Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Pioneer Hi-Bred International, Inc. Petition (20-203-01p) for the Determination of Nonregulated Status for Insect Resistant and Herbicide-Tolerant DP23211 Maize

OECD Unique Identifier: DP-Ø23211-2

Draft Environmental Assessment

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TABLE OF CONTENTS **Page**

LIST OF TABLES   Page

LIST OF FIGURES   Page

ACRONYMS and ABBREVIATIONS

a.i.	active ingredient
AOSCA	Association of Official Seed Certifying Agencies
Bt	bacillus thuringiensis
CAA	Clean Air Act
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations (United States)
CO	carbon monoxide
CO_2	carbon dioxide
CRW	corn rootworm
CWA	Clean Water Act
DNA	deoxyribonucleic acid
dsRNA	double stranded ribonucleic acid
dvssj1	Gene encoding for the smooth septate junction protein 1
DvSSJ1	smooth septate junction protein 1
EA	Environmental Assessment
EFSA	European Food Safety Agency
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
FONSI	Finding of No Significant Impact
FQPA	Food Quality Protection Act
FWS	U.S. Fish and Wildlife Service
HR	herbicide resistant
ipd072Aa	gene from *Pseudomonas chlororaphis* encoding IDP072a protein
IPD072Aa	protein for control of corn rootworm
IR	insect resistant
IRM	insect resistant management
IWM	integrated weed management
lb	pound
mRNA	Messenger ribonucleic acid

N_2O	nitrous oxide
NAAQS	National Ambient Air Quality Standards
NEPA	National Environmental Policy Act of 1969 and subsequent amendments
NHPA	National Historic Preservation Act
NMFS	National Marine Fisheries Service
NOP	National Organic Program
NPS	non-point source (pollution)
NRC	National Research Council
NWQI	National Water Quality Initiative
OECD	Organization for Economic Cooperation and Development
PAT	phosphinothricin N-acetyltransferase (enzyme)
pat / mo-pat	gene from *Streptomyces viridochromogenes* that encodes the PAT enzyme
PIP	plant-incorporated protectant
PMI	phosphomannose isomerase
PPRA	Plant Pest Risk Assessment
PPA	Plant Protection Act
RNA	ribonucleic acid
siRNA	small interfering ribonucleic acid
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-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
WCR	Western corn rootworm
WPS	Worker Protection Standard (40 CFR part 170)

1 PURPOSE AND NEED FOR AGENCY ACTION

1.1 Background

In July 2020, Pioneer Hi-Bred International, Inc. (Pioneer) submitted a petition (20-203-01p) to the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), requesting that DP23211 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). As described in more detail below under Section 1.4–Requirement to Issue to Regulatory Status Determination, APHIS regulations at 7 CFR part 340 provide that any person may submit a petition to APHIS requesting that an organism should not be regulated, and APHIS must respond to petitioners with a regulatory status decision.

As part of evaluation of Pioneer's petition APHIS has developed this Environmental Assessment (EA) to consider the potential impacts of a determination of nonregulated status for DP23211 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 DP23211 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 on 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).**Purpose of DP23211 Corn**

There are three varieties of corn rootworm in the United States that are problematic crop pests; southern corn rootworm (SCR; *Diabrotica undecimpunctata*), northern corn rootworm (NCR; *Diabrotica barberi*), and western corn rootworm (WCR; *Diabrotica virgifera virgifera*). Pioneer developed DP23211 corn for resistance to WCR, and the herbicide active ingredient (a.i.) glufosinate-ammonium.[3] WCR resistance is conferred by DP23211 corn production of a double-stranded ribonucleic acid (dsRNA), termed DvSSJ1 dsRNA, which interferes with production of DvSSJ1 protein in WCR via RNA interference (RNAi) and causes WCR death. WCR resistance is also conferred by DP23211 corn production of an insecticidal

[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. Both terms are used interchangeably in this document. 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.

[2] Human environment means comprehensively the natural and physical environment and the relationship of present and future generations of Americans with that environment. Impacts/effects include ecological (such as the effects on natural resources and on the components, structures, and functioning of affected ecosystems), aesthetic, historic, cultural, economic (such as the effects on employment), social, or health effects (40 CFR §1508.1).

[3] Note that "Resistance" to herbicides is defined by the Herbicide Resistance Action Committee (HRAC) as the inherited ability of a plant population to survive and reproduce following repeated exposure to a dose of herbicide normally lethal to the wild type. "Tolerance" is distinguished from resistance and defined by HRAC as the inherent ability of a plant to survive and reproduce following exposure to an herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant. In reference to crops developed using genetic engineering, the terms "resistance" and "tolerance" are often used interchangeably. Throughout this EA, APHIS will use the term "resistance" and "resistant", and "herbicide-resistant" (HR), when referring to biotechnology-derived corn.

IPD072Aa protein. Hence, WCR resistance in DP23211 corn is conferred via two different methods; RNAi, and a protein toxic to WCR.

The nucleotide sequence in DvSSJ1 dsRNA is targeted to match a portion of the smooth septate junction protein 1 messenger RNA (mRNA) in WCR, and down-regulate expression of the DvSSJ1 protein in the mid-gut epithelial cells via RNAi. Smooth septate junctions are intercellular junctions unique to invertebrate epithelial cells that support cell to cell adhesion, contribute to the epithelial cell barrier, and serve other physiological functions (Faivre-Sarrailh et al. 2004; Izumi et al. 2019). When DP23211 corn tissue is ingested by WCR, the DvSSJ1 dsRNA results in suppression of production of DvSSJ1 protein in the cells of the intestinal lining—it interferes with mRNA translation. Reduction in DvSSJ1 protein production results in the loss of smooth septate junction and gut epithelium integrity, which is lethal to WCR (Hu et al. 2019).

Most current insect resistant (IR) crops—those produced using genetic engineering—utilize insecticidal Cry toxins derived from the soil bacterium *Bacillus thuringiensis* (Bt).[4] Cry toxins, when ingested by WCR, cause cell lysis in the mid-gut epithelium and WCR mortality (Bravo et al. 2007). IPD072Aa is also orally active against WCR exhibiting a similar lethal effect when ingested (Schellenberger et al. 2016). Though its exact insecticidal mechanism of action is still under investigation, IPD072Aa appears to have functional similarities to Bt-derived insecticidal proteins, albeit with a differing mechanism of action (Schellenberger et al. 2016). Field test data show that WCR resistant to Cry3Bb1, mCry3A, and Cry34Ab1/Cry35Ab1 were not cross-resistant to the insecticidal activity of IPD072Aa. Thus, the ability of IPD072Aa to kill WCR larvae resistant to these Cry toxins implies that its mechanism of action differs from Cry toxins (Schellenberger et al. 2016).

