and current corn varieties. Phosphorus, phytate, and phytase inputs to soils from PY203 corn would have negligible effects on phosphorus cycling.
Water Resources	Denial of the petition would have no effect on water resources in the United States. Regulated field trials are limited on a spatiotemporal scale, and present negligible risks to water resources.	Because PY203 corn is agronomically similar to currently cultivated corn, and phytase and PMI occur naturally in terrestrial and aquatic environments (plants, microorganisms), approval of the petition and subsequent commercial production of PY203 would present the same potential impacts to water resources as currently cultivated corn varieties. Relative to the extent of use of PY203 corn in livestock feed: As is the case with current microbial phytase additives, there could be reductions in phosphorus

Table 3-1. Summary of Potential Impacts of the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate PY203 Corn**	**Preferred Alternative: Approve the Petition for Nonregulated Status for PY203 Corn**
		runoff from agricultural facilities utilizing PY203 corn (GRAINZYME® phytase) in feed, and cropping systems utilizing manure from animals reared on PY203 corn based feed, which would benefit sustaining water quality. PY203 corn production could increase total U.S. corn acreage by around about 10,000 acres. If there was an incremental increase in corn production resulting from PY203 corn planting, the small increase in planting could contribute to minor increases in runoff of pesticides, topsoil, and fertilizers. The potential increase in phosphorus runoff from PY203 cropland would be expected to be negligible relative to the potential reductions in phosphorus runoff discussed above.
Air Quality	Emission sources, namely tillage and machinery combusting fossil fuels, and the level of emissions associated with corn production, to include regulated field trials, would be unaffected by denial of the petition.	Because the agronomic practices and inputs used for PY203 corn production would remain unchanged, no changes to emission sources (e.g., planting and harvesting equipment) are expected. The area required to produce sufficient PY203 corn (GRAINZYME® phytase) to meet the demands of the poultry and swine production markets is anticipated to be about 10,000 acres. Annual acreage planted to corn normally varies on the order of millions of acres annually. Thus, there would be no substantive increase in the acreage of U.S. corn crops (0.01%). If PY203 corn were produced, air quality would be affected along current trends by emission sources such as tillage (PM), pesticide application (aerosols, spray drift), and use of farm equipment that combusts fossil fuels (CO2, NO2, SO2). The EPA and USDA efforts to limit/reduce emissions, along with state and local efforts, would likewise continue.
Biological Resources		
Soil Biota	Potential impacts of corn production/regulated field trials on soil biota would continue along current trends.	Commercial production of PY203 corn is not expected to present any risks to soil biota. Phytase enzymes naturally occur in soils; produced by plants, animals, bacteria, and fungi. Because the ear (kernel) of the plant, where phytase is primarily expressed, is harvested, negligible amounts of phytase

Table 3-1. Summary of Potential Impacts of the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate PY203 Corn**	**Preferred Alternative: Approve the Petition for Nonregulated Status for PY203 Corn**
		would enter soils relative to amounts already present in the soil. PMI likewise naturally occurs in soils, expressed in various taxa including enteric bacteria, fungi, insects, some species of plants and nematodes, and mammals (de Lonlay and Seta 2009; Hu et al. 2016).
Animal Communities	Regulated field trials of PY203 corn would present negligible risk to animal communities.	Approval of the petition, and subsequent commercial production of PY203 corn, would not be expected to affect animal communities adjacent to or within PY203 corn cropping systems any differently from that of current corn cropping systems. To the extent reductions in phosphorus runoff from agricultural facilities (discussed above) help reduce eutrophication of surface waters, benefits to fish and other aquatic biota would be expected. As detailed in this EA, the phytase and PMI expressed in PY203 corn present negligible risk to wildlife.
Plant Communities	Regulated field trials of PY203 corn would present negligible risks to plant communities.	Because the agronomic practices and inputs that will be used for PY203 corn production are the same as for other corn varieties, potential impacts on plant communities would be the same as that for other corn varieties currently cultivated.
Gene Flow and Weediness	*Tripsacum* species are the only sexually compatible plants found in the United States. The potential for corn (*Zea mays*) to hybridize with wild relatives of *Tripsacum* is low; hybridization and successful introgression of *Z. mays* genes into *Tripsacum* is rare (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995). Due to permit requirements, gene flow to *Tripsacum* species during regulated fields trials of PY203 corn is highly unlikely.	PY203 corn, if grown for commercial purposes, would be cultivated as are current corn varieties and present the same potential risk for gene flow, specifically the propensity for and frequency of gene flow, as current corn varieties. In the unlikely event pollen flow from PY203 corn to *Tripsacum* were to occur, it is unlikely the phytase trait in PY203 corn would present any risk to communities of *Tripsacum* species in terms of plant fitness, or their ecological role in the communities of other plants. Successful introgression of *Zea mays* genes into *Tripsacum* populations, successful gene flow in this direction, has not been observed in the wild (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995).
Biodiversity	Denial of the petition, and further regulated field trials of PY203 corn, would present negligible risks to biodiversity in an around PY203 corn crops.	Because PY203 corn is agronomically the same as currently cultivated corn varieties, commercial production of PY203 corn would affect biodiversity in and around PY203 corn crops no differently than other corn

Table 3-1. Summary of Potential Impacts of the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate PY203 Corn**	**Preferred Alternative: Approve the Petition for Nonregulated Status for PY203 Corn**
		cropping systems. Any reductions in phosphorus runoff from agricultural facilities utilizing PY203 corn (GRAINZYME® phytase) in feed, and cropping systems utilizing manure from animals reared on PY203 corn (GRAINZYME® phytase) based feed, would be of benefit to aquatic ecosystems.
Human and Animal Health		
Human Health and Worker Safety	Denial of the petition would no effect on human health.	Approval of the petition for PY203 corn would not present any risks to public health. The intended use of PY203 corn is for animal feed. Agrivida completed an Early Food Safety Evaluation (New Protein Consultations; NPC) for the Phy02 phytase protein (NPC 000015) on August 7, 2015 (US-FDA 2020b). The FDA had no food safety concerns. A Pre-market Biotechnology Notification (PBN) consultation for PY203 corn was submitted to the FDA in June 2018 (Agrivida 2019; US-FDA 2020a).
Animal Health and Welfare	Denial of the petition would have no effect on animal health and welfare. PY203 corn would remain regulated and unlikely to be utilized for animal feed.	Grain and forage derived from PY203 corn is intended for use as animal feed to facilitate livestock phosphorus assimilation, and animal development and health. Agrivida submitted GRAS Notices AGRN 21 and AGRN 27 to the FDA in 2016 and 2018, respectively. The FDA Center for Veterinary Medicine (CVM) issued response letters indicating that CVM had no questions regarding the Agrivida's conclusion that the ground corn grain containing Phy02 phytase derived from PY203 corn is GRAS under its intended conditions of use in poultry and swine feeds, respectively (US-FDA 2020c).
Socioeconomic		
Domestic Economy	Denial of the petition would have no effect on the U.S. domestic corn feed markets.	PY203 corn grain will be ground into a coarse meal (GRAINZYME® phytase) that will be sold as a feed additive for poultry and swine. PY203 corn could also be used as silage. Relative to food animal rearing, the domestic economic impacts associated with the introduction of PY203 corn into commerce would be considered potentially beneficial; it may reduce the need for the addition of inorganic phosphorus and microbial phytase products to poultry and

Table 3-1. Summary of Potential Impacts of the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate PY203 Corn**	**Preferred Alternative: Approve the Petition for Nonregulated Status for PY203 Corn**
		swine feed, and thereby reduce costs to livestock producers.
International Trade	Denial of the petition would have no impacts on trade. Currently available corn products would be exported subject to market demand.	The commodity itself, GRAINZYME® phytase, could see demand in foreign markets. To the extent that PY203 feed corn emerges as a valued commodity that facilitates food animal rearing, its introduction may enhance the competitiveness of U.S. feed grain producers in global markets. GRAINZYME® phytase crops are grown using identity preservation methods, which reduce the likelihood of commingling with other grain commodities.
Coordinated Framework		
U.S. Regulatory Agencies	Denial of the petition would have no effect on the roles of the FDA and EPA in the oversight of PY203 corn. Introductions of PY203 corn would be regulated by USDA.	Agrivida completed an Early Food Safety Evaluation (New Protein Consultations; NPC) for the Phy02 phytase protein (NPC 000015) on August 7, 2015 (US-FDA 2020b). A Pre-market Biotechnology Notification (PBN) consultation for PY203 corn was submitted to the FDA in June, 2018 (BNF 000167; (Agrivida 2019; US-FDA 2020a)). Pesticide use with PY203 corn, as with all other crops, will be subject to EPA registration and label use requirements.
Regulatory and Policy Compliance		
ESA, CWA, CAA, SDWA, NHPA, 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/effects means changes to the human environment that could result from approval of the petition, subsequent commercial production of PY203 corn, and market utilization of feed commodities derived from this variety.

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 effects on the components and functioning of ecosystems), historic, cultural, social, and effects on human health. Economic effects, such as on corn growers and corn commodities markets, are also considered.

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 biotechnology-derived crop occur within the context of agriculture's general contribution to environmental change (NRC 2010). Crop production has historically converted biologically diverse natural grasslands, wetlands, and native forests into less diverse agroecosystems to produce food, feed, fiber, and fuel. Potential effects on the environment are relative to the intensity and scale of crop production over time, the agronomic inputs applied (e.g., fertilizers, pesticides used to control pests, weeds, and pathogens, irrigation water), the effective management of inputs, and tillage. Corn is used to produce a variety of food products, animal feed, fuel ethanol, and a wide range of industrial products. Meeting market demand for these products requires around 90 million acres of corn production per year (USDA-NASS 2020a). In general, tillage, crop monoculture, and fertilizer and pesticide inputs can have adverse effects on topsoils, water quality, and aquatic ecosystems; fertilizer and pesticide inputs can also present risks to human health and wildlife (NRC 2010). Agriculture is a leading cause of water-quality impairment in the United States (US-EPA 2020g). No-tillage systems, crop rotations, integrated pest and weed management, and other environmentally beneficial management practices can help ameliorate some of the adverse impacts, although a tradeoff between the production food, feed, fiber, and fuel, and some degree of environment impacts, will always remain (NRC 2010; NAS 2016). Due to the scale of crop production in the United States, developing and implementing environmentally sound, sustainable agricultural management practices is a primary goal of federal and state programs—applicable to biotechnology-derived, conventional, and organic cropping systems alike (e.g., (USDA-NRCS 2019a; US-EPA 2020d; USDA-NIFA 2020; USDA-NRCS 2020a), and others).

Gene flow, movement of a transgene to sexually compatible species, has also been a topic of concern, more so in terms of potential economic, as opposed to ecological impacts. For corn, gene flow to wild relative species has not been an issue to date because sexually compatible relatives of corn do not exist in the United States. However, gene flow of approved transgenic traits into conventionally bred corn varieties, or other transgenic varieties, is a concern for farmers and markets that depend on adhering to strict non-transgenic trait presence and identity preservation standards for certain food and feed commodities. Such gene flow can result in adverse economic impacts to the transgenic trait-sensitive market.

It is within this context that APHIS evaluates the potential impacts of PY203 corn on the human environment if cultivated on a commercial scale for animal feed purposes.

PY203 Corn: Assumptions Used in Analysis

It assumed that, in the event Agrivida's petition is approved, PY203 corn would be produced commercially, and the grain and silage used for animal feed. PY203 has no herbicide resistant properties, thus, there is no specific herbicide intended for use with this crop. For this reason, weed and herbicide resistant weed management with an herbicide resistant trait is outside the scope of analysis for this EA. It is further assumed, for the purposes of this EA, that the only potential impacts that could derive from production and marketing of PY203 that could be considered unique, as compared to other corn varieties, are relative to the trait genes and gene products, which are summarized below.

Phytase

Phytases naturally occur in commonly consumed cereals, legumes, oilseeds, and nuts (Greiner et al. 2000; Viveros et al. 2000; Reddy 2001; Kumar et al. 2010; Gupta et al. 2015; Secco et al. 2017) and vegetables such as cabbage, spinach and lettuce leaves, mushrooms, radishes and onions (Phillippy and Wyatt 2001). In animals, to include humans, phytases are produced endogenously by the small intestinal mucosa and microflora associated with large intestine, although endogenous phytase activity in animals is much less than that found in plants and microbes (Weremko et al. 1997).

Bioinformatic analyses of the Phy02 phytase amino acid sequence and comparison with a database of allergenic proteins revealed no similarities to known or putative allergens (Agrivida 2019). Agrivida completed an Early Food Safety Evaluation (New Protein Consultations; NPC) for the Phy02 phytase protein (NPC 000015) on August 7, 2015 (US-FDA 2020b). Agrivida submitted an Animal Food Generally Recognized As Safe (GRAS) Notice (AGRN21) in May 2016 (US-FDA 2017). Based on the information Agrivida provided to the FDA, as well as other information, the FDA stated they had no questions regarding Agrivida's conclusion that grain obtained from corn expressing an altered *appA* 6-phytase gene obtained from *Escherichia coli* strain K12 (that in PY203 corn) is GRAS under its intended use—for animal feed (US-FDA 2020b).

Phosphomannose Isomerase (PMI)

PMI is an enzyme involved in carbohydrate metabolism and it, or a highly homologous/orthologous enzymatic proteins, are expressed in various taxa including enteric bacteria, fungi, insects, some species of plants and nematodes, and mammals—including monkeys, mice, and humans (de Lonlay and Seta 2009; Hu et al. 2016). PMI served as a plant selectable marker in the development of PY203 corn. In 2004, the EPA issued an exemption from the requirement of a tolerance for residues in or on plant commodities comprised of PMI, and the genetic material necessary for its production, in all plants when applied/used as plant-incorporated protectant inert ingredients (See 40 CFR § 174.527–Phosphomannose isomerase in all plants; exemption from the requirement of a tolerance). There are no identifiable risk to human health, livestock health, or wildlife presented by PMI.

4.2 No Action Alternative – Deny the Petition

Because APHIS concluded in its PPRA that PY203 corn is unlikely to pose a plant pest risk (USDA-APHIS 2020b), denial of the petition for nonregulated status 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 policies embodied in the Coordinated Framework. Because it would be unreasonable to implement an alternative absent any jurisdiction to do so, this alternative is not a practicable option.

While implementing the No Action alternative is not feasible, APHIS provides a summary evaluation for denial of the petition, where PY203 corn would remain regulated and require APHIS authorization for importation, interstate movement, or release into the environment.

APHIS' regulation of PY203 corn would have no effect on the acreage used for U.S. corn production, nor the current practices and inputs used for the commercial production of corn. Likewise, denial of the petition would have no effect on the physical environment, biological resources, human or animal health, or domestic or international corn markets. Any field testing or interstate movement of PY203 corn would require APHIS authorization, which would be provided via permit, pursuant to 7 CFR part 340, described in more detail below. For permits, APHIS Biotechnology Regulatory Services prescribes criteria and conditions that must be met in order to ensure that the regulated organism is introduced in such a way that it is not inadvertently released beyond the proposed introduction site, and it or its progeny do not persist in the environment. Applicants submit documents for releases, such as design protocols, that address how the required conditions will be met. Permit applicants must describe how developers will perform field testing, including specific measures to keep the organism confined to the authorized field site and measures to ensure that it does not persist after completion of the field test. The permitting provisions found in 7 CFR part 340 describe the information required for permit applications, the standard permit conditions, and administrative information. Standard permit conditions are listed in the regulation, and APHIS can supplement these with additional conditions or requirements, as necessary.

Actions taken by APHIS on permit applications are subject to NEPA. APHIS ensures compliance of permits issued with NEPA, and CEQ and USDA implementing regulations.[8] Issuance of permits are typically authorized under a categorical exclusion from the requirement to conduct an EA or EIS, consistent with APHIS' NEPA implementation regulations (7 CFR part 372). APHIS conducts EAs or EISs for permits as applicable to the permit request. This process complies with CEQ and USDA regulations for implementing NEPA.

There are no anticipated impacts on the human environment that would derive from denial of the petition. To the extent individuals comply with APHIS permit requirements, EPA requirements for pesticide use, and ESA requirements, there would be little risk of harm to wildlife or natural resources as a result of APHIS authorized field testing of PY203 corn. Interstate movement of PY203 corn would present negligible environmental risks.

4.3 Preferred Alternative – Approve the Petition

4.3.1 U.S. Corn Production

4.3.1.1 Acreage and Area of U.S. Corn Production

There are three primary varieties of corn cultivated in the United States: Dent (or field) corn (*Zea mays* var. *indenata*), sweet corn (*Zea mays* var. *saccharata*), and popcorn (*Zea mays* var. *everta*). To a lesser extent flour (*Zea mays* var. *amylacea*) and waxy corn (*Zea mays* var. *ceratina*) varieties are produced. PY203 corn is a dent corn variety. Dent corn, at maturity, has an obvious depression (or dent) at the crown of the kernels—thus its name. Dent corn is primarily used for animal feed and fuel ethanol stock, and comprises the bulk of U.S. production, in excess of 90% of corn acres annually. Among dent corn commodities, animal feed accounts for around 40% – 48% of use, and stock for the production of fuel ethanol for around 30% – 35% (PRX 2019; USDA-ERS 2019e; NCGA 2020). The remainder is processed into a variety of food and industrial products such as starch, sweeteners, corn oil and syrup, and beverage and industrial alcohol (Figure 4-1).

[8] CEQ regulations for implementing NEPA at 40 CFR 1500; USDA regulations implementing NEPA at 7 CFR part 1b; and APHIS regulations at 7 CFR part 372.

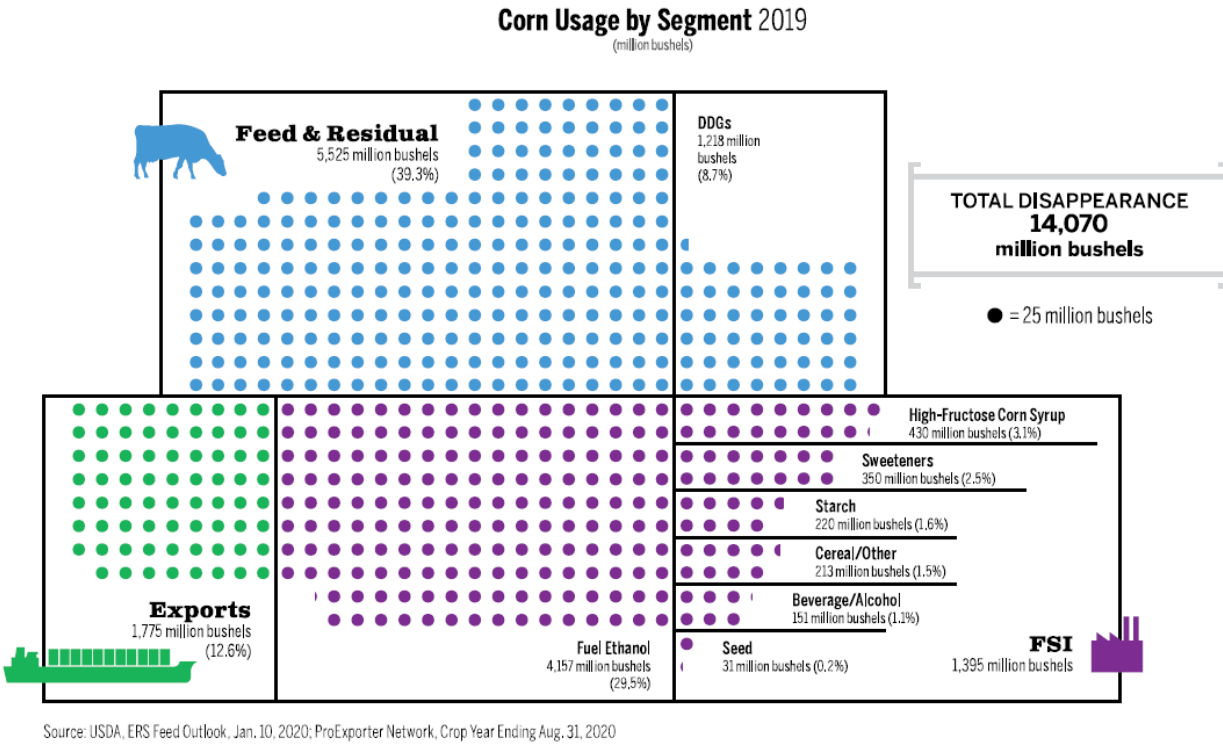

Figure 4-1. Corn Uses in the United States, 2019
Corn has food, seed, and industrial uses (FSI). Note that feed is comprised of grain, and distillers' dried grains with solubles (DDGS). Feed for both dairy and beef has been the primary use of DDGS, but increasingly larger quantities of DDGS are making their way into the feed rations of hogs and poultry. The statistics/percent allocations of corn, provided here for 2019, vary slightly on an annual basis. Source: (PRX 2019; USDA-ERS 2019e; NCGA 2020)

Over the last ten years a total of around 85 to 95 million acres of corn have been planted in the United States on an annual basis (USDA-NASS 2019a). This comprises approximately 25% of total U.S. cropland (~394 million acres). Production of popcorn and sweet corn comprise about 0.2 and 0.5 million acres, respectively (< 1% of corn acreage on an annual basis) (USDA-NASS 2014, 2019b). While commercial corn crops are grown in all states to some extent (except Alaska), the majority of production occurs in the Corn Belt, generally defined as Illinois, Iowa, Indiana, southern and western Minnesota, eastern South Dakota and Nebraska, western Kentucky and Ohio, and the northern two-thirds of Missouri. The leading corn-producing states of Illinois, Iowa, and Nebraska account for approximately 40 % of the annual U.S. harvest (USDA-NASS 2019b). Production also occurs in the Pacific Northwest, California's Central Valley, along the Mississippi River, Texas, and up the Eastern Seaboard from Georgia to Upstate

New York (Figure 4-2).

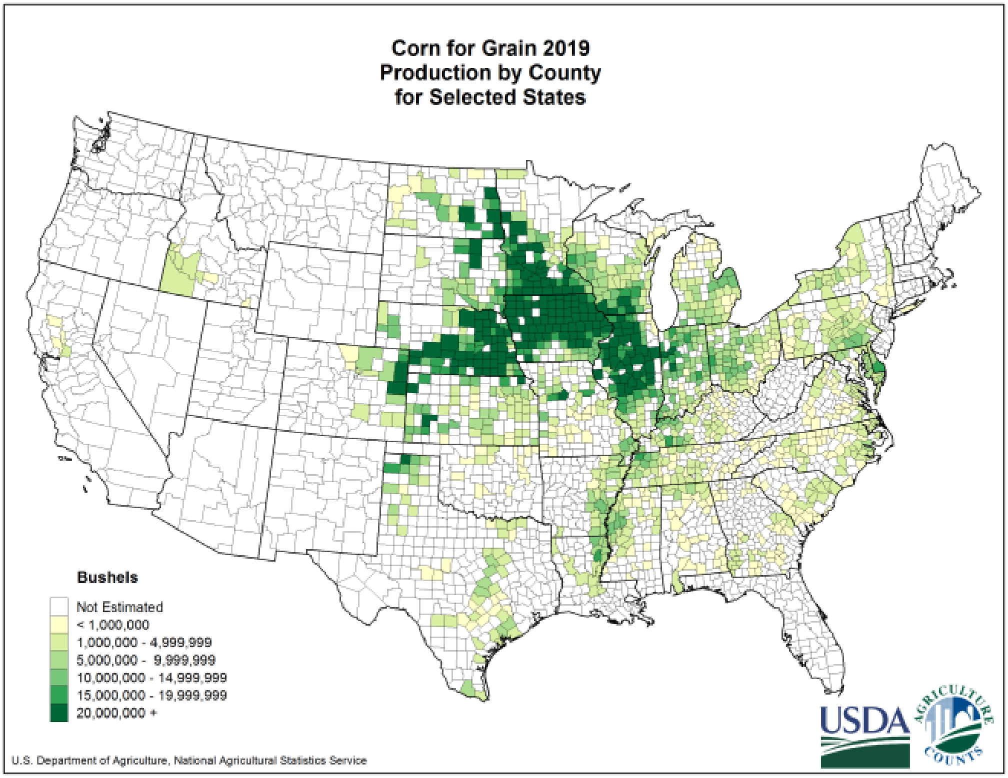

Figure 4-2. Corn Cultivation in the United States by County, 2019
Source: (USDA-NASS 2020b)

Around 92% of the corn produced in the United States is comprised of biotechnology-derived varieties (Figure 4-3), the majority of this corn is dent/field corn, with limited quantities of biotech sweet corn being grown. Only about 1% of the corn grown in the United States is sweet corn—that consumed as canned, frozen, or fresh ears—and of that, only about 10% of sweet corn acreage is comprised of biotech varieties. Most corn varieties are stacked-trait herbicide-resistant (HR) and insect-resistant (IR). Stacked-trait varieties with HR and IR traits accounted for 79% of the 2020 crop. Only 10% contained a single HR trait, and 3% a single IR in 2020. Of the ~90 million corn acres planted in 2020, around 7.27 million were conventionally bred.

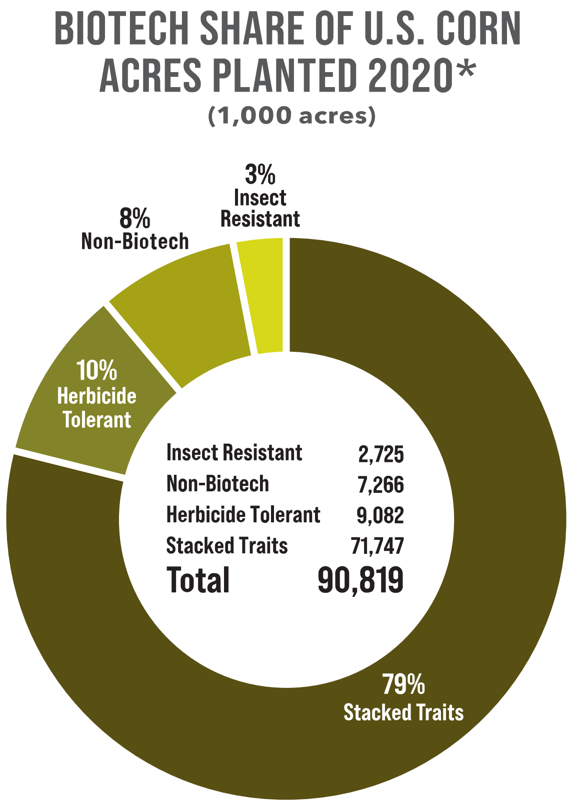

Figure 4-3. Biotech Corn Traits Planted in the United States, 2019
Source: (NCGA 2020)

4.3.1.2 Agronomic Practices and Inputs

Corn production utilizes a variety of agronomic practices and inputs that aim to achieve optimal yield, product quality, and grower net returns. These include the occasional or regular application of manure or synthetic fertilizers; pesticides; tillage; crop rotation; and cover crops. Organic farming systems are required to exclude certain inputs, such as use of synthetic pesticides. Some of these practices (e.g., tillage) and inputs (e.g., fertilizers, pesticides) can, when applied in excess or improperly, or as a result of aggregate effects, present environmental challenges in maintaining air, soil, and water quality. Pesticide and fertilizer use can also present risks to wildlife and human health. The relationship between these practices and inputs and air, soil, and water quality, biological resources, human health, as well as the socioeconomic aspects of corn production, are discussed in the subsequent sections of this chapter.

4.3.1.2.1 Agronomic Practices

Growers employ several practices for the management of pests and weeds, summarized below (Table 4-1), such as scouting for weeds, crop rotation, and maintaining ground cover or mulching. Tillage is the primary practice that can have environmental impacts, and this topic, in relation to PY203 corn, discussed in more detail below.

Table 4-1. Top Practices in Pest Management, 2018 Crop Year	
	% of Corn Acres
Monitoring: Scouted for weeds	94

Avoidance: Rotated crops during last three years	84
Prevention: Used no-till or minimum till	65
Suppression: Maintained ground cover, mulched, or used other physical barriers	45

The USDA-NASS survey asked growers in 18 states that accounted for 93% of the planted corn acreage to report on the practices they used to manage pests, defined as weeds, insects, or diseases. Corn growers reported practices in four categories: prevention, avoidance, monitoring, and suppression. Only the top practice in each category is shown. Source: (USDA-NASS 2019c)

4.3.1.2.1.1 Tillage

Tillage is used to control weeds and soil-borne pests and disease, and prepare the seedbed. Tillage types are classified as conventional, reduced, and conservation tillage (e.g., no-till and mulch-till), which are characterized in part by the amount of plant material left on the field after harvest and the degree of soil disturbance they cause. Conventional tillage involves intensive plowing leaving less than 15% crop residue in the field; reduced tillage leaves 15% to 30% crop residue; conservation tillage, such as mulch-till, involves leaving at least 30% of crop residue. No-till systems leave all crop residue on the field (Claassen et al. 2018; OSU 2019).

Decisions concerning the amount, timing, and type of tillage to employ involve consideration of a wide range of interrelated factors such as the variety and extent of weeds and crop pests present, soil erosional capacity, fuel and other input costs, anticipated weather patterns, and potential air and water quality issues. Over the long-term conventional tillage reduces soil quality, and results in soil erosion and runoff that can adversely affect surface waters (Wallander 2015). Conservation tillage systems are the least intensive and, as the name implies, aim to conserve topsoil and soil quality. Conservation tillage provides a variety of agronomic and economic benefits, such as preservation of soil organic matter, reductions in soil erosion and water pollution, as well as reductions in fuel use and crop production costs (Fernandez-Cornejo et al. 2012; Claassen et al. 2018). However, conservation tillage, especially no-till, can also cause production problems such as increased soil compaction, perennial weeds or weed shifts, buildup of plant pathogens or pests in crop residue, and slow early crop growth due to cooler soil temperatures (Roth 2015). A systematic use of crop rotations can improve the success of conservation tillage by eliminating some of these stresses observed in continuous no-till corn (Roth 2015).

The use of conservation tillage increased steadily throughout the 1980s and 1990s and continues to do so. While approximately 33% of corn acres were produced using conservation tillage systems in 1990, 65% of corn acres were produced using conservation tillage systems in 2016 (Claassen et al. 2018). No-till accounted for around 42% of conservation tillage on U.S. corn acres in 2016 (27% overall) (Figure 4-4). An increase in conservation tillage has been facilitated by the availability of post-emergent herbicides (since the 1980s), which can be applied over crops throughout the growing season—not just before planting, as had previously been the case (Fernandez-Cornejo et al. 2012). Another factor has been the implementation of soil conservation programs that began in the mid-1980s, which encourage/incentivize implementing conservation tillage practices (USDA-NRCS 2006). Continued increases in conservation tillage since the late 1990s have also been attributed to, in part, the use of herbicide resistant crops, which can facilitate effective weed management and reduce the need for mechanical weed control (Towery and Werblow 2010; USDA-ERS 2012).

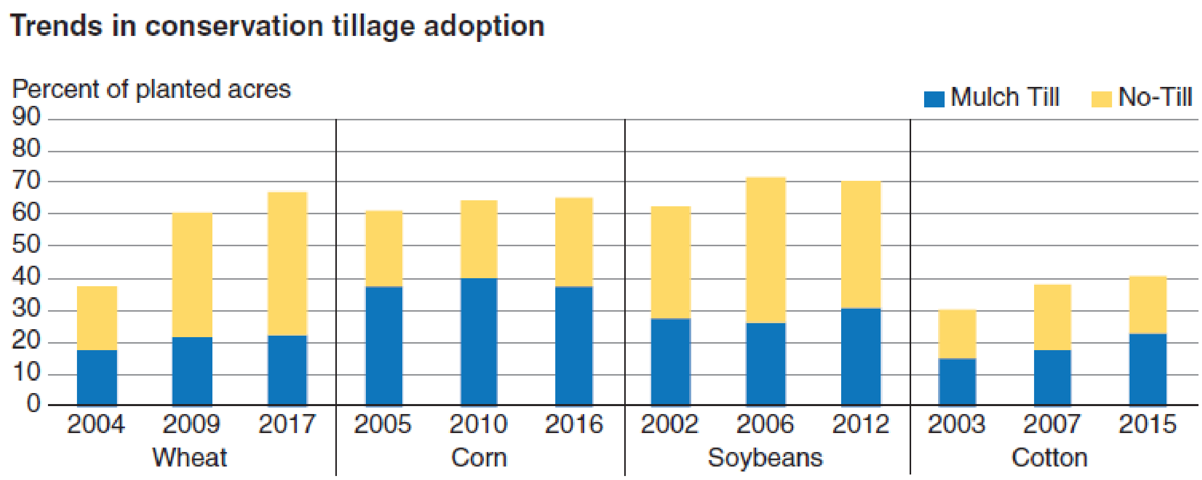

Figure 4-4. Conservation Tillage Practices in Corn, 2005 – 2016
Source: (Claassen et al. 2018)

4.3.1.2.2 Agronomic Inputs

In addition to the agronomic practices described, chemical inputs for control of insect pests, nematodes, pathogens, weeds, and the addition of plant nutrients to soils are an integral aspect of corn production—biotech, conventionally bred, and organic cropping systems alike. These inputs are used to maximize yield, product quality, and grower net returns. Agronomic inputs relative to PY203 corn production are discussed following.

4.3.1.2.2.1 Fertilizers

The majority of corn acreage is treated with fertilizer. Soils in many areas of the United States where corn is produced are naturally deficient in nitrogen, phosphorus, and other nutrients, requiring fertilizer inputs, to include manure, to produce crops efficiently, and the yields necessary, to meet market demand. Given the importance of nutrient availability to corn growth, fertilization with nitrogen, phosphorus, and potassium is practiced widely in the United States.

Since 1975, around 94% to 99% of corn acreage has been treated with nitrogen, with the average rate of application increasing from around 105 to 149 lbs/acre from 1975 to 2018 (USDA-ERS 2019a). Phosphate use has fluctuated from 79% to 86% of acreage, at an average rate of 56 to 60 lbs/acre (USDA-ERS 2019a). The acreage treated with potash has slightly declined since 1975 to around 65% to 84% of acres in 2016, while application rates ranged from 67 to 87 lbs/acre (USDA-ERS 2019a). Inputs for the 2018 crop year (latest data) are provided in Table 4-2.

While nitrogen and phosphorus are important agricultural inputs in crop production, the introduction of amounts exceeding recommended thresholds can have a number of undesirable impacts on water and air quality (discussed in the following relevant sections).

Table 4-2. Fertilizer Applied to Corn Planted Acres, 2018 Crop Year

Fertilizer	% of Planted Acres	Avg. Rate for Year (lbs/acre)	Total Applied (billion lbs)

Nitrogen (N)	98	149	12.0
Phosphate (P2O5)	79	69	4.5
Potash (K2O)	63	87	4.5
Sulfur (S)	32	18	0.5

Source: (USDA-NASS 2019c)

4.3.1.2.2.2 Manure

Manure is widely used as a crop fertilizer and soil amendment. It contains not only nutrients—such as nitrogen, phosphorus, and potassium—but can improve soil quality by neutralizing acidity, increasing organic matter, decreasing compaction, and increasing water-holding capacity. Manure is used as a substitute for commercial fertilizers relative to pricing, proximity of a crop field to sources of manure production, and cost of transport (MacDonald et al. 2009). The option to use manure is primarily limited by the cost of transport, which can be expensive for even short distances (MacDonald et al. 2009).

In 2017 (latest data), approximately 297,297 farms utilized manure on a total of 23.8 million acres (USDA-NASS 2019b). Corn, which is planted on about one-quarter of U.S. cropland, accounts for around 45% of U.S. crop acreage receiving manure, and 65% of the nitrogen applied by farmers based on estimates for 2006 (Ribaudo et al. 2011), with the Corn-Belt receiving the bulk of fertilizer application. Most manure applied to corn comes from dairy cattle and hog operations (MacDonald et al. 2009).

While beneficial to crop production manure can pose environmental and human health risks when stockpiled or applied in excessive amounts (discussed further in subsequent sections on the physical environment, biological resources, and human health). Most manure producing operations store manure prior to application, in pits and lagoons, which can pose environmental risks from seepage, flooding, or catastrophic failure of containment structures (MacDonald et al. 2009). Manure from crop fields, animal feeding operations, and storage sites can also be transmitted to surface waters through the runoff, carrying nutrients, organic matter, and potentially, pathogens. Leaching of nutrients and enteric bacteria to ground water, and volatilization of gases and odors to the atmosphere, can also occur (MacDonald et al. 2009). For example, in 2018, at least 50 lagoons in North Carolina overflowed in the wake of Hurricane Florence. For two lagoons that failed completely, it is estimated that 7 million gallons of untreated swine feces were unleashed into flood waters and surrounding waterways, becoming part of the surrounding ecosystem (Davis 2018). Several community systems in the state stopped supplying drinking water while the EPA monitored for potential contamination. Crops and commodities exposed to floodwaters were considered adulterated by the FDA and could not be used for human food purposes, nor used for animal feed unless they passed a testing protocol (Davis 2018).

Because manure can present risks to water and air quality, federal, state, and local authorities regulate manure production facilities and manure storage. The EPA's Clean Water Act regulations, discussed further in 4.3.2.2–Water Resources, prohibits discharges from certain animal feeding operations to waters of the United States without a National Pollutant Discharge Elimination System (NPDES) permit. Federal and state regulations also require many large operations to develop and implement nutrient management plans (NMPs) as a part of manure production and application (MacDonald et al. 2009; US-EPA 2019d).

4.3.1.2.2.3 Pesticides

Pesticides contribute to higher yields, optimal product quality, and grower net returns by controlling weeds, insects, nematodes, and plant pathogens. However, some pesticides may be potentially harmful to humans and wildlife, as well as other crops, when not properly used. Common corn pests include *Coleoptera* species (beetles), *Lepidoptera* species (moth and butterfly larvae), pathogenic fungi (e.g., corn leaf blight), bacteria (e.g., stalk rot), and viruses (e.g., dwarf mosaic virus). There are around 50 species of weeds that occur among U.S cornfields, requiring annual control (Jhala et al. 2014). Weeds have been and will remain a problem in corn crop production; they are difficult to manage, competitive, and use up resources — soil moisture, nutrients, access to sunlight — that would otherwise be available to the corn plant. In corn production, herbicides are the most widely used, followed by fungicides and insecticides (Figure 4-5), exemplary of the significance of weed control in corn production.

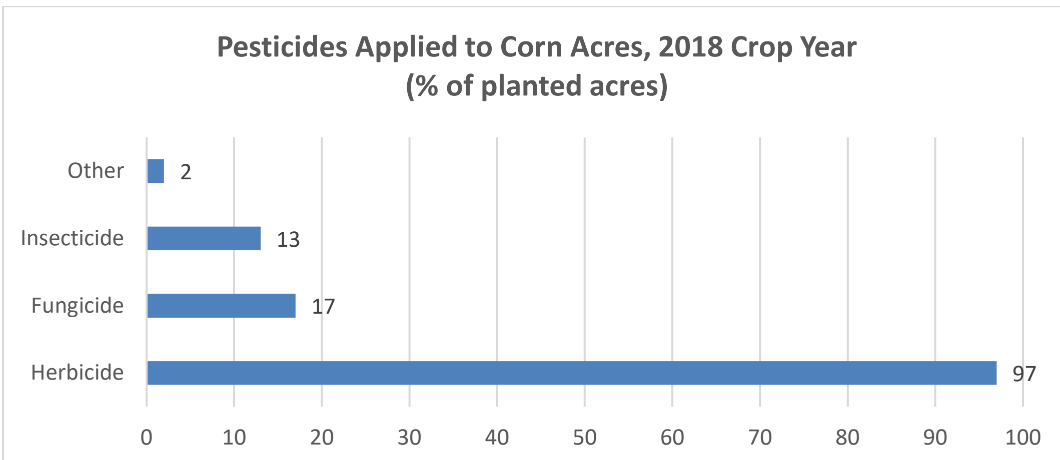

Figure 4-5. Pesticides Applied to Corn, 2018
Source: (USDA-NASS 2019c)

4.3.1.3 Potential Effects on U.S. Corn Production

Acreage and Area of Corn Production

Approval of the petition could result in a minor increase in U.S. corn acreage, relative to market demand and the level of adoption of PY203 corn. Agrivida states that the area required to produce sufficient PY203 corn to meet the demands of the poultry and swine production markets is about 10,000 acres, around 0.01% of current U.S. corn acreage. Acreage for corn production normally fluctuates on the order of millions of acres/year (USDA-NASS 2020a). Thus, any increased acreage used for PY203 corn production would be comparatively small. Grain produced from PY203 corn would be ground into a meal that would be added to the feed of poultry and swine (non-ruminants) at a ratio of one to four pounds of PY203 meal to one ton of feed.

Agronomic Practices and Inputs

The agronomic practices and inputs used for PY203 corn production would be similar to/same as that for other field corn varieties (Agrivida 2019). PY203 has no herbicide or insect resistant properties, thus, no effect on the herbicides or insecticides used with this crop. Corn growers implement production practices and select pesticide inputs based on weed/HR weed populations, insect/resistance-insect populations, and disease pressures present; the efficacy of pesticides; costs of pesticide inputs; worker safety considerations; and ease and flexibility in management of pests and weeds. APHIS did not identify any significant changes to agronomic practices or inputs that would have effects on plant diseases, insect pests, or their management.

4.3.2 Physical Environment

4.3.2.1 Soil Quality

Overview

Relative to crop production, concerns regarding soils are the potential for agronomic practices and inputs to affect soil fertility; erosional capacity; off-site transport of topsoil (sediments), pesticides, and fertilizers; and disturbance of soil biodiversity. While soil erosion occurs through natural processes, tillage, cover crops, crop rotation, and pesticide and fertilizer inputs can influence the biological, physical, and chemical properties of soil, and have a substantial impact on soil fertility and erosional capacity (Baumhardt et al. 2015). Soil quality loss occurs through declines in soil organic matter (SOM), minerals (magnesium, calcium), nutrients (nitrogen, phosphorus, potassium), soil biota, and physical alteration of soil structure (compaction).

Soil Erosion on U.S. Croplands

Due to the rate of soil formation, which is on the order of millimeters per year, soil is considered a nonrenewable resource that requires conservation for sustainable crop production. Soil erosion not only increases fertilizer requirements and production costs, it leads to impaired air and water quality (USDA-NRCS 2010; Baumhardt et al. 2015). Excessively eroding cropland soils are concentrated in the Midwest, Southern High Plains of Texas, and Northern Plain States, to include the Corn Belt (Figure 4-6).

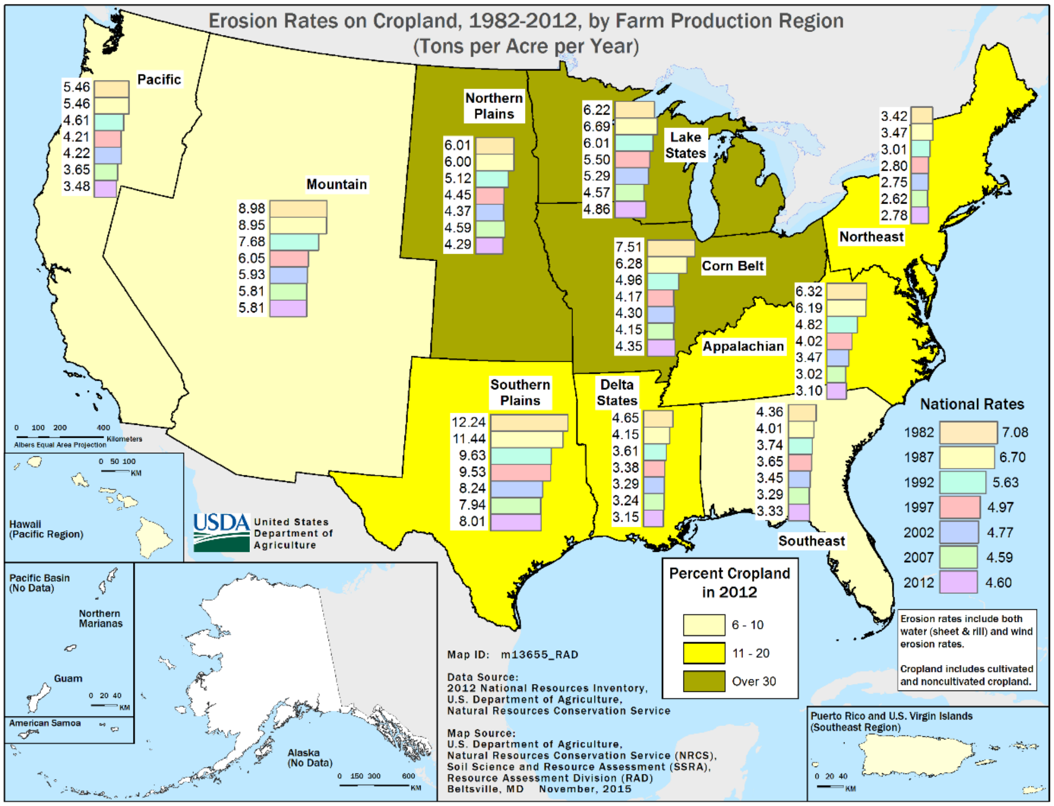

Figure 4-6. Locations and Status of U.S. Croplands Subject to Water and Wind Erosion
*Cropland in this figure includes both cultivated and uncultivated cropland.
Source: (USDA-NRCS 2018b)

Since 1985, conservation programs have specifically targeted highly erodible lands in the United States; as conservation tillage and cover cropping practices increased, soil erosion has declined (USDA-NRCS 2010, 2018a). In 1982, total annual water erosion (sheet and rill) on cultivated cropland was 3.82 tons per acre per year, versus 2.71 in 2015 (USDA-NRCS 2010, 2018a). For wind erosion, erosion rates reduced from 3.21 to 1.91 tons per acre over the same time period (USDA-NRCS 2010, 2018a). Any decrease in erosion of cropland soils carries with it a corresponding decrease in run-off and introduction of non-point source pollution (NPS) pollutants such as sediments, fertilizer, and pesticides into surface waters.

A 2017 survey conducted by the Sustainable Agriculture Research and Education (SARE) program and the Conservation Technology Information Center (SARE/CTIC 2017) found that 41% of surveyed farmers who were cover-crop users applied continuous no-till practices, 14% rotational no-till, 27% reduced tillage, 4% vertical tillage (a type of conservation tillage), with only 14% using conventional tillage. A 2017 USDA-NASS survey showed that overall, surveyed farmers applied reduced tillage on 97.7 million acres, conventional tillage on 80 million acres, and no-till on 104.5 million acres (Table 4-3). In addition, farmers are adopting the use of cover crops primarily to conserve soils and soil quality (SARE/CTIC 2017). An increase in conservation tillage has been facilitated by the availability (since the 1980s) of post-emergent herbicides (Fernandez-Cornejo et al. 2012), which can be applied over crops throughout the growing season—not just before planting, as had previously been the case. The increasing use of conservation tillage is also attributed to an increased use of HR crops, which provide for effective chemical means of weed control, and can reduce reliance on tillage for control of weeds (Fernandez-Cornejo et al. 2014). However, the availability of HR crops is not the only driving factor in adoption of conservation tillage practices, as many growers adopted conservation tillage well before HR varieties were introduced to the market (Givens et al. 2009).

Table 4-3. Tillage Practice on U.S. Cropland, 2012 – 2017				
	Cropland with reduced tillage, excluding no-till	**Cropland with no-till practices**	**Cropland with intensive tillage practices**	**Cropland planted to a cover crop (excluding CRP)**
2017	97,753,854	104,452,339	80,005,292	15,390,674
2012	76,639,804	96,476,496	105,707,971	10,280,793
Total Harvested Cropland (acres)		**320,041,858**		

Source: (USDA-NASS 2019b)

All growers producing crops on highly erodible land are required to maintain and implement a soil conservation plan that substantially reduces soil loss, and is approved by the USDA National Resources Conservation Service (NRCS). These plans are prepared by the grower pursuant to the Food Security Act of 1985 (P.L. 99-198, Farm Bill), which included a number of provisions designed to conserve soil and water resources, and minimize erosion. The 2014 and 2018 Farm Bills have continued the requirement that producers adhere to conservation compliance guidelines to be eligible for conservation programs administered by USDA-FSA and USDA-NRCS. State agencies likewise provide assistance in development and implementation of soil conservation plans.

4.3.2.1.1 Potential Effects on Soils

Agronomic Practices and Inputs

The agronomic practices and inputs used for PY203 corn production that can impact soil quality would be the same as/similar to those used for other corn cultivars. PY203 corn differs only in the trait genes and gene products; modified levels of phytase, phytate, and expression of PMI, which are unlikely to affect soil quality for the reasons discussed following. The introduced traits in PY203 corn would have no effect on weed management or tillage practices.

Phytase and Phytate

Soil microorganisms are an integral component of the soil phosphorus cycle (Hyland et al. 2005; Singh and Satyanarayana 2011). Various species of bacteria, fungi, and micromycetes produce phosphatase enzymes, to include phytase, that break down organic phosphorus into more bioavailable, inorganic forms of phosphorus (Singh and Satyanarayana 2011). Thus, phytases, and other phosphatases, are ubiquitous in microbial rich soils. Phytase from PY203 corn, which occurs primarily in the seed with little expression in leaves, stems, and roots, can potentially enter soils via degrading plant material. The average amount of phytase protein (Phy02) in the kernel of PY203 corn ranges from 4,548 to 9,079 µg/g DW. In leaves, Phy02 ranges from 0.003 to 0.039 µg/g DW, in stems from 0.0 - 0.220 µg/g DW, and in roots from 0 to 1.028 µg/g DW (Agrivida 2019). Because there is very little production of phytase in the stems, leaves, and roots, deposition of phytase into soils via degrading plant material post-harvest would be of negligible quantities.

Total phosphorus in PY203 corn grain is comparable to the conventionally bred corn variety used in Agrivida field trials; 3,130 ± 260 ppm fresh weight (FW) in PY203 corn vs. 2,830 ± 220 ppm FW for the control corn, and within the lower and upper bounds of total phosphorus measured in other corn varieties (ILSI-CERA 2020). For corn grain, phosphorus levels have been found to range from 1,260.4 ppm FW to 4,090.5 ppm FW, with a mean of 2,671.2 ppm FW. Phytate content in PY203 corn grain is about half that of conventional varieties (e.g., 3,800 ± 800 ppm versus 7,600 ± 900 ppm, or 0.38 ± 0.08 versus 0.76 ± 0.09 %FW), although comparable to other cereals—the phytate content of cereals varies from around 1,000 ppm to 22,000 ppm (0.1% and 2.2 %FW) (Sanz-Penella and Haros 2014).

Apart from increased phytase expression, and decreased levels of phytate, PY203 is compositionally and agronomically similar to other field corn varieties (Agrivida 2019). There is no indication the rate of phosphorus uptake in PY203 corn is significantly different than that of other dent corn varieties. A substantive amount of phosphatase/phytase activity naturally occurs in soils (Singh and Satyanarayana 2011). Based on these factors, the uptake of phosphorus, and deposition of phosphorus, phytate, and phytase during PY203 corn cultivation would not be expected to have any significant effect on soil phosphorus cycling, plant-growth-promoting rhizobacteria, soil fertility, or soil nutrient availability (Singh and Satyanarayana 2011; Singh et al. 2014; Gerke 2015).

PMI

Phosphomannose isomerase (PMI), used as a selectable marker in PY203 corn development, is an enzyme involved in carbohydrate metabolism, and naturally expressed in various taxa including enteric bacteria, fungi, insects, some species of plants and nematodes, and mammals—including monkeys, mice, and humans (de Lonlay and Seta 2009; Hu et al. 2016). Examples of PMIs that have been identified in soil associated bacteria and yeast include; *Escherichia coli* (vertebrate gut bacterium (Tenaillon et al. 2010)), *Bacillus subtilis* (soils, and the gastrointestinal tract of ruminants and humans (Earl et al. 2008)), *Corynebacterium glutamicum* (a gram-positive soil bacterium (Nishimura et al. 2007)), *Saccharomyces cerevisiae* (brewer's yeast (Duina et al. 2014)), and *Aspergillus fumigatus* (soil bacterium (Latgé 1999)). Considering the ubiquity of naturally occurring PMIs in soils, the PMI in PY203 corn, derived from *Escherichia coli,* is unlikely to present any risk to soil quality.

4.3.2.2 Water Resources

Agronomic inputs, and in many areas tillage and irrigation, are necessary for efficient crop production. These practices and inputs can, however, potentially lead to the impairment of surface and coastal waters through runoff of pesticides, fertilizers (nutrients), and topsoils (Bricker et al. 2008; CENR 2010). Groundwater can potentially be impacted by agronomic inputs via leaching, as well as through irrigation withdraw. In many areas of the Midwest corn yields can either be increased by irrigation, or is necessary for production. Irrigated corn accounts for 58% of total annual corn production in the Western Corn Belt (Grassini et al. 2011).

While pollutants come from various sources, the National Water Quality Assessment finds that agricultural nonpoint source (NPS) pollution is a leading cause of impairment of surveyed rivers and streams, the third largest source for lakes/ponds, the second largest source of impairments to wetlands, and a major contributor to contamination of surveyed estuaries, coastal areas, and ground water (US-EPA 2020c). The most common NPS contaminants in agricultural runoff are sediment, nutrients such as nitrogen and phosphorus, and pesticides (Table 4-4), all of which can adversely affect aquatic ecosystems.