Currently, there are four Bt Cry toxins (termed plant incorporated protectants, or PIPs) that are used in IR crops to manage WCR; Cry3Bb1, Cry34/35Ab1, mCry3A, and eCry3.1Ab, which were registered by the U.S. Environmental Protection Agency (EPA) in 2003, 2005, 2006, and 2012, respectively. As with synthetic chemical pesticides, insects are capable of developing resistance to Cry toxins. Field-evolved resistance by WCR to Cry3Bb1 corn, mCry3A corn, and eCry3.1Ab corn has been documented in multiple Midwestern states (Gassmann et al. 2016; Jakka et al. 2016). Additionally, cross-resistance among Cry3Bb1, mCry3A, and eCry3.1Ab has been reported (Jakka et al. 2016).

Sustaining the agricultural and environmental benefits of Bt based crops—the efficacy of Cry toxins—is a primary objective among crop producers, industry, and EPA (US-EPA 2020h). Bt Cry toxins, in the form of powder and sprays, are also used in organic and conventional crops for control of insect pests—Bt preparations have been used as an insecticide in conventional crops for over 40 years. To help counter the development of insect resistance to Bt (Cry) toxins in transgenic crop plants the EPA has mandated the implementation of an Insect Resistance Management (IRM) plan for each commercially registered Bt PIP (US-EPA 2020h).

DP23211 corn is intended to diversify strategies used for WCR control through utilization of an RNAi based mechanism, and the IPD072Aa toxin, both of which function through mechanisms of action that

[4] *Bacillus thuringiensis* (Bt) are gram-positive spore-forming bacteria that produce insecticidal proteins during the sporulation phase that occur as parasporal crystals (Cry proteins/toxins).

differ from Cry toxins (Schellenberger et al. 2016; Hu et al. 2019; Pioneer 2020). The introduction of insecticidal traits that have mechanisms of action that differ from Cry toxins is intended to provide growers an additional corn variety for control of WCR in corn production, and help alleviate selection pressure for development of Cry resistance in WCR populations.

Resistance to glufosinate-ammonium in DP23211 corn is conferred by introduction of a modified gene (*mo-pat*) derived from the soil bacterium *Streptomyces viridochromogenes.* The *mo-pat* gene encodes for expression of the enzyme phosphinothricin acetyl transferase (PAT), which acetylates and disables the herbicidal activity of glufosinate-ammonium, rendering DP23211 corn resistant to the herbicide. The PAT protein has been previously used in biotechnology-derived varieties of corn, soybean, cotton, and canola crops to confer glufosinate resistance (USDA-APHIS 2022a). The herbicide-resistance trait in DP23211 corn is intended to facilitate weed management.

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 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 an update to the Coordinated Framework (USDA-APHIS 2020b), and an 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 biotechnology-derived products 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, 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 (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, and certain egg products that are regulated by the USDA—to ensure the safety of the nation's food supply (US-EPA 2020i; USDA-AMS 2020). Under its National Residue Program, the USDA's Food Safety and Inspection Service (FSIS) monitors meat, poultry, and processed egg products for pesticide residue and takes enforcement actions if it finds pesticide residues that exceed EPA tolerance levels (USDA 2020b).

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 (FSMA). The FDA created the Plant Biotechnology Consultation Program in the 1992 to cooperatively work with plant developers to help them ensure foods made from their new plant varieties are safe and lawful (US-FDA 1992b, 2006). In this program, the FDA evaluates the safety of food/feed from the new crop variety before it enters the market. Although the consultation program is voluntary, plant developers routinely participate in it before bringing a new plant variety to market. The FDA completed its first plant biotechnology consultation in 1994. Thus far, the FDA has evaluated more than 150 new plant varieties through this program.

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

1.4 Requirement to Issue a Regulatory Status Determination

Under the authority of the plant pest provisions of the Plant Protection Act, the regulations in 7 CFR part 340, "Movement of Organisms Modified or Produced Through Genetic Engineering," regulate, among other things, the importation, interstate movement, and environmental release of organisms modified or produced through genetic engineering that are plant pests or pose a plausible plant pest risk.[5] 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).[6] The final rule was implemented in phases. APHIS' new Regulatory Status Review (RSR) process, which replaced 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 became effective for all crops as of October 1, 2021. However, as noted in the final rule, "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).

Pioneer's petition for a determination of nonregulated status subject of this EA is being evaluated in accordance with the legacy regulations at 7 CFR 340.6, as it was received by APHIS in July, 2020, prior to full implementation of the final rule. 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

[5] 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 biotechnology-based techniques, 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 biolistics, and chemical and radiation based mutagenesis—as long as they are not plant pests or developed using plant pests.

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

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.

2 SCOPING AND PUBLIC INVOVLEMENT

APHIS seeks public comment on draft 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 [7] and on the APHIS website (USDA-APHIS 2020a).

2.1 Public Involvement for Petition 20-203-01p

On November 3rd, 2020 APHIS announced in the *Federal Register* that it was making Pioneer's petition available for public review and comment to help identify potential environmental and interrelated economic issues that APHIS should consider in evaluation of the petition.[8] APHIS accepted written comments on the petition for a period of 60 days, until midnight, January 4th, 2021. At the end of the comment period APHIS had received four comments on the petition. One comment was from an individual, which stated opposition to biotechnology-derived (biotech) crops in general. Three comments were received from industry organizations, which generally supported approval of the petition. A full record of each comment received is available online at www.regulations.gov [Docket No. APHIS-2020-0098].

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 the impacts analysis in this EA (40 CFR § 1501.9–Scoping):

- 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

[7] 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]
[8] Federal Register, / Vol. 85, No. 213 / Tuesday, November 3, 2020, p. 69564. Pioneer Hi-Bred International, Inc.; Availability of a Petition for the Determination of Nonregulated Status for Insect Resistant and Herbicide Tolerant Maize [Docket No. APHIS–2020–0098]. Available at https://www.federalregister.gov/documents/2020/11/03/2020-24267/pioneer-hi-bred-international-inc-availability-of-a-petition-for-the-determination-of-nonregulated

- 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 are involved in insect pest and weed management, the primary focus of this EA is on: (1) insect and insect resistance management, (2) weed and herbicide resistant weed management, (3) potential effects of exposure to the introduced trait genes and gene products on human health and wildlife, and (4) gene flow and potential weediness of DP23211 corn.

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, and (2) Preferred Alternative, approval of the petition

3.1 No Action Alternative: Deny the Petition Request

One of the alternatives that APHIS considers is a "No Action Alternative". APHIS discusses this alternative as required by CEQ NEPA implementing regulations at 40 CFR § 1502.14. Under the No Action Alternative APHIS would deny the petition request for nonregulated status and DP23211 corn would remain regulated under 7 CFR part 340. Permits issued by APHIS would be required for the environmental release or shipment of DP23211 corn. Because APHIS concluded in its PPRA that DP23211 corn is unlikely to pose a plant pest risk (USDA-APHIS 2022b), 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–Issue a Determination of Nonregulated Status for DP23211 Corn

Under this alternative APHIS would approve the petition and DP23211 corn would no longer be subject to regulation under 7 CFR part 340. APHIS permits would no longer be required for the environmental release or shipment of DP23211 corn. Because it was determined that, based on the scientific evidence before the Agency, DP23211 corn is unlikely to pose a plant pest risk (USDA-APHIS 2022b), 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 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 draft EA. Detailed analysis of the affected environment and environmental impacts is discussed in Chapter 4.