Table 4-4. Causes of Impairment in Assessed Waters, 2019								
	Rivers, Streams		**Lakes, Reservoirs, Ponds**		**Bays, Estuaries**		**Wetlands**	
	Miles	*Rank*	*Acres*	*Rank*	*Miles*	*Rank*	*Acres*	*Rank*
Nutrients	118831	3rd	3943395	2nd	18279	2nd	67849	6th
Sediment	138874	2nd	502200	12th	400	18th	1237	15th
Pesticides	18069	16th	412672	13th	7543	8th	202	21st

Shown are national water quality data reported by the States to EPA under Section 305(b) and 303(d) of the Clean Water Act. The data shown is the most current available, which varies widely among states, spanning the years from 2004 to 2016. The EPA lists around 34 different factors that are the cause impairment of U.S. waters. For rivers and streams, the EPA lists sediments as the second most frequent cause of impairment, nutrients third, and pesticides sixteenth. For lakes, reservoirs, and ponds, nutrients are second, sediments twelfth, and pesticides thirteenth. For bays and estuaries, nutrients are second, sediments eighteenth, and pesticides 8th. For wetlands, nutrients are sixth, sediments fifteenth, and pesticides twenty-first. Source: (US-EPA 2020c)

Excess sediment in runoff can adversely affect aquatic ecosystems by covering fish breeding substrates, increased turbidity, and impairing growth of aquatic plants. Nutrient runoff (e.g., nitrogen and phosphorus) from agricultural fields can contribute to eutrophication of surface waters. Nearly two-thirds of the U.S. estuaries have moderate to high levels of eutrophication. Eutrophic conditions cause impairments to human uses and living resources as a result of harmful algal blooms and hypoxic/anoxic conditions,[9] which lead to algal and invertebrate imbalances, fish kills, fish consumption warnings, declines in tourism, and impacts on fisheries (Bricker et al. 2008; CENR 2010). Based on a USGS study (Munn et al. 2018), some the most impaired streams, based on algae or invertebrate conditions, are in those areas with the greatest agricultural land use—primarily in the central United States, to include the Corn Belt (Figure 4-7). Watersheds with a high potential to discharge nutrients from agricultural areas to estuaries are located primarily in the Heartland, Mississippi Portal, and Southern Seaboard regions (Wiebe and Gollehon 2006; CENR 2010; US-EPA 2020b).

[9] Hypoxia means low dissolved oxygen concentrations. Anoxia means a total depletion of dissolved oxygen. Both conditions are harmful to aquatic biota.

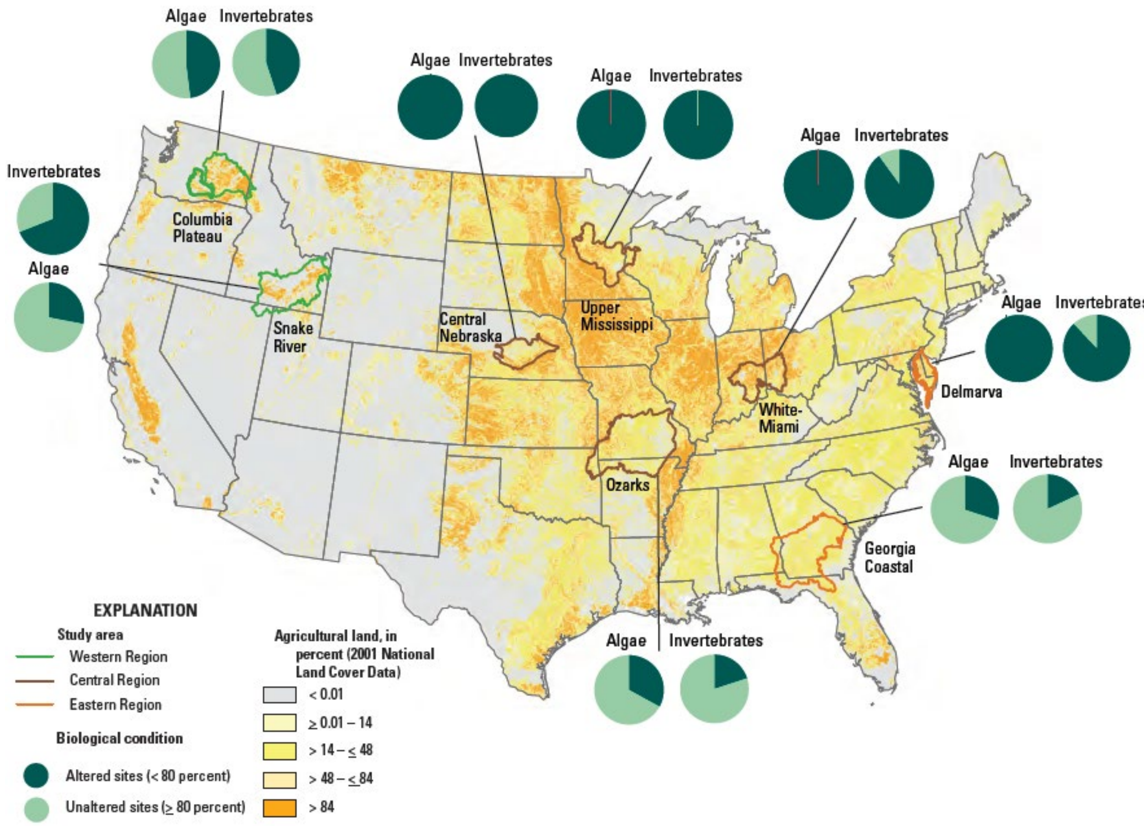

Figure 4-7. Areas of Impaired Rivers and Streams in the United States
Based on USGS surveys conducted from 2003 to 2011, biological conditions in streams decreases as agricultural intensity increases in a watershed. Generally, biological condition was highest in the Western Region where the agricultural intensity is the lowest; conversely, biological conditions were lowest in the Central Region where agricultural intensity is highest. Assessing biological condition involves comparing the observed number of taxa at a site to the number of taxa expected based on a set of regional reference sites. A stream with a score greater than 80 percent implies an unaltered stream, whereas a stream with a score less than 80 percent implies an altered biological condition. Source: (Munn et al. 2018)

Human uses impacted by impairment of surface waters include commercial and recreational fishing, shellfish harvesting, fish consumption, swimming, aesthetics, and tourism (CENR 2010). The overall top four causes of these use impairments were listed as agriculture (crops and animal operations), wastewater treatment plants, urban runoff, and atmospheric deposition (Bricker et al. 2008; Boesch 2019). Animal operations and crop agriculture were noted mostly for systems in the mid- and South Atlantic regions while exurban development (outside boundaries of urban areas) was reported in the South Atlantic region. Excess nutrients have a major economic impact—causing an estimated $2.2 billion per year in damages related to recreational water usage, waterfront real estate, drinking water treatment, and recovery of threatened and endangered species (Dodds et al. 2009). In all regions except for the North Atlantic, controlling non-point sources remain a primary focus (CENR 2010).

Over the last 50 years the Midwest has been re-engineered with tile drainage systems that allow farmers to control subsurface water levels, which can increase yields. Tile drainage systems can however negatively affect water quality by facilitating run-off of water and its solutes—such as nitrogen, phosphorus, pesticides, and sediment—into streams and rivers without allowing natural attenuation of run-off to occur (CENR 2010; Ribaudo 2011). USDA-ARMS data indicate that nearly 26% of treated cropland is tiled, most of this in corn production, and that about 71% of tiled acres do not meet nitrogen management criteria (Ribaudo 2011).

The U.S. corn belt lies within the Mississippi River Basin, which spans 1,245 million square miles across 31 states. Nitrogen and phosphorus run-off in the Mississippi River basin is particularly problematic for Gulf of Mexico ecosystems and fisheries (Wiebe and Gollehon 2006; US-EPA 2019c, 2020c). Much of the tile-drained cropland is located in the Mississippi River Basin; the most heavily tile-drained areas are also the largest contributing source of nitrate to the Gulf of Mexico, leading to seasonal hypoxia (David et al. 2010). Agricultural sources contribute around 70% of the nitrogen and phosphorus delivered to the Gulf of Mexico, versus a 9% to 12% contribution from urban sources (Alexander et al. 2008). Corn, specifically, accounts for about 45% of U.S. crop acreage receiving manure, and 65% of the 8.7 million tons of nitrogen fertilizer applied by farmers each year (Ribaudo et al. 2011). Nitrogen run-off from cornfields in the Mississippi River Basin is the single largest source of nutrient pollution to the Gulf of Mexico's "dead zone" (Figure 4-8, Figure 4-9).

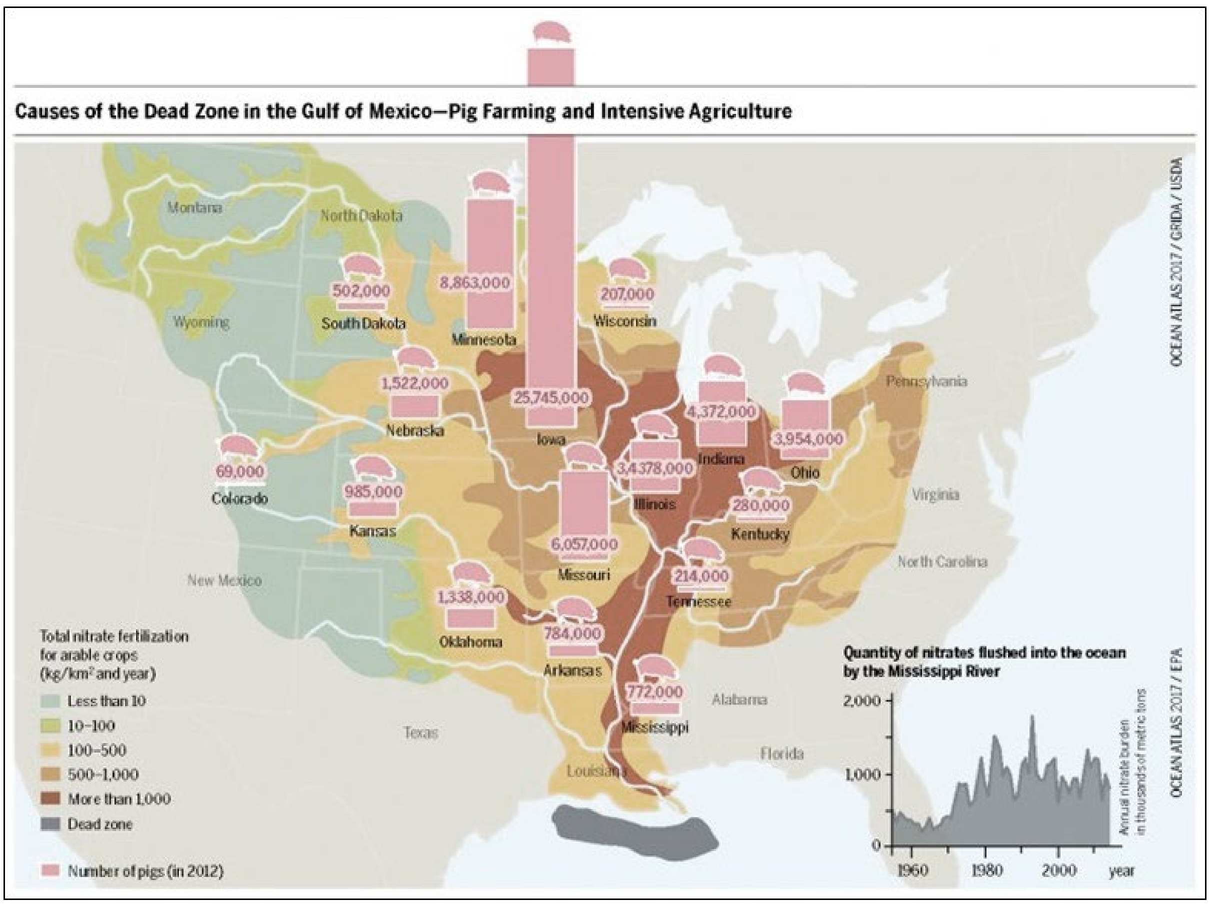

Figure 4-8. Agricultural Runoff: Mississippi River Watershed

Artificial fertilizer and hog manure are used to fertilize commercial corn crops. The Midwest is the heart of U.S. pork production. Waste products and fertilizer/manure enter the Mississippi-Missouri watershed surface waters through runoff, which empties into the northern Gulf of Mexico.
Source: (Bähr 2017; NOAA 2019)

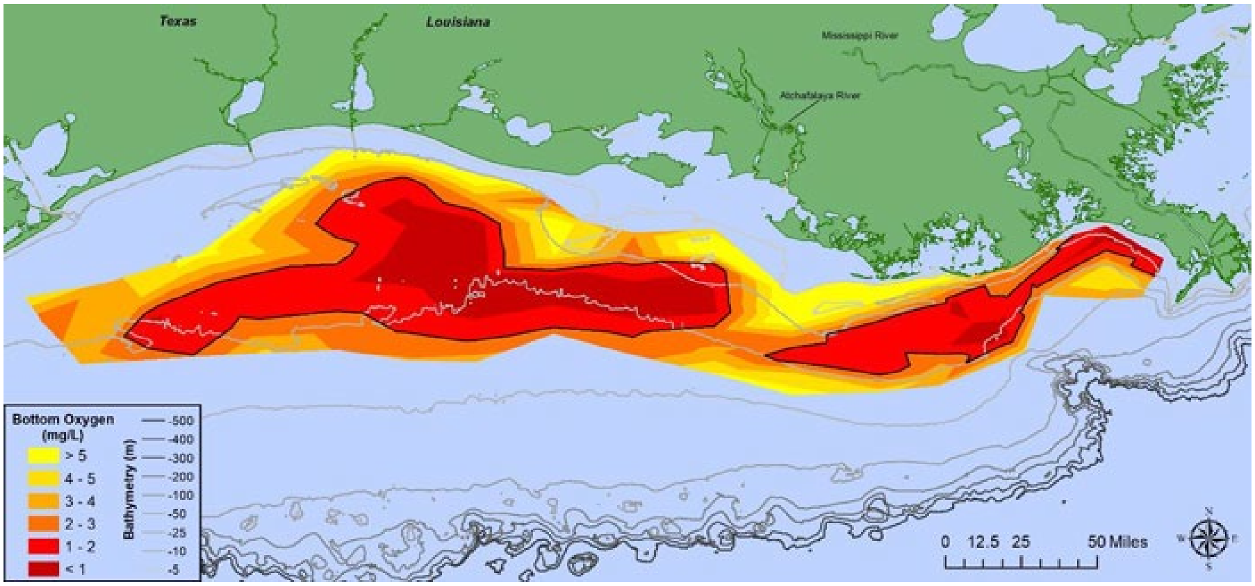

Figure 4-9. Gulf of Mexico Dead Zone, 2019
Excess nitrates and phosphates in agricultural and other runoff cause the formation of a hypoxic/anoxic "dead" zone in the Northern Gulf of Mexico that adversely affects coastal resources and habitats. This dead zone occurs on annual basis. The 2019 dead zone was 18,006 km^2 (6,952 $mi.^2$), an area about the size of New Jersey. It was the 8th largest measured since dead zone mapping began in 1985.
Source: (Conners 2019)

Phytate and Phytase in Relation to Agriculture and Water Quality

As discussed in Section 1.2–Purpose of PY203 Corn, phytase is commonly added to animal feed to help breakdown phytate during digestion, and assimilation of dietary phosphorus. The presence of phytate in animal feed, and fortification of animal feed with inorganic phosphorus, can however present environmental issues. Phytate in feed that is not digested passes through the gastrointestinal tract of livestock resulting in manure with high levels of organic phosphorus (Turner et al. 2002). About 70% of total phosphorus in feed is excreted in feces due to inefficient breakdown of phytate by monogastric animals (Turner et al. 2002). The addition of inorganic phosphorus to animal feeds can further elevate phosphorus concentrations in manure and liquid wastes (i.e., manure ponds)[10] (Nahm 2002; Turner et al. 2002). Elevated levels of phytate and inorganic phosphorus in manure and liquid wastes can, through runoff from agricultural sites, contribute to the loading of phosphorus in surface waters (eutrophication), and associated water quality and aquatic wildlife/fisheries issues discussed above (e.g., anoxic/hypoxic conditions, death of fish and other aquatic biota) (Ribaudo and Johansson 2006; Alexander et al. 2008; Fanelli et al. 2019; US-EPA 2019c). Run-off of phosphorus from animal rearing facilities is in fact one of

[10] A manure pond or manure lagoon is a man-made outdoor basin filled with animal waste that undergoes anaerobic respiration as part of a system designed to manage and treat refuse created by concentrated animal feeding operations (CAFOs).

the leading causes of water pollution in the United States (US-EPA 2019c). Consequently, the presence of phytate in feed, and fortification of feed with inorganic phosphorus, are undesirable from an environmental and water quality standpoint.

4.3.2.2.1 Water Quality Regulation

Point and Non-Point Source Discharges

Impacts on water resources derive from point source and NPS pollutants. Under Section 404 of the Clean Water Act (CWA) it is unlawful to discharge any pollutant from a point source into navigable waters, unless a permit authorized under the CWA was obtained. The EPA's National Pollutant Discharge Elimination System (NPDES) permit program controls point source discharges. NPS pollution, which is the primary type of discharge from cropping systems, is not regulated under the CWA; rather, it is left largely to voluntary controls implemented by states and local authorities. Thus, many crop production activities do not require a Section 404 permit. To be exempt, the farming activity must be part of an ongoing farming operation and cannot be associated with bringing a wetland into agricultural production or converting an agricultural wetland to a non-wetland area.

Animal Feeding Operations (AFOs) are sites where animals are reared and kept in confined facilities. AFOs that meet the regulatory definition of a concentrated animal feeding operation (CAFO) are regulated under the CWA as point source dischargers [Section 502(14)]. This is because manure and wastewater from CAFOs have the potential to contribute excess pollutants such as nitrogen and phosphorus, organic matter, sediments, pathogens, hormones, and antibiotics to surface and groundwater (US-EPA 2020k). The EPA's NPDES CAFO regulations prohibit discharges from a CAFO to U.S. waters without an NPDES permit (US-EPA 2020n). NPDES CAFO regulations were issued, specifically, because animal manure is a primary source of nitrogen and phosphorus found in surface and groundwater (Table 4-5). The NPDES regulation describes which operations qualify as CAFOs and sets forth the basic requirements that are included in CAFO discharge permits. NPDES CAFO permits generally apply to severe precipitation events that can cause an overflow of manure, litter, or process wastewater, and/or pollutants, subject to various provisions stipulated in the permit (US-EPA 2020l). The EPA and state permitting authorities monitor compliance with NPDES permits. Large livestock operations are also required to have nutrient management plans (NMPs), which require balancing nutrient applications with the nutrient utilization of crops (US-EPA 2020i).

Table 4-5. Estimated Nitrogen (N) and Phosphorus (P) Produced from Animal Manure in 2007

State	Estimated Animal Manure (1000 kg of N)	Estimated Animal Manure (1000 kg of P)	Estimated Animal Manure per Farm Land Area (kg of N/km^2)	Estimated Animal Manure per Farm Land Area (kg of P/km^2)
Alabama	133,956	41,438	3,678	1,138
Alaska	796	225	221	62
Arizona	50,998	12,309	483	117
Arkansas	179,024	56,005	3,183	996
California	327,287	75,388	3,184	733
Colorado	136,460	38,852	1,074	306
Connecticut	3,493	749	2,105	451

Table 4-5. Estimated Nitrogen (N) and Phosphorus (P) Produced from Animal Manure in 2007

Delaware	20,080	5,944	9,729	2,880
Florida	101,939	30,901	2,709	821
Georgia	158,802	48,575	3,810	1,165
Hawaii	7,957	2,485	1,756	548
Idaho	115,094	27,493	2,473	591
Illinois	105,906	36,690	976	338
Indiana	103,411	35,432	1,727	592
Iowa	398,551	144,981	3,198	1,163
Kansas	293,838	84,863	1,568	453
Kentucky	144,122	43,414	2,544	766
Louisiana	59,630	18,259	1,819	557
Maine	6,109	1,391	1,118	255
Maryland	37,297	10,548	4,474	1,265
Massachusetts	3,672	818	1,745	389
Michigan	75,204	19,574	1,858	484
Minnesota	211,302	68,684	1,941	631
Mississippi	112,038	34,567	2,517	777
Missouri	261,450	84,045	2,228	716
Montana	131,048	41,155	532	167
Nebraska	314,619	96,219	1,705	521
Nevada	22,792	6,765	955	283
New Hampshire	2,676	581	1,407	305
New Jersey	4,394	1,114	1,487	377
New Mexico	80,695	20,699	462	118
New York	85,755	17,913	2,943	615
North Carolina	215,818	80,115	6,201	2,302
North Dakota	88,069	27,324	550	171
Ohio	108,025	32,516	1,907	574
Oklahoma	283,852	87,463	1,998	616
Oregon	74,777	21,237	1,127	320
Pennsylvania	125,555	32,946	3,978	1,044
Rhode Island	495	120	1,747	424
South Carolina	47,205	15,054	2,381	759
South Dakota	189,425	59,013	1,071	334
Tennessee	124,787	38,148	2,803	857
Texas	699,431	206,361	1,325	391
Utah	56,209	17,083	1,251	380
Vermont	15,934	3,047	3,201	612
Virginia	102,834	30,895	3,137	943
Washington	63,537	16,069	1,054	266
West Virginia	27,580	8,304	1,842	555
Wisconsin	191,761	42,098	3,117	684
Wyoming	69,123	21,070	566	172

Table 4-5. Estimated Nitrogen (N) and Phosphorus (P) Produced from Animal Manure in 2007				
Total	**6,174,812**	**1,846,939**	**110,862**	**31,984**

Source: (US-EPA 2019c)

Due to the potential impacts of agriculture on water resources, there are, in addition to the CWA and NPDES program, various national and regional efforts to reduce NPS contaminants in agricultural runoff, and runoff itself, such as the EPA's Mississippi River/Gulf of Mexico Hypoxia Task Force (US-EPA 2020d) and USDA-NRCS National Water Quality Initiative (NWQI) (USDA-NRCS 2020e). Through the NWQI, and other conservation programs, the NRCS and partners (e.g., local and state agencies, nongovernmental organizations) work with producers and landowners to implement voluntary conservation practices that improve water quality, such as comprehensive nutrient management planning (USDA-NRCS 2020d). The NWQI program is in its 9th year and extended through 2023. It provides funding and technical assistance for conservation practices, and in 2019 invested $30 million in targeted assistance to help farmers and ranchers improve water quality in high priority watersheds (USDA-NRCS 2020e). State water quality agencies and other partners contribute additional resources for watershed planning, program implementation, and outreach, and for monitoring efforts to track water quality improvements over time.

Several other legislative drivers also influence how Federal agencies address water quality including the Food, Conservation, and Energy Act of 2008 ("Farm Bill"); the Energy Independence and Security Act of 2007; the Coastal Zone Management Act; and The Harmful Algal Bloom and Hypoxia Research and Control Act. Responsibility for resolving hypoxia spans several Federal agencies (U.S. Department of Agriculture, U.S. Geological Survey, U.S. Environmental Protection Agency, and National Oceanic and Atmospheric Administration), which oversee research and management/control programs. States play a critical role in monitoring and managing eutrophication (CENR 2010).

Phosphorus Regulation and Phytase

Many states now regulate phosphorus fertilizer use and discharges, to include manure use and management (Key et al. 2011). Manure nutrient testing, including phosphorous content, is a practice required as part of many State-mandated manure management plans, and the percent of farms testing manure for phosphorous increased from 17% in 1998 to 48% in 2009 (Key et al. 2011). During this same time period, the percent of farms practicing comprehensive nutrient management plans increased, and the percent of farms adding microbial phytase to feed increased at least five-fold (Key et al. 2011). For example, Maryland requires the use of phytase in certain livestock feed to reduce manure phosphorus levels (MDA 2019). All contract feed produced in Maryland for chickens must include phytase or some other enzyme or additive that reduces phosphorus to the maximum extent that is commercially and biologically feasible. Maryland efforts are in part for protection of the Chesapeake Bay watershed. Iowa passed legislation requiring any animal operation with more than 500 animals to have a phosphorus-based manure management plan (IPPA 2019). Nebraska requires manure plans for all sites with 300 or more animal units, which includes mandatory phosphorus reporting (USDA-NRCS 2020c). Nebraska authorities do a case-by-case evaluation on whether manure can be applied (NDE 2019).

Pesticides

The EPA determines use requirements for pesticides that are intended to be protective of water quality, including drinking water, and to protect aquatic life (US-EPA 2019a, 2020e). The EPA provides label use restrictions and guidance for product handling that is intended to prevent impacts to surface and groundwater.

4.3.2.2.2 Potential Effects on Water Resources

The potential impacts of crop production on water quality primarily derive from the collective/aggregate inputs from crop fields into regional surface waters. Total U.S. corn acreage comprises around 90 million acres annually (USDA-NASS 2019b). Certain pesticides—depending on mobility and persistence characteristics—can leach into groundwater at sites where pesticides are mixed or applied. Because the agronomic practices and inputs utilized for PY203 corn production would not substantially differ from other corn varieties, the sources of potential impacts on water resources, namely NPS pollutants in agricultural run-off, would not substantially differ (e.g., sediments, fertilizers, insecticides, herbicides, fungicides). Agrivida states that given the relatively low inclusion rate of the Phy02 phytase in animal feed, the area required to produce sufficient product to meet the demands of the poultry and swine production markets is about 10,000 acres (Agrivida 2019). Relative to total U.S. corn acreage, there could be a minor contribution to increases of NPS pollutants in runoff from U.S. corn fields.

However, PY203 corn, with reduced levels of phytate and increased expression of phytase could potentially contribute to reductions in the runoff of phosphorus from AFOs/CAFOs utilizing PY203 corn for animal feed, and agricultural operations using manure from such AFOs/CAFOs (manure lower in phosphorus). The phytate content of PY203 corn grain is about half that of non-modified corn comparators, around 0.38 ± 0.08 vs. 0.76% ± 0.09 fresh weight (FW), respectively (Table 4-6). The intrinsic phytase activity of corn is very low (0 to 0.190 FTU/g) (Eeckhout and De Paepe 1994; Rodehutscord et al. 2016; Ingelmann et al. 2018), while ground grain from PY203 corn has around 3500 units of phytase activity units per gram of grain[11] (US-FDA 2017). Through both decreased levels of phytate and increased phytase activity PY203 corn could increase bioavailable forms of inorganic phosphorus in feed, and help reduce phytate/phosphorus levels in manure. Factors affecting potential manure phosphorus reduction are discussed in the below subsections.

Table 4-6. PY203 Corn Phytate and Phosphorus Composition

Analyte	Unit	PY203	Control	ILSI Data
Grain				
Phosphorus	ppm Fresh Weight	3130 ± 260	2830 ± 220	1260.4 – 4090.5
Phytate	% Fresh Weight	0.38 ± 0.08	0.76 ± 0.09	0.1 – 1.44
Forage				
Phosphorus	% Dry Weight	0.25 ± 0.03	0.24 ± 0.02	0.07 – 0.44

Source: (Agrivida 2019)

Areas and Volume of Manure Production and Use in the United States

As of 2017, U.S. livestock inventory included approximately 94 million cattle, 73.2 million swine, 10.4 billion poultry, generating an estimated 1.1 billion tons of manure, annually (Table 4-7).