Table 3-1. Summary of Potential Impacts for the Alternatives Considered

Analysis	No Action Alternative: Deny the Petition Request	Preferred Alternative: Approve the Petition for Nonregulated Status for DP23211 Corn
Meets Purpose and Need	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. Regulated field trials would be conducted on lands allocated for this purpose.	Approval of the petition is unlikely to have any effect on an increase or decrease in total U.S. corn acreage. DP23211 corn, if adopted by growers, would be expected to replace other insect resistant (IR) and herbicide resistant (HR) corn varieties

Analysis	No Action Alternative: Deny the Petition Request	Preferred Alternative: Approve the Petition for Nonregulated Status for DP23211 Corn
		currently cultivated, as opposed to augmenting current corn crops.
Agronomic Practices and Inputs	Agronomic practices and inputs used in corn crop production, to include regulated field trials, would remain unchanged. Denial of the petition would have no effect on insect and weed management in corn.	Agronomic practices and inputs would be similar to other varieties of corn. DP23211 corn would facilitate use of glufosinate-ammonium, in lieu of other herbicides. Thus, adoption of DP23211 corn could entail increased use of glufosinate-ammonium in the United States, with a decrease in use of other herbicides with similar spectrums of weed control. As an IR corn variety comprised of plant incorporated protectants (PIPs) with specify for western corn rootworm (WCR), cultivation of DP23211 would likely entail less use of soil- and foliar-applied insecticides to control WCR. Because the DvSSJ1 dsRNA and IPD072Aa protein provide novel mechanisms of action for control of WCR, DP23211 corn may also prove useful in helping deter development of Bt Cry resistant WCR pest populations, and sustaining the efficacy of current and future Bt Cry based IR crops.
Use of Biotechnology-Derived Corn	Denial of the petition would have no effect on grower choice in the planting of biotechnology-derived or conventionally bred corn.	Approval of the petition would provide for cultivation of a corn variety resistant to WCR, an economically damaging insect pest, and the herbicide active ingredient glufosinate-ammonium. These traits are expected to facilitate insect pest and weed management.
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 similar for both DP23211 corn and conventional corn varieties. Because DP23211 corn production could facilitate less insecticide use (~11% per crop cycle), potential impacts on soils could be potentially reduced, relative to non-IR corn varieties.
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 the agronomic practices and inputs utilized for DP23211 corn production would be similar, sources of potential impacts on water resources, namely NPS pollutants in agricultural run-off, would not be expected to substantially differ. Any reduced insecticide use would reduce the risk of insecticides in run-off. The EPA provides label use restrictions and

Analysis	No Action Alternative: Deny the Petition Request	Preferred Alternative: Approve the Petition for Nonregulated Status for DP23211 Corn
		guidance for pesticides, to include glufosinate based herbicides, that are intended to be protective of surface water and groundwater.
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 DP23211 corn production would be similar to conventional corn, and acreage would remain unchanged, significant changes to emission sources nor level of emissions are not expected. There would be minor reductions in National Ambient Air Quality Standards (NAAQS) emissions as a result of reduced insecticide applications.
Biological Resources		
Soil Biota	Potential impacts of corn production/regulated field trials on soil biota would continue along current trends.	Commercial production of DP23211 corn or progeny is not expected to present any risks to soil biota. The introduced HR/IR transgenes and gene products are not expected to have any effects on the long-term viability and function of soil biota or community structures.
Animal Communities	Regulated field trials of DP23211 corn would present negligible risk to animal communities.	Approval of the petition, and subsequent commercial production of DP23211 corn, would not be expected to affect animal communities adjacent to or within DP23211 corn cropping systems any differently from that of current corn cropping systems. The DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI proteins do not pose any hazard to wildlife.
Plant Communities	Regulated field trials of DP23211 corn would present negligible risks to plant communities in proximity to DP23211 corn fields.	Because the agronomic practices and inputs that will be used for DP23211 corn production are similar, save for reduced insecticide use, potential impacts on plant communities would be similar as for other corn varieties currently cultivated. The EPA regulates and determines the use of glufosinate. Pesticide use requirements are intended to be protective of non-target plant communities, such as those in adjacent fields.
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	DP23211 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. Conceptually, the IR trait could

Analysis	No Action Alternative: Deny the Petition Request	Preferred Alternative: Approve the Petition for Nonregulated Status for DP23211 Corn
	Tripsacum is rare (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995). Gene flow to *Tripsacum* species during regulated fields trials of DP23211 corn is highly unlikely.	confer a competitive advantage to wild *Tripsacum* species. While conceptually possible, the likelihood of *Tripsacum* populations comprising the IR trait genes developing is considered unlikely. First, the potential for hybridization and successful introgression of *Z. mays* genes into *Tripsacum* populations is remote (de Wet and Harlan 1972; de Wet et al. 1978; Eubanks 1995). Successful introgression of *Zea mays* genes into *Tripsacum* populations, successful gene flow in this direction, has not been observed in the wild (USDA-APHIS 2007; FAO 2014)). 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%.
Biodiversity	Denial of the petition, and any regulated field trials of DP23211 corn, would present negligible risks to biodiversity in an around DP23211 corn crops.	Commercial production of DP23211 corn would affect biodiversity in and around DP23211 corn crops no differently than other corn cropping systems. The DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI proteins are unlikely to present any hazards to plant, animal, fungal, or bacterial communities. All pesticide use would be subject to EPA registration and use requirements.
Human and Animal Health		
Human Health and Worker Safety	Denial of the petition would have no effect on human health. DP23211 corn would remain regulated and would not be available for food uses.	Approval of the petition would provide for the use of DP23211 corn products in the food and feed industries. Pioneer completed a food safety consultation with the FDA in 2022; the FDA had no questions concerning human food derived from DP23211 corn (US-FDA 2022). The EPA's regulation of pesticides, and worker protection standards, would remain unchanged.
Animal Health and Welfare	Denial of the petition would have no effect on animal health and welfare. DP23211 corn would remain	DP23211 corn would provide for animal feed products. Pioneer completed a an animal feed safety consultation with the

Analysis	No Action Alternative: Deny the Petition Request	Preferred Alternative: Approve the Petition for Nonregulated Status for DP23211 Corn
	regulated and would not be available for feed uses.	FDA in 2022; the FDA had no questions concerning feed derived from DP23211 corn (US-FDA 2022).
Socioeconomic		
Domestic Economy	Denial of the petition would preclude DP23211 corn being available for food, feed, and fuel uses. This, however, would have no effect on domestic markets.	DP23211 corn, a field (dent) corn variety, is expected to be used for provision of standard corn-based food, feed, fuel, and industrial products. The potential impacts of DP23211 corn on domestic markets would be considered largely beneficial. DP23211 corn would provide farmers with an additional option for management of CRW pests and protection of corn grain yield. From 1996-2018, IR corn targeting corn boring pests provided a 7% increase in yield, and increased farm income of $81/ha ($32.8/acre). IR corn targeting CRW provided a 5% increase in yield, and increased farm income of $78/ha ($31.6/acre) (Brookes and Barfoot 2018a). Aggregate U.S. farm income benefit from 1996-2018 for IR corn was $45.6 billion (Brookes and Barfoot 2018a). In 2018, the global gross farm income gains from using IR corn was $4.53 billion (Brookes and Barfoot 2018a).
International Trade	Denial of the petition would have no impacts on the trade of corn commodities.	Approval of the petition is unlikely to have a substantial effect on the trade of U.S. corn 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 DP23211 corn.	As cited above Pioneer consulted with the FDA on the food and feed safety of DP23211 corn. Glufosinate and other pesticide use will be subject to EPA registration and label use requirements.
Regulatory and Policy Compliance		
ESA, CWA, CAA, SDWA, NHPA, EOs	Fully compliant (*not compliant with the Plant Protection Act (PPA) and 7 CFR part 340)	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 DP23211 corn, and market utilization of 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, economic, and effects on human health. Impacts/effects include those resulting from actions that may have both beneficial and detrimental effects.