Table 4-7. Livestock and Manure Production and Use in the United States, 2017

[11] Phytase activity is expressed as phytase units or FTUs. One FTU is the activity of phytase required to liberate 1 µmol of inorganic phosphorus per minute at pH 5.5 from an excess of 15 M sodium phytate at 37°C.

	Farms	Number of Animals	Total Manure	Nitrogen	Phosphorus
			(lbs/day/1000-lb animal)		
All Cattle		**94,399,000**			
Beef cows	727,906	31,723,000	59.1	0.31	0.11
Milk cows	64,098	9,399,600	80	0.45	0.07
Cattle on Feed	28,209	14,006,000	59.1	0.31	0.11
All Poultry		**10,444,500,000**			
Chickens (Layers)	198,272	582,000,000	60.5	0.83	0.31
Chickens (Broilers)	42,226	9,620,000,000	80	1.1	0.34
Turkey	19,956	242,500,000	43.6	0.74	0.28
All Hogs and Pigs	63,246	**73,229,000**	63.1	0.42	0.16

Cattle include beef cattle, dairy cattle, and other cattle and calves (such as breeding stock). Swine include market hogs, which are sent to slaughter after reaching market weight, and breeder hogs, which are used for breeding purposes. Poultry includes chickens as broilers (raised for meat) and as layers (produce eggs), and turkeys. Manure from non-dairy cattle, swine, and poultry is relatively high in phosphorus (manure estimates are as excreted, wet-weight). Source: (Zhang and Schroder 2014; USDA-NASS 2017)

In 2017, approximately 297,300 farms utilized manure on a total of 23.8 million acres (USDA-NASS 2019b). Corn, which is planted on about one-quarter of U.S. cropland, accounts for around 45% of U.S. crop acreage receiving nitrogen, and 65% of the nitrogen applied by farmers based on estimates for 2006 (Ribaudo 2011). Most of the cropland receiving manure was used to grow corn (72%) (Ribaudo 2011). Manure use in the United States is illustrated in (Figure 4-10).

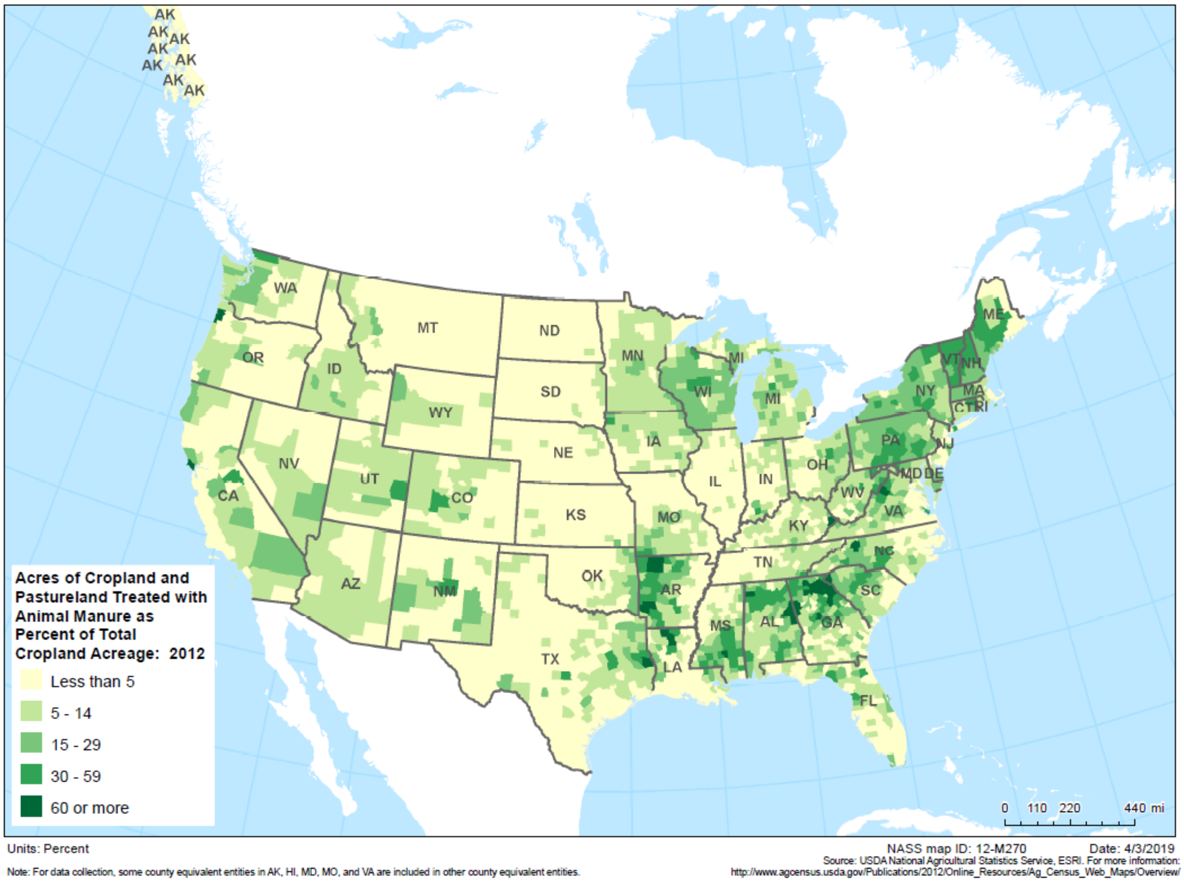

Figure 4-10. Cropland and Pastureland Treated with Manure in the United States, 2012
Source: (USDA-NASS 2014)

Manure Phosphorus as a Point-Source and Non-Point Source Pollutant

Most manure applied to corn comes from dairy and hog operations (MacDonald et al. 2009). Use of manure is influenced by the size, crop/livestock mix on a farm, and the location and proximity of crop and livestock farms. There is a direct association between farm size and the production, concentration, and use of manure. With the exception of the beef cow-calf sector, livestock production—namely swine, dairy cattle, and poultry—has been shifting to larger operations, as economies of scale provide larger operations with lower costs and better net returns (MacDonald and McBride 2009). Large-scale operations inherently consolidate manure on limited acreage and, due to the scale of livestock production, manure production typically exceeds the nutrient needs of on-farm and nearby crops (MacDonald et al. 2009; MacDonald et al. 2012). Centers of large-scale livestock production occur in areas such as the Southeast (poultry and hogs), the High Plains (fed cattle, dairy, and hogs), and the West (dairy).

The consolidation of livestock/cropping operations has meant that an increasing volume of manure is often produced on farms with insufficient cropland for spreading the manure, which presents challenges to effective manure/nutrient management and protecting water quality in these areas (Key et al. 2011; USDA-NRCS 2020d). Because industrialized livestock production concentrates manure on limited land, livestock producers with crops may apply manure at intensities above the agronomic needs of crops, thereby increasing pollution risks (MacDonald and McBride 2009). Intensive swine and poultry facilities, in particular, produce phosphorus rich manure. This surplus of manure phosphorus can lead to excessive losses of phosphorus from lands amended with these manures, or facilities storing the manure (Maguire et al. 2005b; Maguire et al. 2005a). In general, a higher manure-to-cropland ratio can increase the risk that manure nutrients (e.g., nitrogen, phosphorous, and potassium) and pathogens will contaminate groundwater and surface water due to over application of manure on crops, or leakage/spillage from manure storage facilities. Contaminants in runoff or spillage can harm aquatic life and affect drinking water (Key et al. 2011).

While there are challenges with manure management in areas with intensive livestock production, there are a several ways to mitigate the risks associated with concentrated manure production (MacDonald and McBride 2009). One example is to reformulate feed to reduce the amount of nutrients excreted by livestock/poultry. Feed management practices such as feeding phosphorus closer to animal requirements, use of feed additives such as phytase, and high available phosphorus (HAP) grains have proven effective at reducing the total phosphorus in manures produced, without impairing animal performance (Maguire et al. 2005b). Use of phytase as feed additive, discussed below, is another method of reducing manure phosphorus.

Phytase Use as a Feed Additive

The use of phytase in the diet of monogastric animals is common practice, with an estimated penetration of over 70% in swine feed and over 80% in poultry feed. Phytase additives not only increase phytate degradation and phosphorus assimilation, they reduce phytate and total phosphorus concentration in manures (Menezes-Blackburn et al. 2013; Hayes 2019). The use of phytase as an additive in swine feed has been found to reduce phosphorus in swine manure by around 15% to 45% (Smith et al. 2004; Maguire et al. 2005b; Applegate and Richert 2008; Abioye et al. 2010). For broilers and turkey, reported reductions in total phosphorus are up to 31% in broiler litter and 38% in turkey litter when phytase was used (Maguire et al. 2007).

There has also been interest in phytase supplementation in ruminant feeds, although data on beef and dairy cows, phytase supplemented diets, and phosphorus excretion (as total phosphorus and phytate-phosphorus) are mixed, with some studies finding decreases in total fecal phosphorus and other studies finding increases (Humer and Zebeli 2015). Variability in the data for ruminants is due to the types/sources of phytase used as feed supplement (e.g., bacterial/fungal, phytase enzymatic activity), amount of phytase added to the diets, and particular species of ruminant livestock (Kincaid et al. 2005; Knowlton et al. 2007; Jarrett et al. 2014; Winter et al. 2015).

In one study examining the effects of phytase supplementation on phosphorus metabolism and digestibility in beef cattle, there was no relationship found between phosphorus assimilation and excretion, and phytase inclusion in the diet (Long et al. 2017). In other experiments, Hurley et al. (2002) reported that use of 250 FTU phytase and 500 FTU phytase in steer diets supplemented with 0.35% phosphorus resulted in higher fecal phosphorus than in steers that received no supplement. In experiments using diets supplemented with 200 FTU and 400 FTU phytase, without any additional dietary phosphorus supplementation, decreases in fecal phosphorus were observed (~25%) (Hurley et al. 2002).

In sheep, contradictory effects have been reported. While reduced fecal phosphorus was observed in sheep fed phytase supplemented soybean meal, the opposite effect was observed in sunflower meal, and no effect observed in rapeseed meal (Humer and Zebeli 2015). In lambs fed sorghum-based diets supplemented with increasing dosages of *Aspergillus niger* phytase Buendía et al. (2010) observed a linear increased apparent phosphorus digestibility and phosphorus retention, whereas phosphorus excretion and growth performance did not differ between treatments (Humer and Zebeli 2015).

Studies by Nyannor et al. (2007) evaluated a corn expressing a similar Escherichia coli-derived phytase to that expressed in PY203 corn (Phy02), and found that pigs fed phytase-supplemented diets excreted less than one-half as much phosphorus as pigs fed diets with no phytase supplementation, thereby reducing the potential environmental impact of manure-related phosphorus. In another study using the same phytase expressing corn variety, there was found to be a 15% reduction in phytic acid (P) concentration in the digesta of broiler chicks (Nyannor et al. 2009).

Summary

There are ongoing issues concerning the impact of synthetic fertilizer and manure derived nutrients on water quality, and various federal and state level initiatives to manage nutrients in agricultural runoff (e.g., (USGS 2016; US-EPA 2020d; USDA-NRCS 2020e)). Many states have passed legislation that regulates phosphorus fertilizer use based on soil phosphorus levels in an attempt to protect surface waters from NPS phosphorus inputs (Miller 2012).

Strategies to address excess manure phosphorus produced in areas with intensive animal production include reducing feed phytate and increasing forms of bioavailable phosphorus in animal diets. Feeding to animal phosphorus requirements, increased use of phase feedings, more accurate assessment of phosphorus availability in feeds, and use of feed additives such as phytase (as with PY203 corn) can significantly reduce the phosphorus concentration in animal wastes (Maguire et al. 2007). Diet modification and use of phytase has been found to reduce phosphorus in swine manure by around 15% to 45%, up to 25% for dairy cows, and 31% to 38% in poultry litter (Maguire et al. 2007). While there are many variables influencing the effect of phytase supplementation on manure/litter phosphorus concentration, PY203 corn could potentially serve as a cost effective means of delivering phytase to livestock feed for the improvement of phytate digestion, contribute to reducing phosphorus levels in manure, and thereby phosphorus runoff from AFOs/CAFOs, as well as phosphorus runoff from cropping systems that may utilize manure from PY203 corn fed animals (Eeckhout and De Paepe 1994; Godoy et al. 2005; Abioye et al. 2010; Dersjant-Li et al. 2015; Cowieson et al. 2017).

To the extent that GRAINZYME® phytase is adopted for use in animal feed, and that AFO/CAFO manure derived from PY203 corn based feeds are utilized for fertilization of cropland, these uses collectively could contribute, to some degree, to overall reductions in phosphorus runoff from agricultural facilities in the United States. Any contribution to reductions in total anthropogenic phosphorus inputs into surface waters could potentially result in improvements in the quality of impaired or threatened water bodies. While there are several variables that affect the efficacy of phytase in reducing manure phosphorus, as discussed above, the potential for reductions in phosphorus inputs to surface waters exists. Any realized reduction in phosphorus runoff from agricultural sites would be beneficial to improving/sustaining local and regional water quality.

As discussed previously, Agrivida states that the area required to produce sufficient PY203 corn is about 10,000 acres (0.01% of total U.S. corn acreage) (Agrivida 2019). Thus, there could potentially be a minor contribution to NPS pollutants in agricultural runoff to surface waters from PY203 cropland. This would be relative of the proximity of surface waters to PY203 crops. The potential increase in phosphorus runoff from PY203 cropland would be expected to be negligible relative to the potential reductions in total phosphorus runoff discussed above.

There are various national and regional efforts to reduce the cumulative effects of NPS contaminants in agricultural run-off, and run-off itself (US-EPA 2020d; USDA-NRCS 2020e). For example, in 2012, the USDA Natural Resources Conservation Service (NRCS) launched the National Water Quality Initiative (NWQI), in collaboration with the EPA and state water quality agencies, to reduce nonpoint sources of nutrients, sediment, and pathogens related to agriculture in high-priority watersheds in each state (USDA-NRCS 2020e).

4.3.2.3 Air Quality

National Ambient Air Quality Standards

Air pollution can adversely affect human health and the environment and maintaining air quality a primary U.S. regulatory goal. The EPA establishes National Ambient Air Quality Standards (NAAQS) pursuant to the Clean Air Act (CAA) that are intended to protect public health and the environment (US-EPA 2020q). NAAQS are established for six criteria pollutants: ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), sulfur dioxide (SO_2), lead (Pb), and particulate matter (PM). In addition to criteria pollutants, the EPA regulates 187 hazardous air pollutants, such as ammonia and hydrogen sulfide, as well as greenhouse gas emissions. To help regulate emissions the EPA has categorized primary emissions sources into: point, mobile, biogenic, and area. Point sources include major industrial facilities such as chemical plants, oil refineries, and power plants. Mobile sources include cars, trucks and buses and off-road equipment such as ships, airplanes, and agricultural and construction equipment. Area sources are defined as smaller operations such as dry cleaners and gas stations. Biogenic sources are comprised of vegetation, soils, and animals.

All areas of the United States are classified as to their consistency with the NAAQS; for example, having attained NAAQS, or not. States enforce the NAAQS through creation of state implementation plans, which are designed to achieve EPA-established NAAQS. The EPA designates a region as being in attainment for a criteria pollutant if atmospheric concentrations of that pollutant are below the NAAQS, or being in nonattainment if criteria pollutant concentrations violate the NAAQS.

Crop production practices, on a regional scale, can generate air pollutants that can contribute to challenges in maintaining NAAQS. Agricultural emission sources include smoke from agricultural burning (PM); fossil fuel consumption associated with equipment used in tillage, pesticide application, and harvest (CO_2, NO_x, SO_x); soil particulates from tillage (PM); soil nitrous oxide (N_2O) emissions from the use of fertilizers/manure; and atmospheric emissions through the volatilization of pesticides, and gases from manure (Aneja et al. 2009; US-EPA 2020o).

While the EPA establishes NAAQS, the standards do not set emission control requirements for any particular industry, including agriculture. The USDA and the EPA provide guidance for regional, state, and local regulatory agencies, and farmers, on how to best manage agricultural emissions sources (USDA-EPA 2012). These measures allow stakeholders the flexibility in choosing which measures are best suited for their specific situation. The EPA has also developed USDA-approved measures to help manage air emissions from cropping systems to help satisfy State Implementation Plan requirements. The EPA recommends that in areas where agricultural activities have been identified as a contributor to a violation of NAAQS, USDA-approved conservation systems and activities be implemented to limit emissions. The USDA Environmental Quality Incentives Program Air Quality Initiative provides financial and technical assistance to help farmers and ranchers limit air pollution (USDA-NRCS 2020b).

Pesticides

Apart from NAAQS emissions, spray drift, and volatilization of pesticides from soil and plant surfaces, can result in the introduction of constituent chemicals into the air; which can present human health risks, and risks to nearby crops. Thus, drift and volatilization of pesticides can be a source of concern to both farmers and the general public in regard to potential environmental and human health effects. Volatilization is dependent on pesticide chemistry, exposed soil structure and wetness, dew, humidity, and temperature (US-EPA 2020h). Drift is dependent on wind conditions, topography, the type of crop sprayed, and applicator practices, to include application equipment features such as nozzle size (US-EPA 2019b).

The EPA's Office of Pesticide Programs, which regulates the use of pesticides, introduced initiatives to help pesticide applicators minimize off-target pesticide drift. The EPA's voluntary Drift Reduction Technology Program was developed to encourage the manufacture, marketing, and use of spray technologies that reduce pesticide drift. The EPA is also working with pesticide manufacturers through the registration and registration review programs on improvements to pesticide label instructions to reduce drift and volatilization (US-EPA 2019b, 2020h).

4.3.2.3.1 Potential Effects on Air Quality

Because the agronomic practices and inputs for PY203 corn production are the same as/similar to other corn varieties, no changes to emission sources (i.e., tillage, fossil fuel burning equipment, the application of fertilizers and pesticides) are expected. As discussed, the area required to produce sufficient PY203 corn (GRAINZYME® phytase) to meet the demands of the poultry and swine production markets is anticipated to be about 10,000 acres. Annual acreage planted to corn normally varies on the order of millions of acres annually (USDA-NASS 2020a). Thus, there would be no substantive increase in the acreage of U.S. corn crops (0.01%). If PY203 corn were produced, air quality would be affected along current trends by emission sources such as tillage (PM), pesticide application (aerosols, spray drift), and use of farm equipment that combusts fossil fuels (CO_2, NO_2, SO_2). The EPA and USDA efforts to limit/reduce emissions, along with state and local efforts, would likewise continue (US-EPA 2020a).

The handling and storage of manure associated with livestock and poultry production facilities generates a wide range of air-borne contaminants including ammonia, carbon dioxide, hydrogen sulfide, and methane (UNL 2019). The potentially reduced phosphorus levels in manures derived from PY203 corn would have no effect on manure associated NAAQS or other emissions. Nitrogen, carbon, hydrogen, and sulfur are the primary constituents of manure derived atmospheric pollutants (USDA-NRCS 2007). The phosphorus cycle differs from the biogeochemical cycles of primary atmospheric pollutants in that it does not include a gas phase; although small amounts of phosphoric acid (H_3PO_4) may make their way into the atmosphere, contributing—in some cases—to acid rain (ELC 2019). Where the biogeochemical water, carbon, nitrogen, and sulfur cycles all include at least one phase in which the element is in a gaseous state, very little phosphorus circulates in the atmosphere because phosphorus does not easily form gases (ELC 2019).

4.3.3 Biological Resources

4.3.3.1 Soil Biota

Soil health, of which soil biota are a primary component, determines the efficacy by which crops can provide food, fiber, fuel, and industrial products, how soils regulate services protecting water and air quality, and soil erosional capacity (FAO 2017a). Soil biota consist of microorganisms (bacteria, fungi, archaea and algae), soil animals (protozoa, nematodes, mites, springtails, spiders, insects, and earthworms), and plants (e.g., algae) living all or part of their lives in or on the soil, or pedosphere (Fortuna 2012). Soil biota play a key role in the formation and turnover of soil organic matter (including mineralization), biodegradation of anthropogenic substances (e.g., pesticides), nutrient cycling, suppression of plant diseases, promotion of plant growth, soil structure formation, and most biochemical soil processes (Gupta et al. 2007; Fortuna 2012; Parikh and James 2012). Plant roots, including those of corn, release a variety of compounds into the soil creating a unique environment for microorganisms in the rhizosphere (root zone). Millions of species of soil organisms exist but only a fraction of them have been cultured and identified (Fortuna 2012).

Some microorganisms can cause plant diseases, which can result in substantial economic losses in crop production, and soils treated to control plant pathogens. Soil borne corn crop diseases include fungal corn rusts, corn leaf blights, ear smuts, ear and kernel rot fungi, and maize mosaic viruses (Strunk and Byamukama 2019).

Potential changes to the soil microbial community as a result of cultivating biotechnology-derived crops has been a primary topic of research since their introduction in the late 1990s (e.g., (Motavalli et al. 2004; Locke et al. 2008; Kremer and Means 2009)). Potential direct impacts could possibly include changes to the structural and functional community near the roots of transgenic plants due to altered root exudation or the transfer of novel proteins into soil, or a change in microbial populations due to the changes in agronomic practices used with biotechnology-derived crops (e.g., pesticides, tillage practices). The majority of these studies have focused in Bt crops due to their insecticidal activity (Kowalchuk et al. 2003; Hannula et al. 2014; Zaman et al. 2015; Xie et al. 2016; Yasin et al. 2016).

Relative to crop production, the main factors affecting soil biota populations and diversity include soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), plant type (providers of specific carbon and energy sources into the soil), and agricultural management practices (crop rotation, tillage, pesticide and fertilizer application, and irrigation) (Kowalchuk et al. 2003; Garbeva et al. 2004; Gupta et al. 2007). Climate, particularly the water and heat content of soil, is a principal determinant of soil biological activity.

Pesticides

The continued use of pesticides are considered necessary to commercial crop production if the projected global demands for food are to be met (FAO 2017b). The capacity of the soil to filter, buffer, degrade, immobilize, and detoxify pesticides is a function of soil biota, particularly soil microbiota.

Some pesticides used on corn crops can, relative to the application rates, mode of action (potential toxicity), and frequency of exposure of soil biota to a pesticide, potentially impact soil communities (Stevenson et al. 2002; Locke and Zablotowicz 2004). Changes in community structure are known to be the most common type effects observed with pesticides (FAO 2017b). A recent global assessment of the impact of plant protection products on soil functions and soil ecosystems concluded that most agricultural inputs can cause changes in the number, activity, diversity, and community structures of soil biota (FAO 2017b).

In general, the effects of pesticides can lead to both decreases and increases in the attributes of soil organisms, such as biomass, enzymatic activity, soil respiration, and species composition. The challenge lies in interpreting these changes relative to whether such changes reflect adaptive responses in soil organisms/communities, or potentially harmful effects, such as decreased species diversity, impeded soil functions, and diminished soil productivity (FAO 2017b). Changes in diversity and community structure might not always lead to changes in ecosystem function, soil processes, as the relationship between the diversity of soil organisms and soil ecosystem functioning is complex, and there exists redundancy among soil organisms and soil processes (Nielsen et al. 2011; Hannula et al. 2014). There is very limited evidence that the observed effects of pesticides on soil organisms have led to significant and long-lasting effects on soil functions (FAO 2017b). However, the inability to clearly link the observed effects of pesticides on organisms with soil functions is a major limitation of the current literature (FAO 2017b).

There is more evidence for harmful effects of pesticides on earthworms. Specifically, the negative effects of copper-based fungicides are well-established, and recent evidence indicates that neonicotinoids are particularly toxic to earthworms (FAO 2017b). Generally, earthworms are subject to pronounced, long-term effects when exposed to fungicides and insecticides (targeting Fungi and Animalia kingdoms), while herbicides (targeting the Planta kingdom) have limited or no effects (FAO 2017b).

While the application of a pesticide may lead to the local suppression of a taxonomic unit of soil organisms, the resilience of soil organisms, or ability to adapt, and functional redundancy across taxa, serve to limit the effects of pesticides on soil ecosystem processes (FAO 2017b). Fundamentally, the vast majority of soil organisms have yet to be identified, and a comprehensive assessment of the effects of pesticides on soil biota is not possible at this time (FAO 2017b).

4.3.3.1.1 Potential Effects on Soil Biota

Because PY203 corn is agronomically equivalent to other field corn varieties, the potential impacts of PY203 corn production on soil biota would be similar. Use of pesticides on PY203 corn is not expected to be any different than that currently used in corn production. All pesticide use on PY203 corn would be subject to FIFRA and EPA requirements (US-EPA 2020j).

While PY203 corn differs from other corn varieties in the phytase, phytate, and PMI content, this difference is not expected to have any adverse effects on soil biota or community structures. Phytase, PMI, and phytate from PY203 corn can potentially enter soils via degrading plant material. Phytase occurs primarily in the seed (kernel), with little expression in leaves, stems, and roots. Phytate and PMI are expressed in all tissues (Agrivida 2019). Because the ear of the plant is harvested, only minor amounts of phytase would potentially enter soils through lost corn ears/seed at harvest. For example, harvest losses in corn of 10% or more are not unusual, although the goal is to limit to the 2% to 4% range (Sumner and William 2012).

Phytase enzymes are ubiquitous in soils, produced by various soil bacteria (e.g., *Bacillus* sp. and *Enterobacter*) and fungi (Konietzny and Greiner 2002; Jorquera et al. 2008a; Mukhametzyanova et al. 2012; Liu et al. 2018). Microbial phytases are actively secreted into soil where they serve to decompose plant debris and free inorganic phosphorus from soil organic compounds, which is used by microbes and plants for growth (Richardson 2001). Microbial phytases are key enzymes in phosphorus cycling in soil (Jorquera et al. 2008b; Mukhametzyanova et al. 2012).

An extensive range of soil bacteria and fungi that are able to solubilize various forms of phosphorus, via phosphatases, have been reported (e.g., (Kucey 1983; Rodriguez and Fraga 1999; Richardson 2001)). Studies have found that 54% of fungi and 76% of bacteria isolated from corn rhizosphere were capable of using phytate as a sole phosphorus source—via production of phosphatases such as phytase. The main fungi genera identified are *Aspergillus*, *Penicillium*, *Eupenicillium*, *Paecilomyces*, and *Fusarium*, and for bacteria *Bacillus* and *Pseudomonas* (Menezes-Blackburn et al. 2013). (Jorquera et al. 2008b) found that the proportion of phytate mineralizing bacteria was from 44% to 54% in the rhizosphere of perennial ryegrass, white clover, wheat, and oat.

The hydrolysis of organic phosphorous compounds by means of phosphatase enzymes is critical for plant and microbial growth. Plants and microorganisms together increase phosphorus availability by solubilizing inorganic phosphorus and mineralizing organic phosphorus, in part by production of phosphatase enzymes. The presence of a significant amount of phosphatase/phytase activity in soil has been reported. Soil microbes expressing a significant level of acid phosphatases/phytases include strains from the genus *Rhizobium*, *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Bacillus*, as well as *Sporotrichum thermophile*, *Emericella rugulosa*, *Discosia* sp. FIHB 571, and some other fungi (see review by (Singh and Satyanarayana 2011)). The importance of inorganic phosphorus to plant growth is evidenced by the extensive use of chemical fertilizers to improve soil fertility and agricultural productivity. For corn, application rates are in the range of 90 lb of P_2O_5 per acre.