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 impacts of commercial production of a biotechnology-derived crop on the human environment 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 sufficient to meet societal needs. Potential effects on the environment depend on the intensity and scale of crop production over time, the agronomic inputs applied (e.g., fertilizers, pesticides, irrigation water), and the effective management of inputs, pests, weeds, and tillage. Corn is used to produce a variety of food products, animal feed, fuel ethanol, and a wide range of industrial products. There are around 90 million acres of land area in the United States planted to corn, and 323 to 340 million acres of total U.S. cropland planted on annual basis. Thus, the scale of potential impacts, namely in an aggregate sense, requires integration of crop production with sustainability and mitigation practices, for both biotechnology based and conventional corn cropping systems. In general, tillage, crop monoculture, and fertilizer and pesticide inputs can potentially have adverse effects on topsoil, water quality, air quality, and local biodiversity. Agriculture is a leading cause of water-quality impairment in the United States as a result of run-off crop protection/production inputs (US-EPA 2020l). No-tillage systems, crop rotations, cover cropping, integrated pest and weed management, and other environmentally beneficial practices can help ameliorate some of the adverse impacts, although a tradeoff between the agricultural production food, feed, fiber, and fuel, and some degree of environment impacts, will always remain (Robertson and Swinton 2005).

Gene flow, movement of a transgene to sexually compatible species, has also been a topic of concern with transgenic crops, 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 transgenic traits in corn into non-modified corn varieties, or other transgenic corn varieties, is a concern for farmers and markets that depend on adhering to strict identity preservation standards for certain food and feed commodities. Such gene flow can result in adverse economic impacts to the transgenic trait-sensitive market.

Due to the scale of crop production in the United States (e.g., 300 to 330 million acres planted with principal on an annual basis), developing and implementing environmentally sound, sustainable agricultural management practices is a primary goal of federal and state programs—applicable to biotechnology-based, conventional, and organic cropping systems alike (e.g., (USDA-NRCS 2015; US-EPA 2020a; USDA-NRCS 2020b), and others).

It is within this context that APHIS evaluates the potential impacts of DP23211 corn on the human environment if deregulated, and cultivated on a commercial scale for corn based commodities.

DP23211 Corn: Assumptions used in Analysis

It assumed that, in the event Pioneer's petition is approved, DP23211 corn would be grown for production of food, feed, fuel, and industrial commodities. It is also assumed that the only potential impacts that could derive from production and marketing of DP23211 corn that could be considered unique, as compared to other corn varieties, are relative to the trait genes and gene products (Table 4-1). DP23211 corn would provide insect resistance traits, and facilitate weed management with the use of glufosinate–ammonium based herbicides.

Table 4-1. Summary of Genetic Elements in DP23211 Corn

Genetic Element	Description	Origin Species	Occurrence
pmi	Phosphomannose isomerase gene	*Escherichia coli*	Soils, foods, and intestines of animals
mo-pat	Maize-optimized phosphinothricin acetyltransferase gene	*Streptomyces viridochromogenes*	Soil bacterium
DvSSJ1	Fragment of the smooth septate junction protein 1 (dvssj1) gene	*Diabrotica virgifera virgifera* (western corn rootworm)	Ubiquitous throughout the United States
ipd072Aa	Insect protection gene	*Pseudomonas chlororaphis*	Soil bacterium

4.2 No Action Alternative: Deny the Petition Request

Because APHIS concluded in its PPRA that DP23211 corn is unlikely to pose a plant pest risk (USDA-APHIS 2022b), 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 (as discussed in 1.4 – Requirement to Issue a Regulatory Status Determination), 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 DP23211 corn would be regulated under 7 CFR part 340 and require APHIS authorization for importation, interstate movement, or release into the environment.

APHIS' regulation of DP23211 corn, which would effectively preclude commercial production of this variety, 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 DP23211 corn would require APHIS authorization, which would be provided via permit, pursuant to 7 CFR part 340, described in more detail below. For both permits, APHIS Biotechnology Regulatory Services prescribes criteria and conditions that must be met in order to ensure that the regulated plant 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 regulated 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.[9] 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 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 DP23211 corn. Interstate movement of DP23211 corn would present negligible environmental risks.

[9] 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.

4.3 Preferred Alternative: Approve the Petition Request

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. Corn varieties are differentiated by the starch, protein, oil, water, or other properties of the kernel, and produced for specific uses; e.g., food, animal feed, industrial products.

DP23211 corn is a dent corn variety (Oestreich 1993; Pioneer 2020). 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 38% – 48% of use, and stock for the production of fuel ethanol for around 25% – 35%, on annual basis (NCGA 2022). The remainder is processed into a variety of food and industrial products such as starch, sweeteners, corn oil and corn syrup, and beverage and industrial alcohol (Figure 4-1).

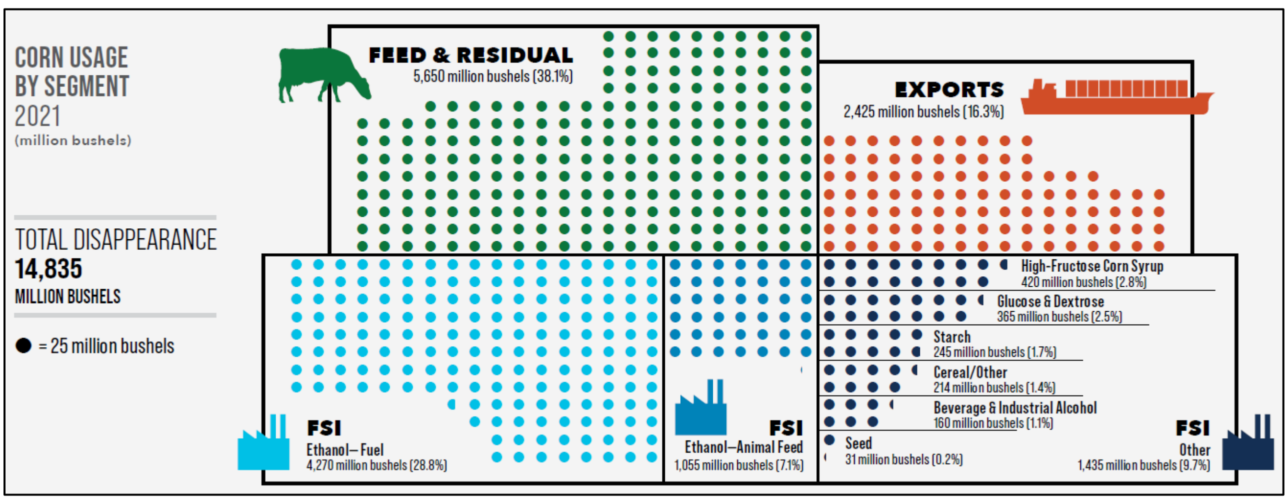

Figure 4-1. Corn Uses in the United States, 2021

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 2020, vary slightly on an annual basis. Source: (PRX 2019; USDA-ERS 2019; NCGA 2020)