Once in soil, phytases must withstand many factors in order to remain functional. These include: 1. deactivation and inhibition by adsorption and immobilization on soil solid particles; 2. proteolytic and microbial mediated degradation; 3. inhibition by interaction with metal ions, anions and metabolites; and 4. denaturation by soil environmental factors (temperature, pH, water content, light) (George et al. 2006). The impact of these factors on phytases found in the soil environment is potentially large, leaving little phytase activity for longer-term hydrolysis of inositol phosphates (George et al. 2006).

Few studies have explicitly measured phytase activity in soil, but 'baseline' activities, if detectable, are in the range of 10–300 pKat/g soil (George et al. 2006). In comparison with the activity of various enzymes released to the soil through biological processes (George et al. 2006), the baseline phytase activity in soil appears insignificant when compared with total phosphomonoesterase activities, which are 1–2 orders of magnitude greater, but is similar to those of soil phosphodiesterases (George et al. 2006).

Considering the abundance of phytase producing soil microorganisms; ubiquity of phytases in soils; potential inhibition of activity via various biotic and abiotic factors; regular fertilization of cropland soils due to insufficient inorganic phosphorus; and that any phytase entering soil via degrading PY203 corn tissue would do so slowly over time; it is unlikely degrading PY203 corn seed or other plant tissue would present any risk to soil biota or soil phosphorus cycling.

PMI is naturally expressed in various taxa including enteric bacteria, fungi, insects, some species of plants and nematodes, and mammals (de Lonlay and Seta 2009; Hu et al. 2016). PMIs that have been identified in soil associated bacteria and yeast include; *Escherichia coli* (vertebrate gut bacterium (Tenaillon et al. 2010)), *Bacillus subtilis* (soils, and the gastrointestinal tract of ruminants and humans (Earl et al. 2008)), *Corynebacterium glutamicum* (a gram-positive soil bacterium (Nishimura et al. 2007)), *Saccharomyces cerevisiae* (brewer's yeast (Duina et al. 2014)), and *Aspergillus fumigatus* (soil bacterium (Latgé 1999)). Considering these factors, and that discussed in Section 4.3.2.1–Soil Quality, it is unlikely that the introduction of PMI from PY203 corn via degrading plant material or root exudates would present any risk to soil biota, soil P cycling, or disturbance of the soil ecosystem.

4.3.3.2 Animal Communities

4.3.3.2.1 Birds and Mammals

Commercial cornfields, which are intensively cultivated, provide less suitable habitat for wildlife than undisturbed lands. As such, the types and numbers of animal species found in and near cornfields will be less diverse. Cornfields can, however, provide food and cover for wildlife, such as for birds, as well as large and small mammals.

Following harvest, it is common to find large flocks of migratory bird species foraging in cornfields, such as Canada geese (*Branta canadensis*), snow geese (*Chen caerulescens*), sandhill cranes (*Grus canadensis*), and various other species (Best et al. 1990; Taft and Elphick 2007; Sherfy et al. 2011). The types and numbers of birds that inhabit cornfields will vary regionally and seasonally.

A variety of mammals forage on corn at various stages of plant growth. Large- to medium-sized mammals that are common foragers of cornfields include those in Table 4-8 (Fleharty and Navo 1983; ODNR 2001). The most notable of these is the white-tailed deer which often inhabit woodlots adjacent to cornfields and frequent fields for both food and cover, especially in mid-summer. Agricultural crops, particularly corn and soybean, comprise a major portion of deer diets in Midwestern agricultural regions; deer are considered responsible for more corn damage than any other wildlife species (MacGowan et al. 2006). Cornfields are vulnerable to deer damage from emergence through harvest, although damage to corn at the tasseling stage most directly impacts yield (Stewart et al. 2007). Losses to crop yield from feeding by raccoons have also been documented (Beasley and Rhodes Jr. 2008). Mature corn has been shown to constitute up to 65% of the diet of raccoons in some areas prior to harvest (MacGowan et al. 2006). As with larger mammals, small mammal use of cornfields for shelter and forage also varies regionally (USDA-NRCS 1999; U-Illinois-Ext 2000; Sterner et al. 2003).

Table 4-8. Animals Commonly Found in Corn Fields

Birds		**Mammals**	
Common Name	**Scientific Name**	**Common Name**	**Scientific Name**
Red-winged blackbird	*Agelaius phoeniceus*	Large Mammals	
Grackle	*Quiscalus quiscula*	White-tailed deer	*Odocoileus virginianus*
Horned lark	*Eremophila alpestris*	Raccoon	*Procyon lotor*

Brown-headed cowbird	*Molothrus ater*	Wild boar	*Sus scrofa*
Vesper sparrow	*Pooecetes gramineus*	Woodchuck	*Marmota monax*
Ring-necked pheasant	*Phasianus colchicus*	Small Mammals	
Wild turkey	*Meleagris gallopavo*	Deer mouse	*Peromyscus maniculatus*
American crow	*Corvus brachyrhynchos*	House mouse	*Mus musculus*
Blackbird	*Turdus merula*	Meadow vole	*Microtus pennsylvanicus*
Various quail species	*Coturnix spp.*	Ground squirrel	*Spermophilus tridecemlineatus*

4.3.3.2.2 Invertebrates

Although certain invertebrates in agricultural settings are considered pests, such as the European corn borer (*Ostrinia nubilalis*) and corn rootworm (*Diabrotica* spp.), the majority are beneficial, performing valuable functions; they pollinate plants, contribute to the decay and processing of organic matter, reduce weed seed populations through predation, cycle soil nutrients, and attack other insects and mites that are considered plant pests (Landis et al. 2005). Some of the beneficial species include the convergent lady beetle (*Hippodamia convergens*), carabid beetles, the caterpillar parasitoids (e.g., *Macrocentrus cingulum*), and the predatory mite (*Phytoseiulus persimilis*) (Landis et al. 2005; Shelton 2011). Earthworms, termites, ants, beetles, and millipedes contribute to the decay of organic matter and the cycling of soil nutrients (Ruiz et al. 2008).

4.3.3.2.3 Potential Effects on Wildlife Communities

Vertebrate Wildlife that May Feed on PY203 Corn

Approval of the petition, and subsequent commercial production of PY203 corn, would not be expected to affect animal communities adjacent to or within PY203 corn cropping systems any differently from that of current corn cropping systems. Conceptually, the only potential risk to wildlife, as a matter of hazard assessment, would be from exposure to the trait protein products via consumption of the plant material, namely the kernel, which is largely limited to birds, granivorous insects, foraging rodents, and larger mammals.

The nutrient composition of grain and forage derived from PY203 corn and a conventionally bred control were determined and compared to conventionally bred corn. Grain samples were analyzed for proximate nutrients, amino acids, fatty acids, minerals, vitamins, and other metabolites (phytate, trypsin inhibitor, p-coumaric acid, raffinose, and ferulic acid). Forage samples were analyzed for nutrients only. Apart from the modified levels of phytase, phytate, phosphorus, and expression of PMI, PY203 corn was determined to be nutritionally comparable to other corn varieties (Agrivida 2019). For most of the analytes there are no significant differences in the composition of grain or forage between PY203 corn and the near isogenic control corn. Where statistically significant differences in amino acids, fatty acids, vitamins, and minerals were observed between PY203 corn grain and the control corn, all analyte values, except for intended increase in phytase, were within the normal range of conventional corn varieties published in the ILSI crop composition database (ILSI-CERA 2020).

Phytase and Phytate

Because phytase enzymes naturally occur in plants (e.g., cereals, legumes, oilseeds, and nuts) (Viveros et al. 2000; Konietzny and Greiner 2002), they are commonly consumed by wildlife. Phytase enzymes serve to degrade phytate to make phosphate, minerals, and myoinositol available for plant growth and development (Greiner et al. 2000; Gupta et al. 2015). Grains, seeds, and pollen contain both constitutive and germination-inducible phytases, with large increases in phytase activity reported in germinating seeds as well as in germinating pollen (Lin et al. 1987; Greiner et al. 2000).

In a subchronic exposure study, rats were dosed orally each day for 90 days with 400 mg (400,000 µg) of purified phytase representing 462,000 FTU/kg body weight—this would be comparable to small mammal consuming an entire ear of PY203 corn (Agrivida 2019). The rats in this study showed no adverse health effects (EFSA 2008). Larger animals consuming an ear of PY203 corn would be exposed to smaller doses of phytase on a per body weight basis. As previously discussed, consumption of grain from PY203 corn may improve the dietary availability of phosphorus and minerals in the animals consuming it.

Phy02 phytase tolerance studies were also conducted in poultry and swine. In the poultry study a feed containing approximately 60,000 units of Phy02 phytase activity (FTU) per kilogram was fed to broiler chickens from 1 to 42 days of age. Chickens showed improved performance from the addition of phytase to the feed and demonstrated no signs of adverse health effects (Agrivida 2016). Blood chemistry and hematology analyses as well as examination of organs after euthanasia demonstrated no signs of toxicity. Swine were fed diets amended with 45,000 FTU Phy02 phytase per kg. All animals exhibited good performance with no adverse health effects (Agrivida 2016). As in the poultry tolerance study there were no hematological or tissue differences between the Phy02-treated and untreated animals.

A commercial phytase feed product named Quantum® (AB Vista) has been used safely and effectively in poultry and swine diets for the past decade. The phytase in Quantum® is also derived from *E. coli* and is similar to the Phy02 phytase, differing in only 12 amino acids (Agrivida 2016). Grain from transgenic corn expressing a gene encoding the Quantum® phytase was used in two independent poultry feeding trials. The feed contained up to approximately 363,000 FTU of phytase activity per kilogram of diet. After 14 days the chickens demonstrated good performance and no adverse health effects (Nyannor and Adeola 2008; Nyannor et al. 2009). High doses of Quantum® phytase, up to 49,500 FTU/kg, were also tested in swine; no adverse effects were observed (Nyannor et al. 2007).

The FDA completed a safety evaluation of PY203 corn and, based on the information contained Agrivida's submission, had no questions regarding Agrivida's conclusion that ground grain obtained from PY203 corn is Generally Recognized as Safe (GRAS) under its intended use—as a feed additive (US-FDA 2017).

Based on all of these factors, it is unlikely the increased phytase production in PY203 corn presents any risk to wildlife that may consume PY203 corn kernels or other plant parts.

Phosphomannose Isomerase

There are no risks to wildlife presented by PMI. The EPA issued an exemption from the requirement of a tolerance for residues in or on plant commodities comprised of PMI (40 CFR § 174.527). Recognizing the limits in extrapolation of food safety data from animals to humans and vice versa (Hartung 2009; Krewski et al. 2010); considering the FDA and EPA determinations, and that PMI is not a synthetic chemical, rather, it is endogenously expressed by various taxa, it is highly unlikely that cultivation of PY203 corn comprised of PMI would present any risk to wildlife that may consume it.

Modified Phytase and Phosphorus Levels in PY203 Corn – Invertebrates

PY203 corn would likely be treated with insecticides to preserve to the quality of the kernel, and to achieve maximum yield. Few insects would likely have the opportunity to feed on PY203 corn. Nevertheless, evaluation of the potential effects of PY203 corn phytase and phosphorus on insect populations is provided.

Many plants on which insect may feed express phytase, although expression levels vary widely (Eeckhout and De Paepe 1994; Cangussu et al. 2018). Of the cereals that have been analyzed, rye (5130 FTU/kg), triticale (1688 FTU/kg), wheat (1193 FTU/kg) and barley (582 FTU/kg) are rich in phytase activity (Eeckhout and De Paepe 1994). Non-transgenic corn has, inherently, negligible levels of phytase activity compared to other cereals—around 66 FTU (Chen et al. 2008)—whereas PY203 corn contains approximately 3500 FTU per gram of grain (Agrivida 2019).

APHIS is unaware of any studies that describe consumption of phytase by insects via plant tissues to be potentially harmful. There are few published papers on the effects of transgenic phytase corn and larval insect development, specifically. Of the studies available, these concentrated on the potential effects of transgenic phytase corn on herbivorous insects, including Asian corn borer (*Ostrinia furnacalis* (Guenée)), corn earworm (*Helicoverpa armigera*), and carabid beetles (Carabidae). Among these studies, no significant differences in survival, weight, or physiology was observed among those reared on diets of transgenic phytase corn and non-modified corn (Zhang et al. 2010; Xu et al. 2018).

In general, natural variation in dietary phosphorus and other minerals is likely to affect, to some degree, the growth rate and population dynamics of insects. Some studies have shown that growth rates of certain species, such as budworm (*Choristoneura occidentalis*), house cricket (*Acheta domesticus*), and tobacco hornworm (*Manduca sexta*) are accelerated when fed diets that contain high concentrations of phosphorus (Clancy and King 1993; Perkins et al. 2004). In vitro studies where phosphorus was added to the diet have been shown to increase the growth of lepidopteran larvae (Clancy and King 1993). Perkins et al. (2004) found that dietary phosphorus content significantly affects the growth of tobacco hornworm (*Manduca sexta*). On both artificial and natural diets, caterpillars given high-phosphorus diets grew significantly faster than those given low-phosphorus diets. Similarly, Janssen (2009) reported increased larval growth of black armyworm (*Spodoptera exempta*) with increased phosphorus concentrations in corn leaves.

The means for all minerals (e.g., calcium, magnesium, phosphorus, potassium) in PY203 grain are within the historical ranges for corn grain, based on the ILSI- Crop Composition Database (ILSI 2019). Total phosphorus in PY203 corn, specifically, is marginally higher than in the conventionally bred corn variety used in Agrivida field trials (e.g., 3,130 ± 260 ppm fresh weight (FW) in PY203 corn vs. 2,830 ± 220 ppm FW for the control corn), although within the boundaries of normal variability for corn, which ranges from 1,260.4 ppm FW to 4,090.5 ppm FW (ILSI-CERA 2020). The phytate content in PY203 corn grain is about half that of conventional varieties (e.g., 3,800 ± 800 ppm versus 7,600 ± 900 ppm, or 0.38 ± 0.08 versus 0.76 ± 0.09 %FW), although comparable to other cereals—the phytate content of cereals varies from around 1,000 ppm to 22,000 ppm (0.1% and 2.2 %FW) (Sanz-Penella and Haros 2014). PY203 forage is around 2,500 ± 300 ppm (0.25 ± 0.03 % dry weight (DW)) in phosphorus, comparable to conventionally bred corn forage.

Depending on the pH of the digestive system of the consuming insect, the Phy02 phytase may or may not demonstrate phytase enzymatic activity. The Phy02 phytase in PY203 corn grain has optimal activity in the range of pH 2 to 7 (Agrivida 2016), so it would not be expected to have significant activity in the digestive tracts of insects; the midgut of most insects (e.g., lepidopteran, dipteran) has a much more basic pH, around 10 to 12 (Dow 1992). Thus, the activity of the Phy02 phytase protein that is consumed would be likely be negligible in most insects, and digested into its constituent amino acids.

Xu et al. (2018) examined the potential toxicity of transgenic phytase corn (maize 10TPY005 expressing an *Aspergillus niger* phyA2 gene) on Asian corn borer using physiological, biochemical, and gut microflora parameters. In agreement with previous studies (Zhang et al. 2010), the survival rates and weights of Asian corn borers were unaffected by consumption of transgenic phytase corn. The authors found no significant differences in two detoxification enzymes (GST and AChE) and three antioxidant enzymes (CAT, POD and SOD), in comparison with the controls.

Qin et al. (2019) found that while high C:P and N:P ratios improve grasshopper (*Oedaleus asiaticus)* performance, high P content worsened grasshopper performance. Their findings suggested that *O. asiaticus* preferred a dietary ratio of C:N:P of 249.8:18.8:1.0. This ratio indicates that *O. asiaticus* not only prefers plants with relative low N, as shown in earlier studies, but also plants with very low P content. The specific dietary habits observed by Qin et al. (2019) suggest that *O. asiaticus* maintains a nutritional balance of C:N:P by changing its feeding behavior. This finding indicated that grasshoppers select food resources to meet their nutritional C:N:P dietary requirements (Qin et al. 2019).

4.3.3.3 Plant Communities

Plant diversity in surrounding areas is an important component of a sustainable agricultural system (Scherr and McNeely 2008; CBD 2019a). Hedgerows, woodlands, fields, and other surrounding habitat serve as important reservoirs for beneficial insects, as well as plant pests. Corn fields and field edges are also habitat for weeds that adversely impact corn production directly through interference and resource competition (discussed below), and can also harbor both beneficial or damaging insects and plant microbes. Most weeds, however, provide valuable ecosystem services. By providing habitat, pollen and nectar resources, and serving as hosts, plants adjacent to corn fields can support a suite of beneficial arthropod species that serve as pollinators of insect-pollinated crops, and biological control agents, insects that prey on corn plant pests, such as lady beetles, spiders, and parasitic wasps (Scherr and McNeely 2008; Nichols and Altieri 2012). Surrounding plant communities can also help regulate run-off, reduce soil erosion, and improve water quality (Reichenberger et al. 2007; Egan et al. 2014). Hence, effective management of surrounding plant communities can provide benefits to corn crop production via control of insect pests and agricultural run-off (Altieri and Letourneau 1982), and support pollinator services to other plants that benefit from insect pollination (Nichols and Altieri 2012).

4.3.3.3.1 Potential Effects on Plant Communities

Because the agronomic practices and inputs that will be used for PY203 corn production would be no different, the potential impacts on vegetation proximate to PY203 corn fields would be the same as that for other corn varieties. Most relevant to the environmental review of transgenic cropping systems are sexually compatible plant communities with which the transgenic crop plant can interbreed, discussed in the following section.

4.3.3.4 Gene Flow and Weediness of Corn

Gene flow as a mechanism for the unintended movement of plant transgenes to conventionally bred crops, other transgenic crops, and wild or feral plants has been a primary topic of interest and research since the advent of transgenic crops in the 1990s. Factors such as the particular type of transgenic plant being grown, adjacent cropping systems, occurrence of wild relative species with which the transgenic plant may crossbreed, and transgenic trait all require consideration when evaluating the potential environmental impacts that could result from gene flow (Warwick et al. 2009; Ellstrand 2014). Gene flow among transgenic crops and conventional and organic cropping systems is of particular interest to farmers, food or feed processors, and international, federal, and state regulators, as such gene flow can adversely affect crop management, net returns on crops and their products, and domestic and international trade. Gene flow from transgenic plants to wild relative species is a topic of interest among ecologists and environmentalists, as well as federal and state regulators, due to concerns that a transgene may confer weediness traits to, or alter the fitness of, wild relative species.

Of particular interest to APHIS is the possible occurrence of gene flow from a transgenic plant to sexually compatible wild relative species that could lead to introgression of the trait gene into a wild population, and development of a phenotype that could adversely affect agricultural interests and/or the environment.

4.3.3.4.1 Factors Governing Gene Flow among Crop Plants and Wild Relative Species

The rate and success of pollen mediated flow is dependent on numerous factors such as the presence, abundance, and distance of sexually-compatible plant species; overlap of flowering times among populations; method of pollination; biology and amount of pollen produced; and weather conditions, including temperature, wind, and humidity.

The salient environmental concern is whether the flow of a transgenic trait gene to a wild relative will have adverse ecological consequences. For a significant environmental impact to occur, gene flow would have to lead to the production of a fertile hybrid plant that produces viable offspring, and the resulting transgenic -wild plant hybrid having some type of competitive advantage that can lead, ultimately, to introgression of the transgene into a wild plant population. The transgene in a wild relative or other crop plant may very well prove detrimental to the hybrid, or have no effect (Ellstrand et al. 2007; Ellstrand 2014; Goldstein 2014). The ecological consequences of a transgene in a wild species depends on the type of trait, the stability of the gene in the genome, the fitness conferred to the hybrid through expression of the trait gene, and ecological factors in the area of the hybrid (Felber et al. 2007; Ellstrand 2014).

It is generally assumed traits that impart increased fitness will persist in populations and those that impart negative effects on plant fitness will not. If a resulting transgenic-wild type hybrid had a competitive advantage over wild populations, it could persist in the environment and potentially disrupt the local ecology. Where the transgenic trait does not provide fitness, and is not deleterious to survival of the hybrid, the transgene may still persist in wild populations with no effects on the local ecology. This could be the case for a number of introduced traits.

In respect to the occurrence of a transgenic-wild type hybrid, gene flow from a transgenic crop plant to wild or weedy relative species does not necessarily constitute an environmental harm in and of itself, nor does it inherently imply environmental damage (Ellstrand 2014). The salient issue is what the resultant ecological consequences of such gene flow to a wild population may be (Ellstrand 2014). Current understanding suggests that the presence of a transgenic trait outside the area of cultivation will likely have little or no adverse consequences unless:

(1) the trait confers novel or enhanced fitness or weediness to the wild relative hybrid, resulting in the evolution of increased weediness or invasiveness in wild type hybrids, or

(2) the trait confers to transgenic-wild relative hybrid progeny reduced fitness, resulting in a selective disadvantage in wild relative populations (Kwit et al. 2011; Ellstrand 2014).

Hence, in evaluating potential environmental impacts it is not the risk of gene flow itself that is the chief concern, but rather the environmental consequences that could occur as the result of such an event; whether the transgene will persist in a wild population, and whether hybrid or introgressed populations will have adverse effects on ecosystem dynamics.

4.3.3.4.2 The Potential for Gene Flow among Corn (*Zea mays* L.) and Wild Relative Species

Corn (*Zea mays* L. subsp. *mays*) is one of the oldest domesticated plants in the world, the origins of which date back to around 5,000 – 3,600 years ago in southern Mexico (de Wet et al. 1978; Eubanks 1995). How corn evolved is still a matter of investigation, although most investigators agree that what we know as cultivated corn most likely descended from an annual species of "teosinte" (*Zea mays* ssp. *parviglumis*), a closely related wild grass endemic to Mexico (Piperno and Flannery 2001). Teosinte is the common name applied to several distinct wild *Zea* species closely related to corn (*Zea mays* L. ssp. *mays*). Cultivated corn (*Zea mays* L. subsp. *mays*) is sexually compatible with teosinte (*Zea* spp.), with a few exceptions. The closest relative of *Zea* in the United States is the genus *Tripsacum*, with which corn does not readily hybridize (OECD 2003).

Teosinte

Wild teosinte relatives of corn comprise a group of annual and perennial species that commonly occur within the tropical and subtropical areas of Mexico, Guatemala, Costa Rica, Honduras, El Salvador, and Nicaragua (Sánchez González et al. 2018). The natural geographic distribution of teosinte extends from the Western Sierra Madre of the State of Chihuahua, Mexico to the Pacific coast of Nicaragua and Costa Rica, including the western part of Mesoamerica. The Mexican annuals *Zea mays* ssp. *parviglumis* and *Zea mays* ssp. *mexicana* show a wide distribution in Mexico, while *Zea diploperennis*, *Zea luxurians*, *Zea perennis*, *Zea mays* ssp. *huehuetenangensis*, *Zea vespertilio* and *Zea nicaraguensis* have more restricted and distinct ranges, representing less than 20% of the total occurrences from published sources for the period 1842-2016 (Sánchez González et al. 2018).

Except for *Z. perennis*, *Zea mays* and teosinte cross readily, and their hybrids are fully fertile (de Wet and Harlan 1972). Hybridization and introgression between *Z. mays* and the subspecies *Z. mays* subsp. *mexicana* occurs in Mexico, and has probably been taking place since the advent of corn domestication wherever these two taxa are sympatric (de Wet et al. 1978; Ellstrand et al. 2007). Hybrids appear to maintain their unity of type in the wild (de Wet and Harlan 1972). In general, humans select in the direction of corn (*Zea mays*), and nature strongly favors teosinte over their hybrid, which is less well adapted for natural seed dispersal (de Wet and Harlan 1972). The rate at which domesticated corn crop genes may enter teosinte populations will be limited by genetic barriers, phenological differences, and the relative fitness of the hybrids (Ellstrand et al. 2007).

Teosinte do not appear to be present in the United States other than in botanical gardens or at research stations. The USDA Plants Database lists *Zea mexicana* (Syn. *Z. mays* ssp. *mexicana*) as present in Florida, Alabama, and Maryland, having been introduced from Mexico (USDA-NRCS 2019b). It has, apparently, occasionally been cultivated in the Southern United States for forage (Hitchcock 1951). The documentation cited for occurrence in Florida only shows distribution of native or naturalized populations in Miami-Dade, Orange, and Levy Counties (Wunderlin et al. 2019). While citations were provided in the Plants database for distribution in Maryland and Alabama, current Maryland plants databases have no listed *Zea* species, other than *Z. mays* (UMD 2005; MPA 2019), nor are any *Zea* species or subspecies other than *Z. mays* (corn) listed in Alabama (Kral et al. 2019).

Zea perennis (Syn. *Euchlaena perennis* Hitchc.) is listed as occurring in Texas and South Carolina. It is described as having been cultivated at academic research stations in Texas, and established on James Island, South Carolina (Hitchcock 1951). It is not known if the James Island population has persisted. There are no *Zea* species found in the comprehensive online South Carolina Plant Atlas (USC 2019); which catalogues over 3000 species.

Teosinte identified as *Zea mays* ssp. *parviglumis* is listed as having occurred in Miami-Dade County, Florida (Wunderlin et al. 2019), an area that is now largely urban. *Zea diploperennis* and *Zea luxurians* are also listed in the USDA Plants database, but there is no information about the presence of any wild populations in the United States.

Experts familiar with the teosinte collections in the United States, some of whom were involved with revision of the Manual of Grasses for North America (Roché et al. 2007), are not aware of any naturalized or native populations of teosinte currently growing in the United States (USDA-APHIS 2013).

Tripsacum

The closest relative of *Zea* in the United States is the genus *Tripsacum* (OECD 2003). Three species have been identified: *T. dactyloides*, Eastern gamagrass, is known to occur in the eastern half of the United States, *T. lanceolatum*, Mexican gamagrass, occurs in the southwest of the United States, and *T. floridanum*, Florida gamagrass, is native to South Florida and Cuba (Wozniak 2002; OECD 2003). *T. dactyloides* is the only *Tripsacum* species of widespread occurrence and agricultural importance in the United States, and has been commonly grown as a forage grass (USDA-NRCS 1996). *T. fasciculatum* and *T. latifolium* occur in Puerto Rico (USDA-NRCS 2019b). *Tripsacum* species (2n=18) can be represented by diploid, triploid, tetraploid, and higher ploidy levels (Lee et al. 2017).