Over the last ten years around 85 to 95 million acres of dent corn have been harvested in the United States on an annual basis (USDA-NASS 2019b). This comprises approximately 25% of total U.S. cropland). Production of popcorn comprises around 0.2 million acres, and sweet and waxy corn around 0.5 million acres, each, on an annual basis (< 1% of corn acreage on an annual basis). While dent corn can be grown in all states to some extent, the majority of commercial 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 dent corn-producing states of Illinois, Iowa, and Nebraska account for approximately 40% of the annual U.S. harvest. Substantial production also occurs in Idaho, California's Central Valley, along the Mississippi River, and up the Eastern Seaboard from Georgia to Upstate New York (Figure 4-2).

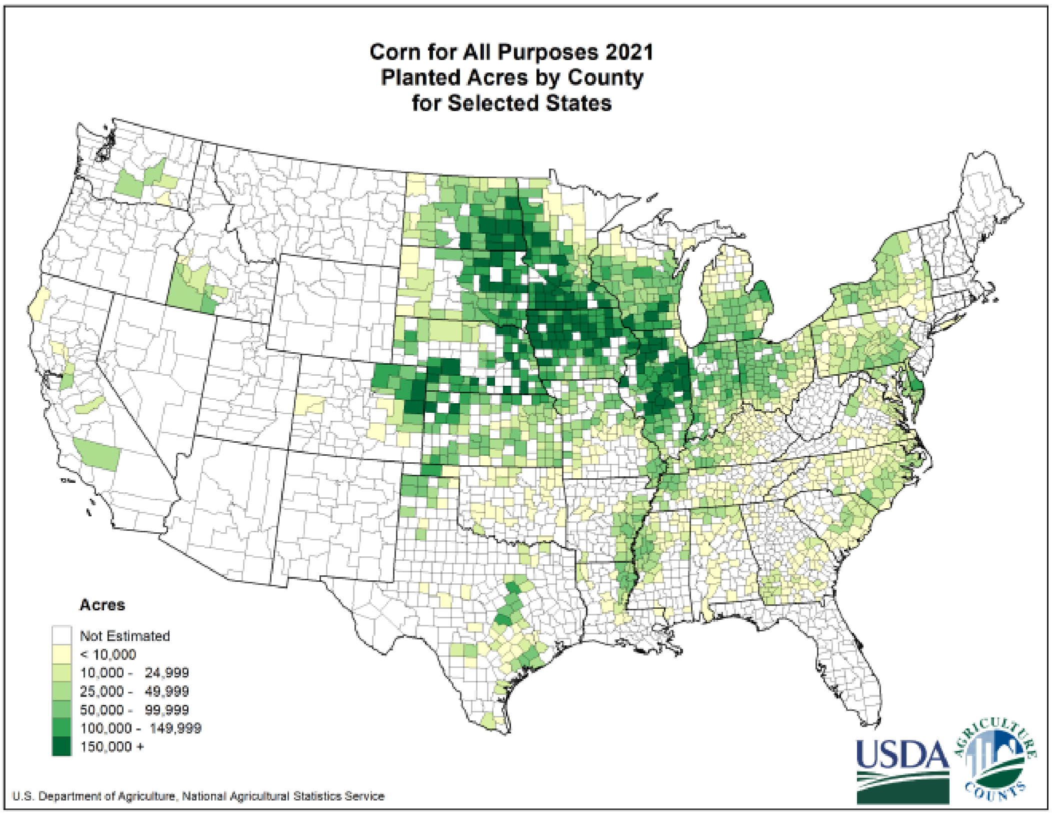

Figure 4-2. Corn Cultivation in the United States by County, 2021

Source: (USDA-NASS 2021)

Around 93% of the corn produced in the United States is comprised of biotechnology-derived varieties (Figure 4-3), the majority of this 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 (Reiley 2019). Most corn varieties are stacked-trait herbicide-resistant (HR) and insect-resistant (IR). Stacked-trait varieties with HR and IR traits accounted for 81% of the 2021 crop. Only 9% contained a single HR trait, and 3% a single IR in 2021. Of the ~90 million corn acres planted in 2021, around 7.3 million were conventionally bred.

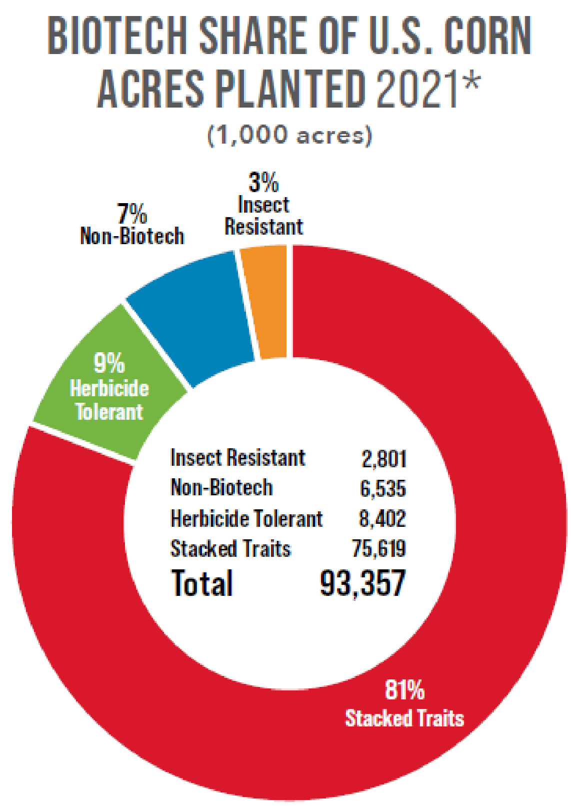

Figure 4-3. Biotech Corn Traits Planted in the United States, 2020

Source: (NCGA 2022)

In addition to the varieties described, there are around 12,000 acres of traditional or Indian corn produced in the United States, primarily on Indian reservations (USDA-NASS 2020a). Traditional or Indian corn is an open-pollinated (nonhybrid), non-biotech cultivar of *Zea mays* that was indigenously developed and consists of many heritage varieties of sizes, color, and drought tolerance. Traditional corn grown on southwest reservations has been passed from generation to generation through seed saving by American Indian and Hispanic communities. Traditional or Indian corn is culturally significant; it is reported to be grown in all states except Alabama, Michigan, Missouri, Montana, Nebraska, South Dakota, Vermont, Virginia, and West Virginia.