Although not closely related cytologically (e.g., differing numbers of chromosomes), gene exchange can take place between *Z. mays* and *Tripsacum* (de Wet et al. 1978). Certain species of *Tripsacum* can and have been crossed with *Zea mays* or at least some accessions of each species can cross under experimental lab conditions, but only with difficulty. The resulting hybrids are male sterile and usually female sterile (de Wet et al. 1978; Leblanc et al. 1996; Lee et al. 2017; Iqbal et al. 2019). Hybrids between *T. dactyloides* and *Z. mays,* however, have been found to be male sterile, but usually female fertile (de Wet and Harlan 1972). Attempts at artificially induced introgression from *Tripsacum* species into *Z. mays* failed to produce either teosinte-like offspring or the combination of characteristics assumed to indicate introgression during the evolution of several South American races of corn (Mangelsdorf and

Reeves 1959; de Wet and Harlan 1972). The probability of natural introgression from *Tripsacum* in the direction of *Z. mays* seems to be low (de Wet et al. 1978).

Hybrid combinations with *Z. mays* (as pollen donor) and *T. dactyloides* are known to give rise to recovered *Z. mays* within three or more further backcrosses with *Z. mays*. It is, however, not too likely that this process commonly occurs in nature (de Wet et al. 1978). With each successive backcross, the offspring become more *Z. mays* like, and less capable of surviving in competition without the help of humans. Hybrids have been observed to not only produce low yields, but are also partially female sterile (de Wet et al. 1978).

In summary, gene exchange is possible between *Zea* and *Tripsacum*, and several South American races of corn, where teosinte is absent, exhibit past evidence of hybridization (de Wet et al. 1978). Natural introgression between *Zea* and *Tripsacum*, however, appears unlikely (de Wet et al. 1978). Hybrids between *Z. mays* and *Tripsacum*, as well as their derivatives when backcrossed with *Z. mays*, are poorly adapted for survival in competition with both their wild and cultivated parents (de Wet et al. 1978). Although hybridization of *Tripsacum* and *Z. mays* has been accomplished in the laboratory using special techniques under highly controlled conditions (Wozniak 2002; Lee et al. 2017), pollen-directed gene flow from corn (*Zea mays*) to wild *Tripsacum* species is considered an unlikely event (Wozniak 2002; Lee et al. 2017). APHIS is unaware of any reported cases of hybridization among naturally occurring *Tripsacum* and *Z. mays* in the United States.

4.3.3.4.3 Corn as a Weed or Volunteer

In the United States, there are no *Zea* species listed on the Federal Noxious Weed List (7 CFR part 360) (USDA-NRCS 2019b). Corn (*Zea mays*), as a highly domesticated crop plant with limited seed dispersal and dormancy, does not readily form persistent feral populations; does not present as a weed outside of areas of cultivation (USDA-NRCS 2019b; USDA-APHIS 2020b).

Corn can and periodically does occur as a volunteer plant in subsequent crops planted in the same field. Corn seed can remain in fields as a result of harvester inefficiency, dispersal by birds and other foraging wildlife, or from fallen ears. When seeds survive to the next growing season, volunteer plants may develop within subsequent crops rotated with corn, such as soybean, dry beans, sugar beets, as well as subsequent corn crops.

Volunteer corn is more of agronomic/economic than environmental concern; the presence of volunteers can result in minor to significant yield impacts on subsequent crops planted in the same field, interfere with harvest, and cause unacceptable levels of contamination in harvested soybean (Nicolai et al. 2019), depending on the density of the volunteer corn (Nicolai et al. 2019; Jhala et al. 2020). In controlled agronomic studies, volunteer corn densities ranging from 800 to 13,000 plants per acre resulted in yield losses of 0 to 54% in soybean and 0 to 13% in corn (Nicolai et al. 2019). Similarly, soybean yield reductions have been found to range from 10% to 41% where early-emerging volunteer corn densities ranged from 0.5 to 16 plants m^2, although no soybean yield loss occurred with a late-emerging cohort of volunteer corn (Marquardt et al. 2012). Thus, the potential impact of volunteer corn on the yield of subsequent crops can be substantial. Volunteer corn can also encourage dispersal and survival of western corn rootworm and gray leaf spot disease limiting the benefits of a corn-soybean rotation (Jhala and Rees 2018). Successful control of volunteer corn is accomplished with the use of various combinations of cultivation practices and use of herbicides with differing modes of action (Jeschke and Doerge 2010; Nicolai et al. 2019).

4.3.3.4.4 Probability and Potential Effects on Gene Flow and Weediness

PY203 corn, if grown for commercial purposes, would be cultivated as are current corn varieties and present the same potential risk for gene flow, specifically the propensity for and frequency of gene flow, as current corn varieties. Accordingly, PY203 corn cropping systems would not be expected to present more or less risk for gene flow to wild relative species, or other corn crops, as do current corn varieties.

As previously reviewed, teosinte (*Zea* spp.) do not appear to be present in the United States other than in botanical gardens or at research stations. The closest relative of *Zea mays* in the United States is the genus *Tripsacum*. Three species have been identified: *T. dactyloides*, Eastern gamagrass, in the eastern half of the United States*, T. lanceolatum*, Mexican gamagrass, occurs in the southwest of the United States, and *T. floridanum*, Florida gamagrass, is native to South Florida and Cuba. *T. dactyloides* is the only Tripsacum species of widespread occurrence and agricultural importance in the United States, and commonly is grown as a forage grass (USDA-NRCS 1996).

While it is possible that *Tripsacum* species may occur in areas where PY203 corn is cultivated, gene introgression from PY203 corn into *Tripsacum* populations under natural conditions is considered highly unlikely, for two reasons. In contrast with corn and teosinte, which may hybridize under certain conditions, as discussed previously, the potential for hybridization and successful introgression of *Z. mays* genes into *Tripsacum* is rare (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995). Special techniques are required to hybridize *Z. mays* and *Tripsacum*; hybrids of *Tripsacum* species with *Zea* species do not commonly occur outside of a laboratory. Offspring are often sterile or have reduced fertility, and are unable to withstand even mild winter conditions (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995).

Second, while corn pollen can travel as far as 1⁄2 mile (800 m) in 2 minutes in a wind of 15 miles per hour (27 km/h) (Nielsen 2016), most pollen is deposited within a short distance of the corn plant. Numerous studies show the majority (84-92%) of pollen grains travel less than 16 feet (5 meters) (Pleasants et al. 2001). At a distance of 200 feet (60 m) from the corn plant, the pollen concentration averages only about 1%, compared with pollen samples collected about 3 feet (0.9 m) from the pollen source (Burris 2002; Brittan 2006). The number of outcrosses is reduced to one-half at a distance of 12 feet (3.6 m) from the pollen source, and at a distance of 40 to 50 feet (12 to 15 m), the number of outcrosses is reduced by 99%. Thomison (2004) showed cross-pollination between cornfields could be limited to 1% or less by a separation distance of 660 feet (200 m), and to 0.5% or less by a separation distance of 984 feet (300 m). However, cross-pollination frequencies could not be reduced to 0.1% consistently, even with isolation distances of 1,640 feet (500 m).

Based on all of these factors, it is unlikely hybridization of PY203 corn and *Tripsacum* species would occur. In the event such hybrids developed, it is unlikely that the phytase trait extant in PY203 corn would present any risk to communities of *Tripsacum* species or their ecological role in the communities of other plants.

Volunteer PY203 Corn

For the reasons discussed below, PY203 is no more likely to occur as a volunteer in subsequent seasons after its planting than conventional corn. For corn, factors contributing the occurrence of volunteer corn include pre-harvest seed loss, stalk and root lodging characteristics, and ear-drop. All of these can contribute to the occurrence of volunteer corn (considered a weed in subsequent crops). Field studies comparing the phenotypic properties of PY203 corn to conventionally bred comparator lines found statistically significant differences in stalk lodging and dropped ears, the values for each of these characteristics lower for PY203 corn as compared to the control (Agrivida 2019). In the case of stalk lodging, PY203 demonstrated 3.7% lodging compared to 6.2% for the conventionally bred control line. PY203 corn exhibited slightly fewer dropped ears compared to the control, 0.78 vs. 1.34 ears per plot. The emergent and final stand count was also slightly lower (62.7 vs. 65.9 plants/plot for emergent stand count and 60.9 vs. 64.2 plants/plot for final stand count), and grain test weights significantly lower for PY203 corn as compared to the control line. There were no observed differences in the incidence of diseases or insect predation between the PY203 corn and the conventionally bred comparator line (Agrivida 2019).

The phenotypic differences described—less stalk lodging, fewer dropped ears, lower grain weight, and lower emergent and final stand count—would not be expected to confer a competitive advantage or disadvantage to *Tripsacum* species that may be pollinated by PY203 corn. There are no weediness characteristics (e.g., increased hardiness, rapid growth, stress tolerance, pest/disease resistance) associated with PY203 corn. In the United States, corn (*Zea mays*) nor *Tripsacum* is listed as a weed, neither are on the Federal Noxious Weed List (7 CFR part 360) (USDA-NRCS 2019c). Corn, domesticated *Zea mays*, has been cultivated throughout the United States without any evidence it forms persistent feral populations.

Various post-emergence herbicides are available to control volunteer corn in each of the major corn rotational crops. However, because of the variety of resistance traits available in both corn and soybeans, choosing an effective herbicide that won't harm the crop requires careful planning. For example, in soybeans, the list includes glyphosate, glufosinate, the FOP ACCase inhibitors (quizalofop, fluazifop-p-butyl, fluazifop + fenoxaprop), and the cyclohexanedione ACCase inhibitors (clethodim, sethoxydim; "DIM" herbicides) (Boehm 2019; Jhala et al. 2019). Imazamox (Raptor®) is an ALS inhibitor and another option for post-emergence control of volunteer corn (at 2-8 inches) in soybean, alfalfa, dry beans, peas, lima bean, snap bean, clover, and edamame. For volunteer control of corn in wheat crops, including PY203 corn, additional post-emergence herbicide options are available such as Powerflex® (pyroxsulam), GoldSky® (florasulam+ pyroxsulam + fluroxypyr), and Perfectmatch® (clopyralid+ fluroxypyr+ pyroxsulam) (Ikley 2020).

These data suggest that PY203 corn is no more likely to become weedy than conventional varieties of the crop. PY203 corn volunteers can be managed using a variety of currently available cultural methods, as well as herbicides.

4.3.3.5 Biodiversity

Biological diversity in the context of agriculture encompasses the variety of species that are capable of existing in a given agricultural setting. Various taxa contribute to essential ecological functions upon which agriculture depends, such as pollinators, soil biota, and predators of crop pests (CBD 2019a). One invaluable function of biodiversity is the support of diverse populations of beneficial insects on farms. In one study of corn farms across the Northern Great Plains, Lundgren and Fergen (2014) found that farms with lower insect biodiversity had more plant pests, and that more biodiverse cornfields had fewer plant pests. The results from their study also suggest that designing cropping systems to sustain a broad array of insect species can require fewer insecticide inputs and save farmers money. Thus, farming practices that promote insect biodiversity can facilitate control of plant pests.

4.3.3.5.1 Potential Effects on Biodiversity

Commercial production of PY203 corn would affect biodiversity in and around PY203 corn crops no differently than other corn cropping systems. As discussed in the sections addressing soil biota and wildlife, the phytase and PMI trait proteins are unlikely to present any risks to plant, animal, fungal, or bacterial communities. The same or functionally similar enzymes are ubiquitous among plants and microorganisms, and commonly consumed by wildlife. The agronomic practices and inputs used for PY203 would be the same as those used for other corn varieties, tramsgenic and conventionally bred crops alike. Consequently, there are no unique risks to biodiversity—beyond that already posed by conventional corn cropping systems—that would likely derive from cultivation of PY203 corn.

As discussed for water resources, PY203 corn, with reduced levels of phytate and increased expression of phytase could potentially contribute to reductions in the runoff of phosphorus from AFOs/CAFOs utilizing PY203 corn for animal feed, and agricultural operations using manure from such AFOs/CAFOs (manure lower in phosphorus). To the extent that GRAINZYME® phytase is adopted for use in animal feed, and that AFO/CAFO manure derived from PY203 corn based feeds are utilized for fertilization of cropland, these uses collectively could contribute, to some degree, to overall reductions in phosphorus runoff from agricultural facilities in the United States. Any contribution to reductions in total anthropogenic phosphorus inputs into surface waters would be beneficial to sustaining biodiversity in aquatic ecosystems.

4.3.3.6 Threatened and Endangered Species

The Endangered Species Act (ESA) of 1973, as amended, is a far-reaching wildlife conservation law. 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. It is the responsibility of the federal agency taking the action to assess the effects of their action and to consult with the U.S. Fish & Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) if it is determined that the action "may affect" listed species or designated critical habitat (a process is known as a Section 7 Consultation).

4.3.3.6.1 Potential Effects on Threatened and Endangered Species

After reviewing the possible effects of a determination of nonregulated status for PY203 corn, discussed in more detail in Appendix 1, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed threatened or endangered species or species proposed for listing. APHIS also considered the potential effect of a determination of nonregulated status of PY203 corn on designated critical habitat and habitat proposed for designation, and could identify no risks to critical habitats. Corn is not considered a particularly competitive plant species and has been selected for domestication and cultivation under conditions not normally found in natural settings. Corn is not sexually compatible with, nor serves as a host species for, any listed species or species proposed for listing. As previously discussed in 4.3.3–Biological Resources, consumption of PY203 corn by any listed species or species proposed for listing would pose negligible health risks.

Based on our evaluation provided in Appendix 1, APHIS has concluded that a determination of nonregulated status of PY203 corn, and subsequent commercial production of this corn variety, 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 are not required.

4.3.4 Human Health

PY203 corn is intended for use as animal feed. However, potential effects on human health via inadvertent consumption is considered. Human health considerations relative to biotechnology-derived crops, specifically, are those related to (1) the safety and nutritional value of foods derived from biotech crops, and (2) the potential health effects of pesticides that may be used in association with biotech crops. As for food safety, consumer health concerns center on 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. Some consumers may be concerned about the potential consumption of pesticide residues on/in foods derived from biotechnology-derived crops. Occupational exposure to pesticides is also considered.

The introduced functional enzyme in PY203 corn, phytase, which occurs widely in currently consumed foods, is reviewed below, along with phytate. Phosphomannose isomerase, another enzyme introduced into PY203 corn, and which naturally occurs in bacteria, insects, and humans, is also reviewed.

4.3.4.1 Food Safety

The safety assessment of biotechnology-derived crop plants includes characterization of the physicochemical and functional properties of the introduced gene(s) and gene products, determination of the safety of the gene products (e.g., proteins, enzymes), and compositional assessment of the plant. Compositional assessments compare the biotechnology-derived crop plant with non-transgenic, conventional varieties of that crop, and evaluate characteristics such as moisture, protein, fat, carbohydrates, ash, minerals, dietary fiber, essential and non-essential amino acids, fatty acids, vitamins, and antinutrients.

Safety and compositional assessments comparing biotechnology-derived and conventionally bred corn are typically performed using the principles and guidelines outlined in the Codex Alimentarius (Codex), established by the World Health Organization and Food and Agriculture Organization of the United Nations, and Organization for Economic Co-operation and Development (OECD) consensus documents for specific crop varieties (e.g., for corn (OECD 2003). The FDA participates and exercises leadership in the Codex Alimentarius Commission. The Codex is a set of international standards, principles, and guidelines for the safety assessment of foods derived from plants that were modified using biotechnology based techniques (WHO-FAO 2009). These standards help countries coordinate and harmonize review and regulation of foods derived from biotechnology-derived plants to ensure public health and facilitate international trade. Currently, the Codex Alimentarius Commission is comprised of over 180 member countries, to include the United States. Most governments incorporate Codex principles and guidelines in their review of foods derived from biotechnology-derived crop plants.

As summarized in Section 1.3–Coordinated Framework for the Regulation of Biotechnology, the FDA regulates the safety of plant-derived foods pursuant to the FFDCA and FSMA. The FDA created a voluntary premarket food safety consultation process in the 1990's. This consultation process enables developers to engage with the FDA on the safety and legality of foods derived from their new plant varieties and helps to ensure that any safety or regulatory issues associated with a food from a new plant variety are resolved prior to commercial distribution (US-FDA 1992, 2006). Agrivida completed a New Protein Consultation (Early Food Safety Evaluation) for PY203 corn with the FDA (NPC 000015) on August 7, 2015 (US-FDA 2020a). The FDA's response was that they had no questions regarding Agrivida's conclusions that the potential inadvertent presence in the food supply of low levels of PHY02 protein would not raise safety concerns. A Pre-market Biotechnology Notification (PBN) consultation for PY203 corn was submitted to the FDA in June 2018 (BNF 000167) (Agrivida 2019).

In addition to the FDA consultation, foods derived from plant varieties developed using genetic engineering undergo a safety evaluation among international agencies before entering foreign markets, such as reviews by the European Food Safety Agency (EFSA 2020) and the Australia and New Zealand Food Standards Agency (ANZFS 2020). These reviews likewise adhere to Codex standards.

4.3.4.1.1 Safety of Phytate, Phytase, and Phosphomannose Isomerase

Phytate

Most humans consume phytate on a daily to weekly basis. Phytate is a common constituent in plants and it follows, in plant-derived foods like cereals or legumes (Gupta et al. 2015; Nissar et al. 2017). Phytate is the major storage form of phosphorous comprising 1–5% by weight of cereals, legumes, oil seeds, and nuts (Vats and Banerjee 2004). It represents 50–85% of total phosphorous in cereals (Gupta et al. 2015). The phytate concentration reported in wheat germ and wheat bran range from 1.9–9.0% and 3.0–9.5% respectively (Kasim and Edwards Jr 1998). In rice bran, the phytate content is represents up around 5.9–9.9% (Lehrfeld 1994; Kasim and Edwards Jr 1998). Legume seeds contain 0.2–2.9% of phytate (see review by (Gupta et al. 2015)). Under heat treatment during cooking phytate is stable up to 100º C/212º F (Schlemmer et al. 2009).

It has been estimated that the daily intake of phytate and other inositol phosphates on the basis of Western diets varies from 0.3 to 2.6 grams (g), with a global range from 0.180 to 4.569 g, depending on the diet (Schlemmer et al. 2009). Usually legume-based food items contain higher amounts phytate than do cereal-based food items (Kumar et al. 2010). Sesame seeds (toasted), soy protein concentrate, rice, cornbread, and peanuts have high amounts of phytate, containing 39–57, 11–23, 13–22, 12–19 and 10–20 mg/g, respectively (Kumar et al. 2010).

Phytase Enzymes

There are four primary sources of phytase: plant phytase, fungal and bacterial phytase, phytase generated by small intestine mucosa, and gut-associated microflora phytase (Kumar et al. 2010). Humans naturally produce low levels of phytase in the gastrointestinal tract and commonly consume phytase in foods (Markiewicz et al. 2013). Bacteria inhabiting the human gastrointestinal tract (normal flora) exhibiting phytase activity include e.g. *Escherichia coli*, *Klebsiella*, *Bifidobacterium*, and *Lactobacillus* (Markiewicz et al. 2013). However, the human intestinal tract normally has very low phytase activity, insufficient to degrade all the dietary phytate consumed (Markiewicz et al. 2013). Many animals, including humans, have the adaptive capacity to increase intestinal phytase and other phosphatase activities under conditions of high phytate diets or phosphorus inadequate diets (see reviews by (Kumar et al. 2010; Markiewicz et al. 2013)).

Phytase enzymes have been isolated and characterized from a number of plant based foods such as rice, canola, soybean, wheat, rye, and peas (Kumar et al. 2010). High phytase activity (relative to other foods) of 121 ± 13 and 97 ± 20 phytase units/g occurs in scallion leaves and avocado, respectively.[12] Cabbage leaves and pear have been found to exhibit 25 ± 7 and 24 ± 6 phytase units/g, respectively. Vegetables such as spinach and lettuce leaves, mushrooms, radishes and onions, contained significant amounts of phytase ranging from 47 units/g in yellow onion bulbs to 153 units/g in whole green onions (Phillippy and Wyatt 2001). White wheat has been reported to exhibit 1.5 to 2.5 phytase activity units/g, whereas hard red wheat cultivars had much higher levels, ranging from approximately 2 to 5.5 phytase activity units/g (Okot-Kotber et al. 2003). Apples, oranges, guavas, and bananas have been found to exhibit little to no phytase activity (Phillippy and Wyatt 2001).

Fungi and bacteria are important sources of phytase relative to human and food animal diets. The most commonly used species for commercial production of phytase, as a feed additive or for food processing, are *Aspergillus niger*, *A. ficuum*, and *A. fumigatus* (Kumar et al. 2010). Among yeast phytases, *Saccharomyces cerevisiae* (commonly known as baker's yeast) is used for bread-making (Kumar et al. 2010). Food processing techniques such as soaking, malting, cooking, and fermentation are used to increase the activity of naturally present phytase enzymes in plants and microorganisms (Hotz and Gibson 2007; Kumar et al. 2010).

Phosphomannose Isomerase

Phosphomannose isomerase (PMI) is an enzyme involved in carbohydrate metabolism and it, or homologous enzymatic proteins, are expressed in various taxa including enteric bacteria, fungi, insects, some species of plants and nematodes, and mammals—including monkeys, mice, and humans (Proudfoot et al. 1994; de Lonlay and Seta 2009; Chiang and Kiang 2011; Hu et al. 2016). The PMI protein produced in PY203 corn is encoded by the native *manA* gene from *E. coli* strain K12 (Agrivida 2019). PMI catalyzes the inter-conversion of mannose-6-phosphate and fructose-6-phosphate, which is required for most glycosylation reactions— glycosylation being involved in various cellular processes (InterPro 2019).

In PY203 corn, the *manA* gene was used as a selectable marker in modification of this corn variety (Agrivida 2019). Plant cells lacking this enzyme are incapable of surviving on a mannose (sugar) based growth medium used to culture plant cells. Plant cells expressing the *manA* gene/PMI are capable of growth in a mannose medium, which utilize mannose as a carbon source.

[12] Phytase activity is expressed as phytase units or FTUs. One FTU is the activity of phytase required to liberate 1 µmol of inorganic phosphorus per minute at pH 5.5 from an excess of 15 M sodium phytate at 37°C.

PMI as a selectable marker has been used in development of other corn and rice crops, which have been evaluated for food use by the FDA. See FDA Biotechnology Consultations 113 and 128, and 158, respectively (US-FDA 2020a). As described in the following section, PMI, due to its safety to human and animal health, was issued an exemption from the requirement for a tolerance for foods in 2004.

4.3.4.2 Pesticides, Tolerance Limits for Foods, and Exemption from the Requirement for a Tolerance

The EPA regulates the sale, distribution, and use of pesticides under FIFRA (Section 1.3–Coordinated Framework). Before a pesticide may legally be used in the United States, the EPA must evaluate the pesticide to ensure that it will not result in an unreasonable risk to human health or the environment. Pesticides that complete this evaluation are issued a "registration" that permits their sale and use according to requirements set by the EPA.

Before a pesticide can be used on a food crop, the EPA, pursuant to the FFDCA and Food Quality Protection Act of 1996 (FQPA), also establishes tolerance limits, which is the amount of pesticide residue allowed to remain in or on each treated food commodity (21 U.S. Code § 346a - Tolerances and exemptions for pesticide chemical residues). Pesticide tolerance limits established by the EPA are to ensure the safety of foods and feed for human and animal consumption (US-EPA 2020p). If pesticide residues are found above the tolerance limit, the commodity will be subject to seizure by the government.

Section 408(c)(2)(A)(i) of the FFDCA allows the EPA to establish an exemption from the requirement for a tolerance if EPA determines that the exemption is “safe.” Safe is defined as meaning that there is a "reasonable certainty that no harm will result from aggregate exposure to the pesticide residue." To make a safety finding, the EPA considers, among other things: the toxicity of the pesticide and its break-down products, aggregate exposure to the pesticide in foods and from other sources of exposure, and any special risks posed to infants and children. Some pesticides are exempted from the requirement to have a tolerance.

In 2004, the EPA issued an exemption from the requirement of a tolerance for residues of phosphomannose isomerase (PMI), and the genetic material necessary for its production, in all plant commodities when applied/used as plant-incorporated protectant inert ingredients (See 40 CFR § 174.527 - Phosphomannose isomerase in all plants; exemption from the requirement of a tolerance). The *manA* gene and associated genetic regulatory sequences introduced into PY203 corn are identical to the genetic sequences for which the EPA granted an exemption for the requirement of a tolerance (Agrivida 2019).

Both the FDA and USDA monitor foods for pesticide residues to enforce these tolerance limits, and ensure protection of human health. By example, the USDA Pesticide Data Program (PDP) collects data on pesticides residues on agricultural commodities in the U.S. food supply, with an emphasis on those commodities consumed by infants and children (USDA-AMS 2019a). The Monitoring Programs Division administers PDP activities, including the sampling, testing, and reporting of pesticide residues on agricultural commodities in the U.S. food supply. The program is implemented through cooperation with state agriculture departments and other federal agencies. The EPA uses PDP data to prepare pesticide dietary exposure assessments pursuant to the FQPA. PDP data:

- enable the EPA to assess dietary exposure;

- facilitate the global marketing of U.S. agricultural products; and
- provide guidance for the FDA and other governmental agencies to make informed decisions.

The EPA also sets limits for potential drinking water contaminants that need to be regulated in order to protect public health (40 CFR part 141). These contaminant limits are required by the Safe Drinking Water Act (SDWA). The EPA works with States, Tribes, and many other partners to implement SDWA standards.

4.3.4.3 Worker Safety

Agriculture is one of the most hazardous industries in the United States. Worker hazards include those associated with pesticide application, and the operation of farm machinery. Agricultural operations are covered by several Occupational Safety and Health standards including Agriculture (29 CFR 1928), General Industry (29 CFR 1910), and the General Duty Clause. Further protections are provided through the National Institute of Occupational Safety and Health (NIOSH).

To address the potential hazards associated with exposure to pesticides during field application and handling, the EPA issued the Worker Protection Standard (WPS) (40 CFR Part 170) in 1992. The WPS contains requirements for pesticide safety training, notification of pesticide applications, personal protective equipment, restricted entry intervals following pesticide application, decontamination supplies, and emergency medical assistance. The Occupational Safety and Health Administration (OSHA) also requires employers to protect their employees from hazards associated with pesticides.

On November 2, 2015, EPA revised the WPS to decrease pesticide exposure incidents among agricultural workers, handlers, and their families (80 FR 211, November 2, 2015, p. 67495). The revised WPS requirements went into effect during 2017–2018. On November 1, 2019, the EPA proposed narrow updates to the WPS regulation to improve the agency's Application Exclusion Zone provisions (US-EPA 2020f).

4.3.4.4 Potential Effects on Human Health

It is unlikely that humans would have any dietary exposure to PY203 corn because its intended use is for animal feed. In the event of inadvertent human consumption of PY203 corn; phytase naturally occurs in cereals, legumes, oilseeds, and nuts, and commonly consumed by humans. There are no health hazards presented by inadvertent consumption of the Phy02 phytase in PY203 corn. The biological activity of phytases is limited to the breakdown—hydrolysis and dephosphorylation—of phytate. This results in bioavailable forms of inorganic phosphorus, and the bioavailability of essential minerals, such as calcium, iron and zinc (Bohn et al. 2008; Kumar et al. 2010; Lei et al. 2013). Thus, phytases facilitate dietary phosphorus, as well as essential minerals assimilation. Because phytate chelates essential minerals, such as iron, zinc, and calcium, its presence in diets can contribute to or aggravates deficiencies of these nutrients (Bohn et al. 2008).

Nyannor et al. (2007) tested the effects of high doses of Quantum® phytase (a commercial feed product) in swine demonstrating that feeding up to 49,500 FTU/kg feed was safe and effective. A No Observed Adverse Effect Level (NOAEL) of 462,000 FTU/kg body weight/day based on an acute toxicity study in rats, the equivalent of one ear of PY203 corn for small mammals, was reported for a similar phytase enzyme (Nov9X phytase) (Agrivida 2016). Larger mammals would be exposed to much less phytase on a

FTU/kg body weight basis, if inadvertent consumption of PY203 corn were to occur. If PY203 corn were consumed, the expressed Phy02 protein in PY203 corn would be broken down during digestion into constituent amino acids.

Agrivida is consulting with the FDA as to the food safety of PY203 corn (Agrivida 2019; US-FDA 2020a). Agrivida completed an Early Food Safety Evaluation (New Protein Consultations; NPC) for the Phy02 phytase protein (NPC 000015) on August 7, 2015. The FDA had no questions regarding Agrivida's conclusion that the potential inadvertent presence in the food supply of low levels of Phy02 protein would not raise food safety concerns (US-FDA 2020b). Agrivida submitted a Pre-market Biotechnology Notification to the FDA for PY203 corn in June, 2018 (BNF 000167; (Agrivida 2019)). On January 27, 2021, the FDA concluded consultation, stating that, based on the information Agrivida presented, the FDA had no further questions concerning the safety of human or animal food derived from PY203 corn (US-FDA 2021).

PMI is expressed in various taxa including enteric bacteria, fungi, insects, some species of plants and nematodes, and mammals—including monkeys, mice, and humans. The EPA has issued an exemption from the requirement of a tolerance for residues in or on plant commodities comprised of PMI (40 CFR § 174.527). The EPA arrived at this conclusion because, based on available scientific data, no toxicity to mammals has been observed for PMI as a plant-incorporated protectant inert ingredient (US-EPA 2004).

Phytase can be a cause of pneumonitis (noninfectious lung inflammation) in workers in the animal feed industry (van Heemst et al. 2009). It can present as an occupational allergen causing an immune response among exposed workers sensitive to this enzyme. Thus, certain protective measures may be required to prevent airborne occupational exposure at sites where phytase is handled, particularly during addition of enzyme preparations to animal feed (Doekes et al. 1999). Phytase would remain a component of ground PY203 corn meal, thus direct airborne respiratory exposure to the enzyme in meal dust would be less than that found in raw bacterial and fungal produced enzyme preparations/additives.

Any pesticides used with PY203 corn would need to comply with EPA requirements (Section 1.3– Coordinated Framework for the Regulation of Biotechnology).

4.3.5 Livestock Health and Welfare

The term livestock is defined in different ways, although for the purposes of this EA livestock means all domesticated animals reared in an agricultural setting to produce commodities such as meat (e.g., pork, poultry, fish), eggs, milk, leather, and wool. Horses, which provide labor, are also considered livestock in the United States.

Dent corn, the variety of PY203 corn subject of this EA, accounts for around 70% to 90% of total feed grain use on an annual basis, a primary feed source for beef and dairy cattle, poultry, and hogs. Animal feed derived from dent corn comes not only from the grain, but also from silage (the above-ground portions of the corn plant), stalk residues in fields that might be grazed, and residuals derived from corn refining and milling, such as corn gluten feed, corn gluten meal, corn germ meal, corn steep liquor, and amino acids.

4.3.5.1 Low-Phytate Crops: Corn

Development of low-phytate crop varieties for use as animal feed have been sought since the 1990s (Raboy 2002, 2007; Raboy 2009). The breeding protocols designed for reducing phytate content in corn rely on the isolation of low phytic acid (*lpa*) mutations that impair the biosynthesis of phytate in the seed (Borlini and Rovera 2019). Note that these are low-phytate, not low-phytase enzyme crops. To date, there are no low-phytate crops widely grown for the reasons discussed below.

Animal nutrition studies over the years confirmed that *lpa* crop plants can provide more bioavailable phosphorus and reduce the levels of phosphorus in animal waste (Raboy 2009). Moreover, *lpa* plants have been found to enhance iron, zinc, and calcium nutrition (assimilation) in animals (see review by (Raboy 2007)). Consequently, numerous *lpa* varieties of corn, wheat, rice, barley, soybean, and Arabidopsis have been developed since the 1990s (Raboy 2007). All of these *lpa* plants were developed using chemical mutagenesis and classical genetics (Raboy 2009). Despite the interest in the low-phytate trait, none of these *lpa* varieties are widely produced commercially (Raboy 2002; Raboy 2009; Cassani et al. 2012; Cowieson et al. 2016). The difficulty with low–phytate crop varieties developed through chemical mutagenesis is that systemic reductions of phytate in the plants has usually resulted in off-target effects on seed and plant performance, such as compromised germination, emergence, seed filling, and stress tolerance (Raboy 2007). In brief, the biosynthetic pathways involving phytate can also impact nutritional quality, germination and emergence, disease susceptibility, and signal transduction important to stress response. Targeted engineering of the low-phytate trait may be able to avoid some or all of these off-target effects, although this has not yet been achieved (Raboy et al. 2000; Borlini and Rovera 2019).

An alternative approach to creating *lpa* plant varieties is to engineer crops to express high levels of phytase in seeds, the enzyme that breaks down phytate (Raboy 2009), as with the corn variety subject of this EA (Agrivida 2019). This can avoid off-target effects associated with modified phytate biosynthesis, such as in germination, yield, and stress tolerance (Raboy 2007; Raboy 2009).

4.3.5.2 Phytase and Animal Feed

Due to issues with dietary phosphorus assimilation by non-ruminant livestock (to include poultry), phytase, as a feed additive, is a widely used, included in ~90% of poultry and ~70% of swine diets (DuPont 2019). Various commercial phytase products are available, such as Axtra® PHY, Ronozyme®, and Natuphos®. Phytase is commonly incorporated into commercial poultry, swine, and fish feed to improve the bioavailability of phytate based phosphorus, as well as minerals and amino acids. Most commercially available feed phytases are of fungal (*Aspergillus niger*) or bacterial (*Escherichia coli*) origin (Nielsen et al. 2013).

The FDA has evaluated several transgenic phytase enzymes for use in feed, which have been designated by the FDA as Generally Recognized as Safe (GRAS) for their intended uses (US-FDA 2020c). These include a phytase enzyme produced by an *Aspergillus oryzae* strain expressing a synthetic gene coding for a phytase from *Citrobacter braakii* (Animal Food GRAS Notice [AGRN] 14 and 15) (US-FDA 2020c). This *C. braakii* derived phytase is intended for use in poultry and swine diets (US-FDA 2020c).

4.3.5.3 Potential Impacts on Livestock Health and Welfare

Apart from the modified phytase and phytate levels, and expression of PMI, the nutrient composition of grain and forage derived from PY203 corn is equivalent to that of other corn varieties (Agrivida 2019;

ILSI-CERA 2020). Neither the increased phytase expression nor introduced PMI trait present any risk to livestock. Phytase is considered essential for enhancing the nutritional value of non-ruminant animal feed—namely for grains/legumes—so as to improve animal development and health (Nielsen et al. 2013; Humer and Zebeli 2015; Cowieson et al. 2016; Ingelmann et al. 2018). Consequently, it is the most widely used feed enzyme in the world, included in ~90% of poultry and ~70% of swine diets (DuPont 2019). As a feed additive, PY203 corn is expected to be of benefit to the rearing of non-ruminant livestock.

While the values for calcium, magnesium, manganese, phosphorus, potassium, and zinc from grain of PY203 corn were significantly greater than the corresponding values from grain of the negative control, the values for copper, iron, sodium, and selenium were not statistically different. For all minerals, the mean values for PY203 corn and the negative control were similar to each other and to the means for maize grain from the ILSI-CCDB database (ILSI-CERA 2020).

Field studies comparing the phenotypic properties of PY203 corn to conventionally bred comparator lines found statistically significant lower grain test weights for PY203 corn as compared to the control line. Lower grain test weights are often seen in kernels that have a greater abundance of non-vitreous, floury (or "opaque") endosperm. In corn, the floury or opaque phenotypes are associated with changes in the formation of protein storage bodies (Gerde et al. 2016). Although opaque or floury phenotypes are not preferred by dry-grind corn processors (fuel ethanol and Dried Distillers Grains (DDG)), this phenotype does provide nutritional benefits. Studies with opaque-2 corn have shown it to be nutritionally preferable to normal dent/field corn varieties (Mertz et al. 1965; Nelson 1966), for swine (Cromwell et al. 1967), chicks (Cromwell et al. 1968), and humans (Kies and Fox 1972; Graham et al. 1980).

GRAINZYME® phytase has been shown to be effective in improving growth performance and P digestibility in pigs (Blavi et al. 2019; Broomhead et al. 2019) and broiler chickens (Agrivida 2016). As previously mentioned, Agrivida submitted GRAS Notices AGRN 21 and AGRN 27 to the FDA in 2016 and 2018, respectively (US-FDA 2020c). The notified substance in both AGRN 21 and 27 was for ground grain obtained from PY203 corn. On May 23, 2017 and July 8, 2019, the FDA Center for Veterinary Medicine (CVM) issued response letters indicating that CVM had no questions regarding the Agrivida's conclusion that the ground corn grain containing Phy02 phytase derived from PY203 corn is GRAS under its intended conditions of use in poultry and swine feeds, respectively (US-FDA 2020c). Agrivida submitted to FDA a summary of its safety and nutritional assessment of PY203 corn on June 19, 2018. On January 27, 2021, the FDA concluded consultation, stating that, based on the information Agrivida presented, the FDA had no further questions concerning the safety of human or animal food derived from PY203 corn (US-FDA 2021).

4.3.6 Socioeconomics

As described in Agrivida's petition and summarized in this EA, the intended use of PY203 corn is for animal feed. Therefore, focus in this section is given to corn based feed commodities.

4.3.6.1 Domestic and International Markets

U.S. Feed Corn

Dent corn (PY203 corn variety) accounts for around 70% to 90% of total feed grain use on an annual basis. Sorghum (~ 2-3%), barley (~ 1%), and oats (~ 0.5%) account for the remainder of feed grains (USDA-ERS 2019e). Feed use is related to the number of animals that are fed corn (e.g., cattle, hogs, and poultry), which in turn derives from domestic and international populations, and demand for dietary meats. The amount of corn used for feed also depends on the crop's supply and price, the amount of supplemental ingredients used in feed rations, and the supplies and prices of competing feed ingredients (e.g., soybean, canola) (USDA-ERS 2020a).

Phytase Market

Commercial phytase—which is produced from bacterial and fungal sources—has food, feed, and pharmaceutical uses. Phytase has several values in the feed market, specifically; it improves the assimilation of dietary phosphorus and other trace elements, improves animal performance in livestock production (Acumen 2019), and can reduce environmental regulatory compliance costs and liabilities by reducing the amount of phosphorus excreted by animals, and thereby environmental introduction of phosphorus from animal rearing facilities (Acumen 2019).

The growth of the phytase market has mainly been driven by two factors: a need to replace inorganic phosphates in animal diets due to increasing costs, and concerns about the impact of animal production on the environment—specifically, the minimization of phosphorus laden waste (Jones 2013). Phytase use also affords the opportunity for food animal producers to switch to alternative energy and protein sources that have higher phytate levels, without compromising the overall nutritional value of the animal's diet (Jones 2013).

The 2017 global phytase market was estimated to be worth approximately $380 million (SBWire 2018). The average use rate of phytase across all diets for swine is approximately 70%; the poultry industry is showing closer to 90% adoption (Jones 2013). The sales volume of phytases increased from 114,235 metric tons (MT) in 2012 to 152, 622 MT in 2016, with an average growth rate of 5.96% (SBWire 2018). Valuation of the future global phytase market varies from $590 million by 2023 (SBWire 2018), to around $1 billion by 2025 (Acumen 2019), with projected compound annual growth rates of around 7.9% to 6.3%, respectively. As of 2018, the main phytase producers were Novozymes, DuPont (Danisco Animal Nutrition), AB Enzymes, DSM, and BASF, which collectively accounted for 40% of the market share (Acumen 2019).

Less reliance on inorganic phosphorus as a feed additive would also be expected to provide economic and environmental benefits. The majority of supplemented feed phosphate is derived from phosphate rock reserves that are a non-renewable resource, and becoming increasingly scarce and expensive (Cordell and White 2011). While estimates range from 30 to 300 years and are shrouded by lack of publicly available data, there is a general consensus that the quality and accessibility of remaining phosphate rock reserves are decreasing and costs will increase over time (Cordell and White 2011). This poses the challenge of securing future phosphorus supplies for national and international feed industries.

International Trade: Animal Feed

The United States provides over a third of the total supply of corn in the world market. Dent corn is the largest component of global coarse grain trade (corn, sorghum, barley, oats, rye, millet, and mixed grains), generally accounting for about two-thirds of the volume of grain trade over the past decade (USDA-ERS 2019e). In the 2018/2019 crop marketing year (Sept. 1- Aug. 31) the United States grew around 14.42 billion bushels (366 million metric tons) of corn. Roughly 14.3% of production, 52.3 million metric tons, was exported to more than 73 different countries (USGC 2020), at an estimated value of around $9.2 billion (USDA-ERS 2019b).

As the global demand for meat increases, so does the demand for livestock feed, and in turn, corn. Projected increase in U.S. corn exports over the next decade is largely due to a strong global demand for feed grains in support of meat production, particularly in those countries where climate and geography restrict local production of these feed materials (Westcott and Hansen 2015; USDA-ERS 2019c).

Specialty Corn

Specialty corn consists of a wide range of corn varieties that yield premium prices for growers and processors, and provide specific traits desired by domestic and international customers. These include a range of food grade (e.g., white corn, blue corn, certain yellow corns), and special feed grade grains (e.g., high lysine corn, high oil corn, waxy corn), that typically require special handling and identity preservation programs that span from the field to the end use markets (Lauer 1998; Dickerson 2003). The PY203 corn subject of this EA would be considered a specialty corn variety. There are no other phytase enhanced corn varieties currently on the market. Typically, these premium specialty corn varieties are grown by producers under contract with processor/trader handlers who have a direct buyer/seller source relationship with customers in special domestic markets, and overseas. Most commonly, such contracts and specialty corn varieties are produced under an identity preservation program (Elbehri 2007).

Identity Preservation

As crops and production systems have diversified to meet market demands, the need for segregation and preservation of agricultural commodity identity has increased. Farmers who grow specialty corn in the same general area need to communicate and plan with their neighbors growing different specialty corn to ensure that crop commodity identities are preserved and premiums can be realized. Identity preservation (IP) refers to a system of production, handling, and marketing practices that maintains the integrity and purity of agricultural commodities (Sundstrom et al. 2002). IP typically involves independent, third-party verification of the identification, segregation, and traceability of their product's unique, value-added characteristic (USDA-AMS 2019b). Verification is provided at every stage, including seed, production, processing, and distribution. Buyers are assured that the identity of the product is preserved from the requested stage of production. For example, Genetic ID, an Iowa based certification firm, specializes in ID-preserved food and feed products for producers, and food or feed manufacturers and retailers, that includes testing, validation, inspection, documentation and certification with a proprietary seal. They are used for specialty traits as well as conventionally bred or organic commodities (Egli et al. 2002).

Seed certification programs such as that used by the Association of Official Seed Certifying Agencies (AOSCA) play a major role in maintaining seed purity standards at levels established by the industry for national and international trade (Sundstrom et al. 2002; Elbehri 2007). Similarly, commodity traders, marketing organizations, and food processors have established purity and quality standards for specific end-product uses.

IP is important in international trade. The low level presence (LLP) or adventitious presence (AP) of transgenic trait material in internationally traded conventional or organic commodities are important considerations in the trade of corn. LLP refers to the unintended presence, at low levels, of biotechnology-derived crop material that is authorized for commercial use or sale in one or more countries, but not yet authorized in an importing country (CBD 2018). AP refers to instances when trace amounts of biotechnology-derived crop material that has not been approved for commercial use by any country is found in the commercial crop or food supply (CBD 2018).

Asynchronous approvals and zero tolerance policies can result in the diversion of trade by some exporters, and rejection or market withdrawals by importers of corn (FOEU 2014; Van Eenennaam and Young 2014; Frisvold 2015). Consequently, incidents of LLP or AP can lead to income loss for exporters and consequently for producers, and consumers in importing countries can potentially face higher domestic prices when an import is deterred or directed to another trading partner (Atici 2014).

In general, LLP/AP or compromise of corn commodity identity can cause disruptions in international trade when biotechnology-derived crop material is inadvertently incorporated into food or feed shipments. As such, countries producing biotechnology-derived crop varieties are required to take those measures necessary in the production, harvesting, transportation, storage, and post-harvest processing of biotechnology-derived crops to avoid the potential for LLP/AP in conventional or organic crop commodities.

4.3.6.2 Potential Socioeconomic Impacts

Domestic Economic Environment

PY203 corn kernels, enriched in phytase, will be ground into a coarse meal that will be sold for use in poultry and swine feed under the brand-name GRAINZYME® Phytase. PY203 corn may also be used as silage. Growers will produce and market PY203 corn under contract with Agrivida (Agrivida 2019).

Relative to food animal rearing, the economic impacts associated with the introduction of PY203 corn into commerce would be considered potentially beneficial. GRAINZYME® phytase could replace the need for the addition of microbial phytases and inorganic phosphorus to poultry and swine feed, and facilitate feed digestibility and animal performance, which could provide economic benefits for producers. Any reduction in such costs would be relative to the price of GRAINZYME® phytase, inorganic phosphorus, and microbial phytase additives.

Reductions in manure phosphorus—via microbial phytases or a product such as GRAINZYME® phytase—can also provide economic benefits in the way of reductions in environmental impacts. This derives from (1) reduce phosphorus in runoff from AFOs/CAFOs using phytase based feed, and (2) reduced phosphorus in runoff from crop producers using manure from these AFOs/CAFOs (US-EPA 2002). As discussed in 4.3.2.2–Water Resources, excess nutrients in runoff lead to eutrophication of surface waters that impair human uses and living resources as a result of harmful algal blooms and hypoxic/anoxic conditions. Eutrophication has a major economic impact, causing an estimated $2.2 billion per year in damages related to recreational water usage, fish kills, waterfront real estate, drinking water treatment, and recovery of threatened and endangered species (Dodds et al. 2009). Human uses impacted by impairment of surface waters include commercial and recreational fishing, shellfish harvesting, fish consumption (warnings), swimming, aesthetics, and tourism.

Many states also regulate phosphorus fertilizer use and discharges, specifically, namely manure use and management (Key et al. 2011). For example, Maryland requires the use of phytase in certain livestock feed (MDA 2019). Corn based transgenic phytase has been found to be comparable to microbial phytase additives in terms of phytate degradation (enzymatic activity) and, phosphorus digestibility (Gao et al. 2013). The manure derived from feed mixed with PY203 corn (GRAINZYME® phytase) may be valued by some crop producers—to reduce nutrients in runoff and associated risks to polluting nearby waterbodies.

During cultivation PY203 corn will remain the property of Agrivida, and after harvest the grower will deliver all of the grain to Agrivida for processing into a phytase feed additive product, GRAINZYME® phytase (Agrivida 2020). As a phytase enhanced grain GRAINZYME® is expected to be of higher value to poultry and swine producers than non-phytase grain. Agrivida states they do not intend to sell or deliver grain from PY203 corn for other uses, such as human food uses or dry milling (Agrivida 2019). In the event that Agrivida markets GRAINZYME® for use in dairy or beef cattle, it may sell a limited amount of PY203 corn to dairy or beef producers to enable them to plant and cultivate PY203 corn for the sole purpose of producing phytase-containing corn silage (Agrivida 2019). The cultivation of PY203 corn for this purpose will not be any different than the cultivation of other corn varieties for the production of silage (Agrivida 2019). In this case, dairy or beef producers will be required to agree that plant material derived from PY203 corn will only be used to produce feed for cattle and that grain will not be saved or used for any other purpose (Agrivida 2019).

PY203 corn would entail entry of another biotechnology-derived corn variety into the agricultural seed and grains markets. While PY203 corn would require segregation from other specialty and IP corn commodities in the supply chain, this would not be considered an event that presented unusual or unique risks in an additive sense. New varieties of corn and specialty commodities are expected to be continually developed and marketed to help crop producers meet demands for food, feed, and fuel commodities. Thus, entry of PY203 corn into the domestic market would require segregation (e.g., prevention of commingling, LLP) no differently than other biotech/specialty/IP corn varieties that have and will enter the market. This type of impact is not considered adverse in nature, rather, segregation and channeling of harvested grain to various supply chains is inherent to the corn commodities markets. Identity preservation certification programs are well developed and an intrinsic aspect of crop production in the United States, to include for biotechnology-derived crops (Sundstrom et al. 2002). PY203 corn and GRAINZYME® phytase will be produced using an identity preservation program (Agrivida 2016).

Trade Economic Environment

To the extent that PY203 corn (GRAINZYME® phytase) emerges as a valued commodity that facilitates food animal rearing, to include aquaculture, it may prove competitive among other phytase products in global markets.

As with all biotechnology-derived crop commodities, there exists the potential for LLP or AP occurring in countries importing U.S. agricultural commodities. The issue of asynchronous approvals (AA), and resulting LLP situations, and occurrence of AP, can lead to trade delays, shipment rejection, and costs to traders (FAO 2014). Countries producing biotechnology-derived crops are required to take the measures necessary in the production, harvesting, transportation, storage, and marketing of biotechnology-derived crop commodities to avoid LLP/AP. International trade is facilitated by the World Trade Organization (WTO) and the Organization for Economic Cooperation and Development (OECD) (FAO 2019; OECD 2019). Standards and guidelines for the safety evaluation and trade of biotechnology-derived crop commodities are established under international policy and agreements such as the Codex Alimentarius (FAO 2009), the WTO International Plant Protection Convention (FAO 2019), WTO Sanitary and Phytosanitary Measures (WTO 2020), WTO Technical Barriers to Trade Agreement (WTO 2019), and the Cartagena Protocol on Biosafety (CBD 2019b).

PY203 corn would be subject to the same international requirements, discussed above, as currently traded corn commodities. In general, developers have various legal, quality control, and marketing incentives to implement rigorous stewardship measures to ensure IP and/or stewardship of the crop commodity, prevent commingling, and avoid AA and LLP. By necessity, all international, and industry standards and requirements must be met before marketing of PY203 corn commodities in other countries. It is assumed that there will be strict adherence to stewardship and CODEX requirements to maintain the integrity of PY203 corn commodities so as to reduce legal exposure, and loss of standing in the market. As discussed above for domestic markets, PY203 corn production and processing will be conducted under contract with Agrivida (Agrivida 2019).

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

4.3.7.1 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 Appendix 1. Compliance with the requirements of NEPA, CWA, SDWA, CAA, and NHPA, are specifically addressed in the following subsections.

4.3.7.1.1 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 EA documents the potential environmental outcomes of the alternatives considered, approval or denial of Agrivida's petition, consistent with the requirements of NEPA and Council on Environmental Quality implementing regulations at 40 CFR parts 1500-1508.

4.3.7.1.2 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. Because PY203 corn is agronomically equivalent to currently cultivated corn varieties, the potential sources of impacts on water resources and air quality are the same under both the No Action and

Preferred Alternatives. PY203 corn production would entail the use of pesticides and fertilizers, and to some extent tillage, which will contribute to potential cumulative impacts on air quality, and potentially water quality. The sources and degree of potential impacts would be no different than that which occurs with current corn production. As discussed in Chapter 4, the transgenes and gene products extant in PY203 corn present no known risks to water or air quality. It is possible that PY203 corn could contribute to reductions in phosphorus runoff from AFOs/CAFOs and cropping systems utilizing manure from PY203 corn fed animals. APHIS assumes use of all pesticides on PY203 corn will be compliant with EPA registration and label requirements. Considering these factors, approval of the petition would not lead to circumstances that resulted in non-compliance with the requirements of the CWA, CAA, and SDWA.

4.3.7.2 Executive Orders

The following executive orders (EO) require consideration of the potential impacts of federal actions on human health, cultural resources, wildlife, and the environment.

- **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.

- **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 policy or actions have potential tribal implications.

Neither alternative evaluated in this EA is expected to have disproportionate adverse impacts on minorities, low-income populations, or children, or adversely affect tribal entities. As reviewed in Chapter 4, it is highly improbable the trait genes and gene products in PY203 corn present any risks to human health, nor to animal health and welfare. PY203 corn would be cultivated as are all other corn varieties, using the same agronomic practices and inputs.

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. Neither approval nor denial of the petition would have any effect on Indian tribal self-governance or sovereignty, tribal treaties, or other rights.

- **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 actions to continue coordinated federal prevention and control efforts related to invasive species. This order maintains the National Invasive Species Council (Council) and the Invasive Species Advisory Committee; expands the membership of the Council; clarifies the operations of the Council; incorporates considerations of human and environmental health, climate change, technological innovation, and other emerging priorities into federal efforts to address invasive species; and strengthens coordinated, cost-efficient federal action.

One concern with the cultivation of certain transgenic crops is their potential dispersal, persistence, and spread into non-agricultural areas. Domestication of *Zea mays* has rendered this cultivar less capable of survival in areas outside of cultivation, it is largely dependent on humans for persistence in the environment (OECD 2003). Due the size and weight of the seed, it is not easily dispersed by wind, water, or wildlife (Mallory-Smith and Zapiola 2008; Mallory-Smith and Sanchez Olguin 2011). Corn seed also lacks dormancy, which limits is ability to persist in soil seed bank. Because of these factors, field corn does not easily establish, naturalize, and spread. Thus, field corn (*Zea mays*) is not typically found outside areas of cultivation.

As part of its PPRA, APHIS evaluated the potential weediness and invasiveness of PY203 corn and concluded that it is unlikely that PY203 corn will become weedy or invasive in areas where it is grown (USDA-APHIS 2020b). As discussed in Subsections 3.3.4 and 4.3.3.4, Gene Flow and Weediness, there are a few populations of closely related species of *Tripsacum* within the U.S.; however, the potential for a weedy or invasive species of corn to develop as a result of outcrossing of PY203 corn with other sexually compatible species of corn, or wild *Tripsacum* species, is considered remote. Hybridization and successful introgression of *Z. mays* genes into *Tripsacum* populations is rare (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995). Special techniques are required to hybridize *Z. mays* and *Tripsacum*; hybrids of *Tripsacum* species with *Zea* species do not commonly occur outside of a laboratory. Offspring are often sterile or have reduced fertility, and are unable to withstand even mild winter conditions (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995). There are no known wild hybrids comprised of *Zea mays* and *Tripsacum* species in the United States.