4.3.1.2 Agronomic Practices and Inputs

Commercial 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. Some of these practices (e.g., tillage) and inputs (e.g., fertilizers, pesticides) can, when applied in excess or improperly, present 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.

Apart from the herbicide-, insect-, or disease-resistant trait(s), there are little differences in the agronomic practices and inputs used for biotech and conventionally bred crops. HR crops will influence the types of herbicides used. IR crops generally require less insecticide use, and disease resistant crops the use rates of fungicides and similar chemicals targeting plant pathogens. The agronomic practices, and current uses of the herbicides that will be used with DP23211 corn, are summarized below.

4.3.1.2.1 Agronomic Practices

Growers employ several practices for the management of plant pests and weeds, summarized below (Table 4-2). Among these, tillage is a practice that can have environmental impacts, and this topic, in relation to HR/IR corn, is discussed in more detail below.

Table 4-2. Top Practices in Pest Management, 2018 Crop Year

Practice	% of corn planted 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

Source: (USDA-NASS 2019a)

4.3.1.2.1.1 Tillage

Tillage is used to control weeds, 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 by the amount of plant material left on the field after harvest and the degree of soil disturbance they cause (Harper 2017). Conventional tillage involves intensive plowing leaving less than 15% crop residue in the field; reduced tillage leaves 15% to 30% crop residue; and 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 soil borne 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 impairs soil quality and results in soil erosion and run-off 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 (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 facilitate the benefits of conservation tillage by eliminating some of the stresses observed in continuous no-till crops (Roth 2015).

The adoption of conservation tillage systems 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 in 2016 (27% overall) (Figure 4-4). In part, an increase in conservation tillage was facilitated by the availability (since the 1980s) of post-emergent herbicides (Fernandez-Cornejo et al. 2012), which can be applied throughout the growing season—not just before planting, as had previously been the case. Another factor has been the implementation of soil conservation programs that began in the mid-1980s, which encourage/incentivize conservation tillage practices to help conserve soils (USDA-NRCS 2006). Continued increases in conservation tillage since the late 1990s have also been attributed to the use of herbicide resistant crops, which can facilitate the chemical control of weeds and reduce the need for mechanical weed control (Towery and Werblow 2010; USDA-ERS 2012).

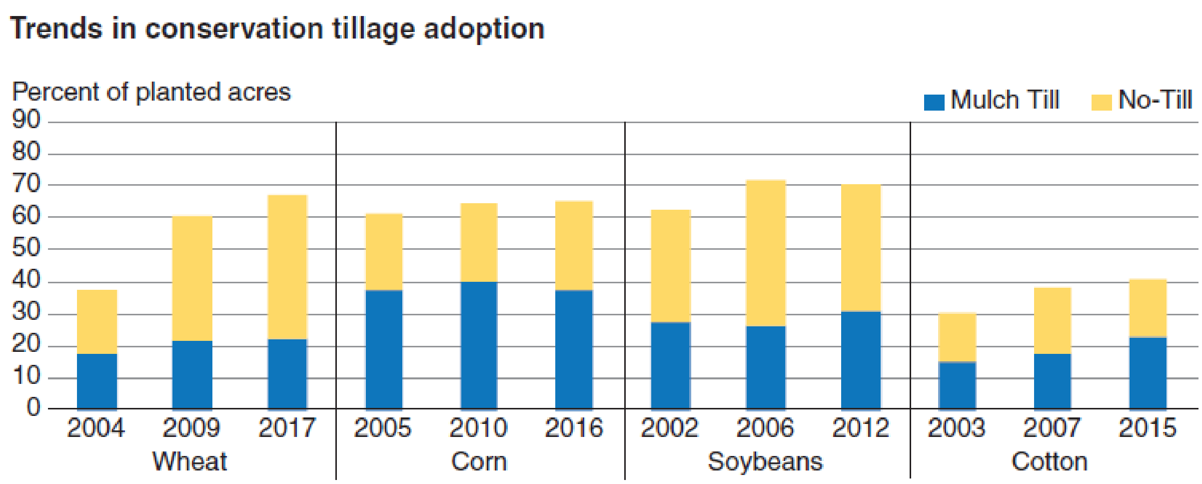

Figure 4-4. Conservation Tillage Practices in Corn, 2005 – 2016

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 DP23211 corn production are discussed following.

4.3.1.2.2.1 Fertilizers

Since 1975, the majority of corn acreage has been treated with nitrogen, phosphate, and potash (potassium), and about a third of planted acres treated with sulfur. Inputs for the 2018 crop year (latest data) are provided in (Table 4-3). 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 the crop 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. While nitrogen and phosphorus are important agricultural inputs, the application of amounts exceeding recommended thresholds can have adverse effects on air and water quality (discussed in Section 4.3.2–Physical Environment).

Table 4-3. Fertilizer Applied to Corn Acres, 2021 Crop Year

Fertilizer	% of Planted Acres	Avg. Rate for Year (lbs/acre)	Total Applied (billion lbs)
Nitrogen (N)	95	150	12.3
Phosphate (P2O5)	75	64	4.1
Potash (K2O)	65	77	4.3
Sulfur (S)	34	19	0.5

Source: (USDA-NASS 2022a)

4.3.1.2.2.2 Pesticides

Pesticides contribute to higher yields and product quality by controlling weeds, insect pests, nematodes, and plant pathogens. Herbicides, in particular, reduce the amount of labor and time required for the manual/mechanical control of weeds. Plant incorporated protectants providing insect resistance, such as the IR traits in DP23211 corn, can likewise reduce labor, as well as fuel and synthetic chemicals costs.

Common corn pests include beetles (*Coleoptera* species), moth and butterfly larvae (*Lepidoptera* species), pathogenic fungi (e.g., corn leaf blight), bacteria (e.g., stalk rot), and viruses (e.g., dwarf mosaic virus) (UMinn 2019). Numerous populations of weed species across the United States require annual management in corn cropping systems (Jhala et al. 2014). 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. Because DP23211 corn is resistant to glufosinate-ammonium and WCR, emphasis in this EA is given to glufosinate use and weed and weed resistance management, and insect pest and insect resistance management, discussed in the following sections.

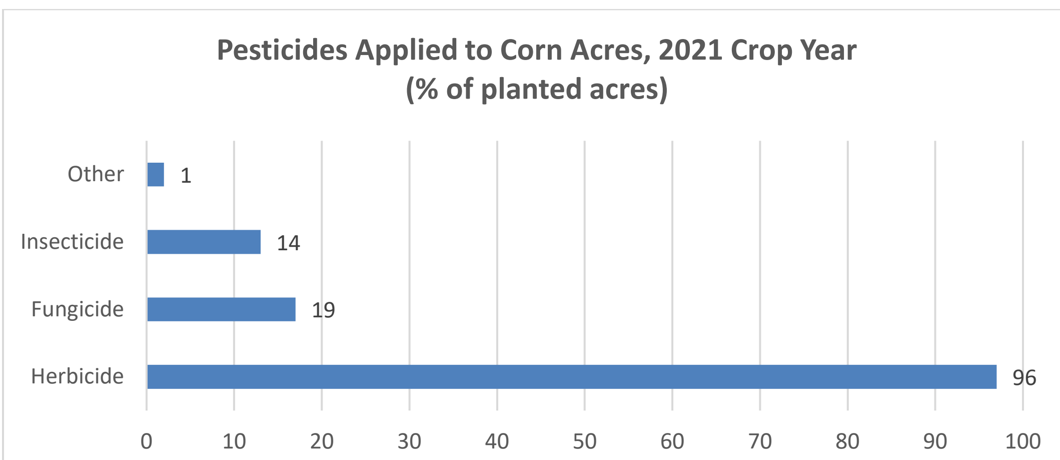

Figure 4-5. Pesticides Applied to Corn, 2021

Source: (USDA-NASS 2022a)

4.3.1.2.2.3 Weed and Herbicide Resistant Weed Management

There are around 50 species of weeds among U.S. cornfields (Jhala et al. 2014). Currently, there are around 23 species that are particularly problematic due to herbicide resistance (Heap 2020). Weeds have been and will remain a problem in corn crop production, especially in the early part of the growing season, due to the slow early growth rate of corn and wide row spacing (Jhala et al. 2014). Their presence can reduce yields and product quality, increase harvest costs, and reduce net economic returns by:

- increasing insect and disease damage in crops by serving as hosts for pests and pathogens;
- reducing seedbed soil moisture and structure as a result of increased tillage needed to kill weeds prior to seeding;
- increasing dockage with higher cleaning and transportation costs; and
- contamination, resulting in reduced grades and quality from similar inseparable size and shape weed seeds (cleavers, for example).

Exemplary of the potential impact of weeds on yield and net returns: On average, weeds cause 52% corn yield loss when using BMPs but no herbicidal weed control. More than half of corn production and value across North America would potentially be lost with weeds left uncontrolled (Page et al. 2012; Dille et al. 2015; Soltani et al. 2017). One study found that, averaged across seven years, weed interference in corn in the United States and Canada caused a 50% yield loss, which equates to a loss of 148 million tons of corn grain valued at over $26.7 billion annually (Soltani et al. 2017). Other studies have found that U.S. corn yield loss from weed interference can range as high as 15% with weed control (Bridges 1992; WSSA 2020).

Weed Management with Herbicide Resistant Corn

Prior to the development of chemical herbicides farmers controlled weeds by tillage, hoeing or pulling by hand, mowing, site selection, crop rotation, and use of crop seed free of weed seeds. U.S. farmers began widescale adoption of chemical herbicides after their commercial introduction in the 1950's because they were inexpensive, effective, easy to apply, reduced labor costs, reduced the need for tillage, and increase crop yields (Fernandez-Cornejo et al. 2014c). Due to their efficacy and affordability synthetic chemical herbicides remain the most commonly used among weed management tools and are expected to remain so for the foreseeable future. Sixty years ago, herbicides accounted for around 18% of pesticide use by volume on U.S. crops, and insecticides 58% percent. Today, herbicide and insecticide use account for approximately 76% and 6% percent of total pesticide applications, respectively (GROi 2018). In the United States, herbicides are currently used on 97% of U.S. corn acres (USDA-NASS 2019a).

In discussing herbicide use and herbicide resistant crops it is important to clarify that simply looking at the total pounds of active ingredient (a.i.) used per year, pounds a.i. per acre used, and trends in increase or decrease in lbs a.i. per year—evaluation of these metrics in isolation of other factors—is not particularly useful for assessment of potential environmental or human health risks. Potential risks to the environment and human health—which are evaluated by the EPA for all herbicides (US-EPA 2020k) — are relative to the specific herbicide a.i., its biological activity (mode of action), the potential toxicity of the a.i. to various taxa, its environmental mobility and persistence, degradation products, as well as the toxicity of herbicide formulations as there may be synergistic effects that derive from such formulations. Bearing these factors in mind, as weight and use rates are some of the most commonly reported metrics, and in response to comments received on EAs and EISs for HR crops requesting evaluation of pesticide use, provided below are usage data on glufosinate and other herbicides that may be used with DP23211 corn (Table 4-4).

Table 4-4. Herbicide Use in U.S. Corn Production – 2021

Herbicide a.i.	lbs a.i./Yr	Application: lbs a.i./acre/Yr (Average)	Treated Acres, % of Area Planted	Portion of Total Herbicide Use (lbs)
ATRAZINE	59,180,000	0.844	65	24.88%
ACETOCHLOR	41,675,000	1.26	34	17.52%
GLYPHOSATE ISO. SALT	32,934,000	0.768	41	13.85%
S-METOLACHLOR	27,002,000	0.995	27	11.35%
GLYPHOSATE POT. SALT	26,812,000	0.992	25	11.27%
GLYPHOSATE	12,691,000	1.037	11	5.34%
METOLACHLOR	7,254,000	2.022	3	3.05%
MESOTRIONE	5,343,000	0.112	47	2.25%
2,4-D, 2-EHE	4,188,000	0.717	6	1.76%
DIMETHENAMID-P	2,891,000	0.566	6	1.22%
DICAMBA, DIGLY. SALT	2,394,000	0.316	8	1.01%
2,4-D, DIMETH. SALT	2,081,000	0.604	3	0.88%
GLYPHOSATE DIM. SALT	2,059,000	0.81	2	0.87%
PENDIMETHALIN	1,163,000	0.98	1	0.49%
DICAMBA, DIMET. SALT	1,096,000	0.223	5	0.46%
CLOPYRALID	1,055,000	0.074	15	0.44%
PARAQUAT	898,000	0.485	1	0.38%
GLUFOSINATE-AMMONIUM	849,000	0.429	2	0.36%
SIMAZINE	642,000	0.912	1	0.27%
TEMBOTRIONE	627,000	0.1	7	0.26%
DICAMBA, SODIUM SALT	446,000	0.103	5	0.19%
CLETHODIM	308,000	0.534	1	0.13%
BICYCLOPYRONE	278,000	0.032	9	0.12%
ISOXAFLUTOLE	251,000	0.071	4	0.11%
CLOPYRALID MONO SALT	214,000	0.074	3	0.09%
METRIBUZIN	208,000	0.172	1	0.09%
FLUMETSULAM	167,000	0.029	7	0.07%
DIFLUFENZOPYR-SODIUM	164,000	0.043	4	0.07%
PYROXASULFONE	120,000	0.081	2	0.05%
SAFLUFENACIL	107,000	0.059	2	0.04%
DICAMBA, POT. SALT	69,000	0.096	1	0.03%
THIENCARBAZONE-METHY	63,000	0.019	4	0.03%
TOPRAMEZONE	62,000	0.012	6	0.03%
DICAMBA	54,000	0.381	(Z)	0.02%
FLUROXYPYR 1-MHE	54,000	0.082	1	0.02%
FLUMIOXAZIN	31,000	0.065	1	0.01%
RIMSULFURON	17,000	0.017	1	0.01%
THIFENSULFURON	10,000	0.012	1	0.00%
HALOSULFURON-METHYL	4,000	0.011	(Z)	0.00%
FLUTHIACET-METHYL	2,000	0.003	1	0.00%
TOTAL	237,818,000			