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

  The United States has recognized the critical importance of migratory birds as a shared resource by ratifying international, bilateral conventions for the conservation of migratory birds. These conventions impose substantive obligations on the United States for the conservation of migratory birds and their habitats. Through the Migratory Bird Treaty Act (Act) the United States has implemented these conventions with respect to the United States. This Executive Order directs executive departments and agencies to take certain actions to further implement the Act.

Migratory birds may transit corn fields and forage on corn, namely residual corn kernels left in the field post-harvest (Sherfy et al. 2011). For example, during migration, about 90% of the sandhill crane diet consists of corn, when corn is available (NGP 2020). As reviewed in this EA, it is unlikely the trait genes and their protein products present any risks to the health of migratory birds. Phytase enzymes occur naturally in the seeds of higher plants, such as cereals, legumes, oilseeds, and nuts, on which birds may forage. Apart from the modified levels of phytase, phytate, and phosphorus, and PMI trait, PY203 is compositionally and nutritionally equivalent to non-modified corn comparators. Phytase is a common additive to poultry diets to improve phosphorus assimilation and animal development/health. There are no known risk to birds via dietary consumption of phytase. Rather, PY203 corn would likely provide a food source for some species of migratory birds.

4.3.7.3 State and Local Requirements

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 regulated organisms 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 biotechnology-derived organisms 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 modified organisms 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 biotechnology-derived organisms within state boundaries.

Neither of the alternatives considered would affect APHIS partnerships with states in the oversight of biotechnology-derived organisms, to include the production of PY203 corn. 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.

4.3.8 Conclusions: Potential Impacts on the Human Environment

As discussed in the Scope of Analysis for this EA (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. APHIS has not identified any significant impacts on the human environmental that would derive from approval or denial of the petition.

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

Commercial crop production of any type, whether a conventional, organic, or biotechnology-based cropping system always has some degree of impact on the environment, as discussed in this EA. The potential introduction of pesticides and fertilizers to surface water or groundwater, soil erosion, emission of air pollutants, and alteration of wildlife habitats—to include aquatic ecosystems—are all impacts that can derive from commercial crop production. These are issues that all farmers, not just those growing biotechnology-derived crops, work with in providing food, feed, fuel, fiber, and industrial products to meet societal needs. The degree of environmental impacts can be minor or noticeably adverse, depending on a variety of factors that include the type and quantity of agronomic inputs and practices employed, geography and proximity of surface waters to crops, local biota, weather, prevalence and diversity of insect pests and weeds, and crop type being produced. With around 360,000 corn farms comprising some 90 million acres of the land in the United States (USDA-NASS 2020a), the scale of potential impacts requires integration of crop production with sustainability and conservation practices—for both biotechnology-derived and conventionally bred crops. While implementing such practices can often result in significant mitigation of environmental impacts, not all impacts can be fully attenuated, and some degree environmental trade-offs in meeting the market demand for corn-based food, feed, fuel, and industrial products are inevitable (Robertson and Swinton 2005).

On approval of the petition, and subsequent grower adoption of PY203 corn, the agronomic practices and inputs that would be used in the cultivation of PY203 corn, and any contribution of these practices and inputs to adverse effects on soils, water quality, or air quality, is expected to be similar to that of other corn crops currently cultivated. To the extent that GRAINZYME® phytase is adopted for use in animal feed, and that manure derived from GRAINZYME® based feeds are utilized for fertilization of cropland, these uses collectively could contribute, to some degree, to overall reductions in phosphorus runoff from agricultural facilities in the United States.

There are various federal, state, and private sector collaborative initiatives to support sustainable agricultural practices and help alleviate the collective impacts of crop production on the physical environment, as well as biological resources—these are described below 4.3.8.9.

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

Long-term agricultural productivity depends on the sustainable use of natural resources—namely topsoils, groundwater, populations of beneficial insects such as pollinators and plant pest predators, and the plants that support beneficial insects. PY203 corn is agronomically equivalent to other dent corn cultivars and utilizes the same types, and same/similar quantities of resources (e.g., groundwater, agronomic inputs), as all other conventional and biotechnology-derived dent corn varieties. The annual production of PY203 corn would face the same challenges in sustaining air and water quality, and top-soils and soil quality as other corn crops. Any groundwater use is expected to be similar to that of other dent corn varieties—there is no indication this variety utilizes more or less water during development.

4.3.8.3 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 involve those where the resources cannot be restored or returned to their original condition. Irreversible commitments entail the loss of future options and applies to the use of resources such as nonrenewable fossil fuels, and resources that are renewable only over long time spans. Irretrievable is a term that refers to those resources that, once used or consumed, would cause the resource to be unavailable for use by others and future generations (e.g., land use).

Corn production involves the irreversible consumption of nonrenewable petroleum based products (e.g., fuels necessary to operate equipment, cleaning agents, pesticide additives/adjuvants). Crude oil cannot be replaced once utilized for energy or other purposes. Some crop production systems may utilize wind or solar energy sources—renewable sources. Topsoil is also considered nonrenewable, its erosional capacity can be affected by the types of tillage and irrigation systems employed on cropland. Over the long-term continued crop production on the same site can contribute to wind and sheet rill erosion. Materials such as aluminum, steel, wood, and plastics would be consumed as part of the process of crop production. Most of these materials are non-renewable and could be irreversibly utilized if not recycled (plastics, metals). Crop production inherently entails the irretrievable removal of natural habitat and associated wildlife from the landscape.

Renewable and nonrenewable resources utilized for PY203 corn production would differ little from that of other dent corn varieties. Any irreversible or irretrievable commitments of resources in PY203 corn production would be the same as or very similar to that of other dent corn cropping systems. It is expected that PY203 corn would be produced on lands already converted and utilized for commercial crop production. Subtle variations in fossil fuel and energy use would occur relative to the frequency and duration of pesticide and fertilizer applications with this crop, and harvesting and facilities efficiencies, relative to other dent corn crops.

4.3.8.4 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 4.3.8, 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. The EPA will regulate the use of pesticides on PY203 corn. Agrivida completed an Early Food Safety Evaluation for PY203 corn with the FDA (NPC 000015) on August 7, 2015 (US-FDA 2020a). A Pre-market Biotechnology Notification consultation for PY203 corn was submitted to the FDA in June, 2018 (BNF 000167) (Agrivida 2019).

4.3.8.5 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 commercial production of PY203 corn, 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 (BLM), Fish and Wildlife Service (FWS), National Park Service (NPS) in the Department of the Interior (DOI), and the Forest Service (FS) in the USDA. A fifth agency, the Department of Defense (DoD), 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 cultivation of PY203 corn on federal lands would require approval by a federal land management agency.

Tribal Nations, State and Local Land Use Plans and Policies

As discussed in Section 4.3.7–Compliance with Federal and State Laws and Regulations, Executive Orders, Policies, and Treaties , approval nor denial of the petition would have any effect on Indian tribal self-governance or sovereignty, tribal treaties, or other rights, nor affect state or local authority in the oversight of organisms developed using genetic engineering, to include the production of PY203 corn on state or county lands. APHIS conducted outreach to tribal nations informing tribes of Agrivida's petition. APHIS received one reply from the Sac & Fox Tribe of the Mississippi in Iowa, stating they had no comments for APHIS on Agrivida's petition request.

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

The energy requirements involved with the full life cycle of PY203 corn production and marketing would differ little from that of other commercial corn crops. USDA-NRCS provides guidance on energy management in crop production via practices such as integrated pest management, precision agriculture, irrigation water and nutrient management, and crop residue management (USDA-NRCS 2020f). Energy conservation estimation tools are also provided to help growers estimate costs and saving associated with irrigation, nitrogen use, and tillage.

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

There are no depletable resource requirements unique to the production and marketing of PY203 corn. Use of natural resources (e.g., irrigation water, soils, fertilizers) would be no different than that of other corn varieties. Natural resource conservation opportunities, whether USDA funded or otherwise implemented by growers or/and state agencies would not differ from that of other conventional and biotechnology-derived corn crops. Available mitigation measures to curtail potential environmental impacts, such as those summarized below in 4.3.8.9, would likewise not differ.

4.3.8.8 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.

PY203 corn production may occur 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. Where PY203 corn was cultivated there may be the potential for increased noise during the operation of machinery and other equipment, as with all corn crop production, however, these activities would have only temporary effects on historic sites in proximity to PY203 corn fields, with no consistent long-term effects on the enjoyment of a historical or cultural resources.

PY203 corn, as other corn production, would occur on lands allocated or zoned for agricultural uses. Considering the areas in which corn is grown in the United States, it is unlikely that urban environments would be affected by PY203 corn production. The design of the built environment in relation to crop production activities would be resolved at the local and state levels of governance (e.g., city, county, and/or state departments governing land use).

4.3.8.9 Means to mitigate adverse environmental impacts.

There are a number of federal, state, and private sector collaborative initiatives to help farmers alleviate the collective impacts of crop production on the physical environment, as well as biological resources. Some of the USDA and partner programs supporting agricultural sustainability and natural resources conservation are summarized below. Practices will vary from region to region and farm to farm, however, some common sets of practices have emerged, which include integrated insect pest and weed management, soil conservation tactics, water resources conservation and protection, cropland biodiversity, and nutrient management. Each contribute in some way to environmental stewardship, long-term farm sustainability, and improved quality of life. For a more detailed description of USDA sustainability and conservation initiatives, see the USDA websites provided in the references below.

The EPA Mississippi River/Gulf of Mexico Hypoxia Task Force (US-EPA 2020d) and USDA Natural Resources Conservation Service (NRCS) National Water Quality Initiative (NWQI) (USDA-NRCS 2020e) aim to reduce NPS contaminants in agricultural run-off, and run-off itself. The purpose of the NWQI, in collaboration with the EPA and state water quality agencies, is to reduce nonpoint sources of nutrients, sediment, and pathogens related to agriculture in high-priority watersheds in each state.

The USDA funded Sustainable Agriculture Research and Education Program (SARE) supports sustainable agricultural practices that are intended to protect the environment, conserve natural resources, and promote cropland biodiversity (USDA-NIFA 2020).

The USDA-NRCS's Natural Resources Conservation Programs help people reduce soil erosion, enhance water supplies, improve water quality, increase wildlife habitat, and reduce damages caused by floods and other natural disasters (USDA-NRCS 2019a).

The USDA-NRCS Environmental Quality Incentives Program (EQIP) provides financial and technical assistance to agricultural producers to address natural resource concerns and deliver environmental benefits such as improved water and air quality, conserved ground and surface water, increased soil health and reduced soil erosion and sedimentation, improved or created wildlife habitat, and mitigation against increasing weather volatility (USDA-NRCS 2020b).

The USDA–NRCS Regional Conservation Partnership Program (RCPP) specifically promotes coordination of NRCS conservation activities with partners that offer value-added contributions to expand USDA's collective ability to address on-farm, watershed, and regional natural resource concerns (USDA-

NRCS 2020a). The 2018 Farm Bill made a number of substantial changes to RCPP: RCPP is now a standalone program with its own funding of $300 million annually.

The USDA National Institute of Food and Agriculture (NIFA) promotes sustainable agriculture through national program leadership and funding for research and extension. It offers competitive grants programs and a professional development program, and it collaborates with other federal agencies through the USDA Sustainable Development Council (USDA-NIFA 2020).

The USDA Conservation Reserve Program (CRP) is a voluntary land retirement program that provides financial compensation to landowners to remove highly erodible and environmentally sensitive land from agricultural production and install resource-conserving practices or preserve wildlife habitat. CRP is the largest federally administered private-land retirement program, with annual outlays approaching $2 billion per fiscal year. CRP enrollment is capped each year, and under the 2014 farm bill, enrollment was limited to no more than 24 million acres during fiscal years 2017 and 2018. The 2018 farm bill expanded CRP acreage to a maximum of 27 million acres by 2023. Nearly 24 million acres are enrolled in CRP as of 2019 (NSAC 2020).

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

Economic considerations have been evaluated in Section 4.3.6–Socioeconomics. Relative to food animal rearing, the economic impacts associated with the introduction of PY203 corn into commerce would be potentially beneficial.

4.3.8.11 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.

Based on APHIS' evaluation provided in Appendix 1 of this EA, a determination of nonregulated status of PY203 corn, and subsequent commercial production of this corn variety, would have no effect on listed species or species proposed for listing, and would not affect designated habitat or habitat proposed for designation.

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

Approval of the petition and subsequent availability of PY203 to commercial markets would not present any risks to public health or worker safety. As reviewed in Section 4.3.4, it is unlikely that humans would have any dietary exposure to PY203 corn because its intended use is for animal feed. In the event of inadvertent human consumption of PY203 corn; phytase naturally occurs in cereals, legumes, oilseeds, and nuts, and commonly consumed by humans. As discussed in the previous sections of this chapter, there are no health hazards presented by inadvertent consumption of the Phy02 phytase in PY203 corn. As reviewed in Section 4.3.5, Agrivida is consulting with the FDA as to the safety of food and feed derived from PY203 corn (Agrivida 2019; US-FDA 2020a).

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

Approval of the petition would provide for the commercial production of PY203 corn, subject to any FDA consultation, and EPA and state requirements. As of October, 2020, APHIS has issued determinations of nonregulated status in response to 38 petitions for biotechnology-derived corn varieties, all but one of these insect and/or herbicide resistant. APHIS maintains a publicly available list of petitions and determinations of nonregulated status on its website (USDA-APHIS 2020c). Seeds 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. Currently, over 90% of U.S. corn, upland cotton, and soybeans are produced using transgenic varieties.

Farmers generally adopt a biotechnology-derived crop 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).

Advances in biotechnologies are expected to refine the precision with which crop varieties will be developed, and lead to a greater diversity of commercial crop varieties (NAS 2016). While it is difficult to predict the scope of improved crop varieties that will emerge in the coming decades, beneficial traits likely to be utilized and adopted by growers include improved tolerance to abiotic stresses such as drought and temperature extremes; increased efficiency in plant physiological processes such as photosynthesis and nitrogen use; resistance to fungal, bacterial, and viral diseases; and new types of herbicide resistance (NAS 2016).

For those biotechnology-derived plants that APHIS has determined are not subject to 7 CFR part 340, which were evaluated for potential plant pest risks, and potential environmental impacts via NEPA analyses: The available science provides little evidence that the cultivation of the presently commercialized biotechnology-derived corn plants have resulted in environmental impacts that are unique or differ from conventional crops and cropping systems (e.g., (Sanvido et al. 2007; NRC 2010; Klümper and Qaim 2014; NAS 2016) and others). Generally, to date, biotechnology-derived crops have been found to have no more or fewer adverse effects on the environment than conventionally bred crops (NRC 2010; NAS 2016).

APPENDIX 1: THREATENED AND ENDANGERED SPECIES

The Endangered Species Act (ESA) of 1973, as amended, is a far-reaching wildlife conservation law. Congress passed the ESA to prevent extinctions facing many species of plants and animals. 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. The U.S. Fish & Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS) together comprise "the Services" and implement the ESA by working with 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, it must be added to the federal list of threatened and endangered wildlife and plants. Threatened and endangered species (TES) are those plants and animals at risk of becoming extinct throughout all or part of their geographic ranges (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 the species to be endangered or threatened because of any of the following factors:

- 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; and
- The natural or manmade factors affecting its survival.

Once a species 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.

1 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 (a process is known as a Section 7 Consultation).

To facilitate the development of its 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 for biotechnology-derived crop lines. By working with USFWS, APHIS developed a process for conducting an effects determination consistent with the Plant Protection Act (PPA) of 2000 (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.

APHIS regulatory authority under the PPA is limited to those organisms that could pose a plant pest risk, or where APHIS does not have sufficient information to determine that the organism is unlikely to pose a plant pest risk. In this case, Agrivida has requested that APHIS consider that PY203 corn is not a plant pest as defined by the PPA. After completing a PPRA, if APHIS determines that PY203 corn seeds, plants, or parts thereof do not pose a plant pest risk, then PY203 corn 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 PY203 corn is not subject regulation. As part of this EA, APHIS analyzed the potential effects of PY203 corn on TES and critical habitat. APHIS thoroughly reviewed data related to PY203 corn to inform the ESA effects analysis. For each transgene 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); and
- Analysis to determine if the transgenic plant is sexually compatible with any threatened or endangered plant species (TES) or a host of any TES.
- 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 TES that may occur from use of pesticides associated with biotechnology-derived crops. 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 biotechnology-derived crops because the 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 corn growers. Under APHIS' 7 CFR part 340 regulations, APHIS only has the authority to regulate PY203 corn or any other biotechnology-derived organism as long as APHIS believes they may pose a plant pest risk. APHIS has no regulatory jurisdiction over any other risks associated with biotechnology-derived organisms including risks resulting from the use of pesticides on biotechnology-derived crop plants.

Relative to pesticide use with PY203 corn, the EPA issues Endangered Species Protection Bulletins as part of the EPA's Endangered Species Protection Program, and pesticide use requirements. Bulletins set forth geographically specific pesticide use limitations for the protection of threatened and endangered (listed) species and their designated critical habitat. These Bulletins contain enforceable pesticide use limitations that are necessary to ensure a pesticide's use will not harm a species listed as threatened or endangered under the Endangered Species Act, or their designated critical habitat (US-EPA 2020m).

2 Potential Effects of PY203 Corn on TES

APHIS evaluated the potential effects that a determination of nonregulated status for PY203 corn may have, if any, on federally listed TES and species proposed for listing, as well as designated critical habitat and habitat proposed for designation. Based on the information submitted by the applicant and reviewed by APHIS, PY203 corn, with the exception of the modified phytase, phytate, and phosphorus expression, and PMI trait, is agronomically and compositionally comparable to conventional corn (Agrivida 2019; ILSI-CERA 2020). The common agricultural practices that would be carried out in the cultivation of PY203 corn are not expected to deviate from current practices, including the use of EPA-registered pesticides. PY203 corn is not expected to directly cause a substantive change in agricultural acreage or area devoted to corn production in the United States (see Section 4.3.1–U.S. Corn Production).

Corn can be grown in all 50 states and U.S. territories. The issues discussed herein focus on the potential consequences of approving the petition for nonregulated status of PY203 corn on TES and critical habitat in the areas where corn is currently cultivated. APHIS obtained and reviewed the USFWS list of TES species (listed and proposed) for all 50 states and U.S. territories where corn is produced from the USFWS Environmental Conservation Online System (USFWS 2020).

For its analysis on TES plants and critical habitat, APHIS focused on the agronomic differences between PY203 corn and corn varieties currently grown; the potential for increased weediness; and the potential for gene movement to native plants, listed species, and species proposed for listing.

For its analysis of potential effects on TES animals, APHIS focused on the implications of exposure to the phytase enzyme and PMI expressed in PY203 corn as a result of the transformation, and the ability of the plants to serve as a host for a TES.

2.1 Threatened and Endangered Plant Species and Critical Habitat

Agronomic data provided by Agrivida were used in the APHIS analysis of the weediness potential for PY203 corn, and evaluated for the potential to impact TES and critical habitat (Agrivida 2019). Two of the agronomic parameters that were assessed in the agronomic comparison study that would be expected to affect weediness potential were emergent stand count (the number of plants in a row 14 days after planting) and final stand count (the number of plants in a row at maturity). In Agrivida's study PY203 corn had similar, but statistically significant lower, emergent and final stand counts compared to the conventional control. Neither of these phenotypic differences would increase the weediness potential of PY203 corn variety. As discussed in APHIS' PPRA (USDA-APHIS 2020b) there are no weedy characteristics associated with PY203 corn (e.g., increased hardiness, rapid growth, stress tolerance, pest/disease resistance). Volunteer corn plants can be easily controlled if needed, either with herbicides or manual removal.

APHIS evaluated the potential of PY203 corn to cross with a listed species. The closest relative of *Zea* in the United States is the genus *Tripsacum*. Three species of *Tripsacum* have been identified: *T. dactyloides*, Eastern gamagrass, is known to occur in the eastern half of the United States, *T. lanceolatum*, Mexican gamagrass, occurs in the southwest of the United States, and *T. floridanum*, Florida gamagrass, is native to South Florida and Cuba. *T. dactyloides* is the only *Tripsacum* species of widespread occurrence and agricultural importance in the United States, and commonly is grown as a forage grass. There are no federally listed *Zea* or *Tripsacum* species in the United States (USFWS 2020). As discussed in Subsection 4.3.3.4–Gene Flow and Weediness of Corn, gene flow from *Zea mays* to *Tripsacum* species in the United States is improbable. Teosinte (wild *Zea* taxa) do not appear to be present in the United States other than in botanical gardens or at research stations (see Subsection 4.3.3.4).

Based on all of these factors, APHIS determined that PY203 corn will have no effect on threatened or endangered plant species or on critical habitat in the United States.

2.2 Threatened and Endangered Animal Species

Threatened and endangered animal species that may be exposed to the gene products from PY203 corn would be those TES that inhabited or transited corn fields and fed on PY203 corn. As discussed in Section 4.3.3–Biological Resources, cornfields are generally considered poor habitat for birds and mammals in comparison with uncultivated lands, but the use of cornfields by birds and mammals is not uncommon. Some birds and mammals use cornfields at various times throughout the corn production cycle for feeding and reproduction. Most birds and mammals that utilize cornfields are ground foraging omnivores that feed on corn seed, sprouting corn, and the corn remaining in the fields following harvest.

For TES birds, whooping crane (*Grus americana*), Mississippi sandhill crane (*Grus canadensis pulla*), piping plover (*Charadrius melodus*), and interior least tern (*Sterna antillarum*) may transit and feed in corn fields during migration (Krapu et al. 2004; Sherfy et al. 2011; USFWS 2011). The whooping crane, in particular, spends the majority of its foraging time during migration in agricultural fields (CWS-USFWS 2007; Jorgensen and Dinan 2016). During migration, about 90% of the sandhill crane diet consists of corn, when corn is available (NGP 2020).

As discussed in Section 4.3.3.2–Animal Communities, many mammals may feed on corn, particularly white tailed deer, raccoons, mice, and voles. There are no listed raccoon species in the United States. There are two listed deer species in the United States. Key deer (*Odocoileus virginianus clavium*) are highly localized in the Florida Keys (USFWS 1999). Listed populations of Columbian white-tailed deer (*Odocoileus virginianus leucurus*) are found in certain areas associated with the Columbia River in Washington (USFWS 2020). These locations are well south and west, respectively, of the regions where corn crops are typically planted (see Section 4.3.1.1– Acreage and Area of U.S. Corn Production). Of the mice, voles, and their relatives in the Cricetidae family, listed species include: the Amargosa vole (*Microtus californicus scirpensis*), which is listed as endangered and occurs in California (USFWS 2020), as well as the Florida salt marsh vole (*Microtus pennsylvanicus dukecampbelli*), which occurs in salt marsh habitat on the Gulf Coast of Florida (USFWS 2020), the endangered Key Largo woodrat (*Neotoma floridana smalli*) of Florida Key's climax hardwood hammocks (USFWS 2020), and the northern and southern subspecies of the endangered, tidal marsh dwelling, salt marsh harvest mouse (*Reithrodontomys raviventris*) (USFWS 2013, 2020).

APHIS considered the risks to threatened and endangered animals from consuming PY203 corn. Agrivida has presented information on the food and feed safety of PY203 corn, comparing the PY203 corn variety with conventional varieties currently grown. There are no toxins or allergens associated with this plant (Agrivida 2019). Compositionally, grain samples were analyzed for proximates, amino acids, fatty acids, minerals, vitamins, and other bioactive metabolites (phytic acid, trypsin inhibitor, p-coumaric acid, raffinose, and ferulic acid). Where statistically significant differences in some of the analytes were detected between PY203 corn and the near isogenic nontransgenic control (e.g., fatty acids, vitamins, and minerals), the analyte values for PY203 corn were within the range of normal values for conventional corn varieties published in the International Life Sciences Institute (ILSI) crop composition database (ILSI-CERA 2020). As discussed in Chapter 4, Agrivida completed an Early Food Safety Evaluation

(New Protein Consultations; NPC) for the Phy02 phytase protein (NPC 000015) on August 7, 2015 (US-FDA 2020b), and submitted an Animal Food GRAS Notice (AGRN21) in May 2016. The FDA stated they had no questions regarding Agrivida's conclusion that grain obtained from PY203 corn is GRAS under its intended use, for animal feed (US-FDA 2020c).

APHIS considered the possibility that PY203 corn could serve as a host plant for a threatened or endangered species (i.e., a listed insect or other organism that may use the corn plant to complete its lifecycle). A review of the species list reveals that there are no members of the genus *Zea* that serve as a host plant for any threatened or endangered species (USFWS 2020).

Considering there are no risk to humans or other animals associated with PY203 corn (discussed in Chapter 4), and the nutritional similarity between PY203 corn to other varieties currently grown, APHIS has concluded that consumption of PY203 corn would have no effect on threatened or endangered animal species.

3 Summary

After reviewing the possible effects of a determination of nonregulated status, and subsequent commercial production of PY203 corn, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. APHIS also considered the potential effect of a determination of nonregulated status of PY203 corn on designated critical habitat and habitat proposed for designation, and could identify no risks to critical habitats. Corn is not considered a particularly competitive plant species and has been selected for domestication and cultivation under conditions not normally found in natural settings. Corn is not sexually compatible with, nor serves as a host species for, any listed species or species proposed for listing. Consumption of PY203 corn by any listed species or species proposed for listing would pose no health risks.

Based on all of these factors, APHIS has concluded that a determination of nonregulated status of PY203 corn, and subsequent commercial production of this corn variety, 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 are not required.

APPENDIX 2: LIST OF PREPARERS

USDA-APHIS	
Name, Title, Project Function	**Education and Experience**
Elizabeth Nelson *Chief, Environmental Risk Analysis Services* Reviewer	▪ Ph.D., Public Health, Capella University ▪ MBA, University of Maryland University College ▪ M.S., Health Care Administration, University of Maryland University College ▪ B.S., Biology, Bowie State University ▪ 16 years of professional experience in environmental compliance, policy, and management, including preparation of NEPA documentation
Lianne Hibbert *Assistant Chief, Biotechnology Environmental Analysis Services* Reviewer	▪ Ph.D., Human Dimension in Natural Resources, University of Missouri ▪ M.S., Fisheries and Wildlife Sciences, University of Missouri-Columbia ▪ B.S., Wildlife Biology, Grambling State University ▪ 18 years of federal service and experience including policy development and review, developing program responses to congressional requests, and program management.
Ron Hardman *Environmental Protection Specialist* EA Team Lead	▪ Ph.D., Environment, Duke University ▪ M.S., Marine Science/Oceans and Human Health, University of North Carolina at Wilmington ▪ B.S., Biology, Adelphi University ▪ 17 years of experience in environmental and human health risk analysis, and environmental regulatory compliance
Marlene Cole *Environmental Protection Specialist* Threatened and Endangered Species Analysis	▪ Ph.D., Ecology & Evolution, Rutgers University ▪ M.F.S, Forest Science (Wildlife Ecology), Yale University, School of Forestry and Environmental Studies ▪ B.A., Biology, Vassar College ▪ 17 years of professional experience in ecological assessment ▪ 10 years of professional experience in environmental regulatory compliance ▪ 2 years of experience in environmental impacts of biotechnology-derived crops

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