*Z = less than one whole percent. Herbicides in bold type are used with HR corn varieties. The data provide is largely based on USDA-NASS statistics for corn. Most of the annual NASS survey data generally captures around 80-90% of acreage for a given crop during a given year. Hence, the data presented likely provides a good approximation of herbicide use in corn production. Source: (USDA-NASS 2022b)

Glufosinate-Ammonium Use

Glufosinate is a non-selective, broad spectrum foliar-applied herbicide that is registered for pre-plant and post-emergence control of over 120 grass and broadleaf weeds on crop and non-crop sites. It is marketed in herbicide formulations such as Basta®, Finale®, Rely®, and Liberty®. Glufosinate is registered for use on both HR corn and conventionally bred varieties of canola, corn, cotton, and soybeans, as well as a variety of other conventionally bred crops, including apples, berries, citrus, currants, grapes, grass grown for seed, potatoes, rice, sugar beets, and tree nuts (US-EPA 2016a). Non-crop use sites include golf course turf, residential lawns, industrial and residential sites, utility and roadside rights-of-way, and timber site preparation for tree plantings. The crops which account for the most glufosinate use are soybean, cotton, and corn (together, over 75% of usage) (US-EPA 2016a); these crops have glufosinate resistant varieties. Currently, corn acres are treated with glufosinate at a rate of 0.4 lbs.a.i./acre (USDA-NASS 2020a), while almond acres are treated at a rate of 1.4 lbs. a.i./acre (US-EPA 2016a; USDA-NASS 2020a). Several other crops, including apples, cherries, grapes (raisin, table, and wine), oranges, peaches, pears, pistachios, and walnuts are treated with glufosinate at rates greater than 1.0 lb a.i./acre (US-EPA 2016a; USDA-NASS 2020a). An overview of glufosinate is in the United States is provided in Figure 4-6.

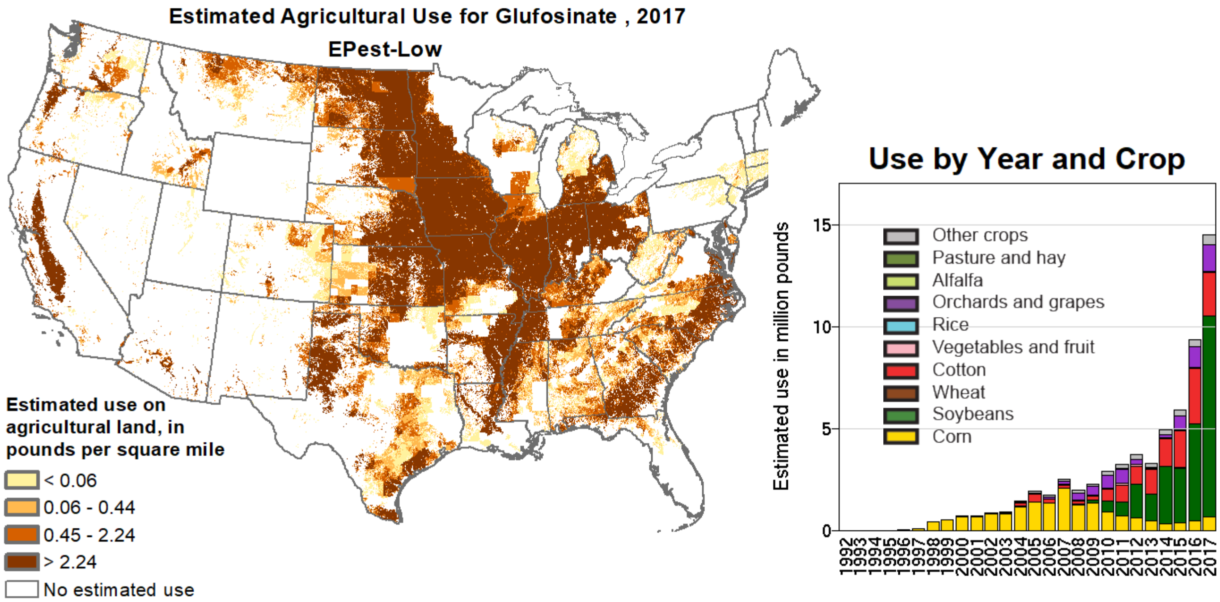

Figure 4-6. Glufosinate Use in the Conterminous United States – 2017

Source: (USGS 2020a)

Glufosinate-Resistant Corn

While glyphosate resistant corn varieties have been the most widely grown, glufosinate and 2,4-D resistant corn varieties have also been available. Glufosinate resistant corn varieties were first deregulated in the mid-1990s (Table 4-5).

Table 4-5. Glufosinate Resistant Corn, Soybean, and Cotton Crops

Crop	Stacked-Trait herbicide tolerance traits	Trade name	Year commercialized in the United States
Corn	Glyphosate and glufosinate	SmartStax	2010
Corn	Glyphosate and 2,4-D	Enlist Duo	2014
Corn	Glyphosate, glufosinate, 2,4-D and ACCase-FOP	SmartStax Enlist	2018
Corn	Glufosinate	Liberty-Link	1996
Soybean	Glyphosate and glufosinate	Liberty-Link	2009
Soybean	Glyphosate and dicamba	Roundup Ready Xtend	2016
Soybean	Glyphosate, glufosinate and 2,4-D	Enlist E3	2019
Soybean	Glufosinate	LibertLink	2009
Cotton	Glyphosate and dicamba	Roundup Ready Xtend	2016
Cotton	Glyphosate, glufosinate and 2,4-D	Enlist	2016
Cotton	Glyphosate and glufosinate	GlyTol Liberty Link	2014

Source: (USDA-APHIS 2022a).

After glufosinate resistant corn varieties were first deregulated (1996) there was a minor increase in glufosinate, up to around 2005. Glufosinate use in corn declined from 2005 to 2014, with a minor increase of an average 0.002 lbs a.i./acre from 2014 to 2018. Overall, use of glufosinate on corn, even with glufosinate resistant corn varieties available, has been relatively limited. For corn, 234,000 lbs were applied in 2016, and 488,000 lbs were applied in 2018 (USDA-NASS 2020a). As of 2018, glufosinate use comprised 0.36% of total herbicides applied to corn (in lbs a.i.), and was used on only around 2% of corn acres (prior Table 4-4).

Glufosinate Mode of Action and Phosphinothricin Acetyltransferase (PAT)

Bialaphos is a naturally occurring herbicidal compound produced by certain species of soil bacterium among the genus *Streptomyces* (Dayan et al. 2009; Dayan and Duke 2014). Bialaphos is a prototoxin, or protoxin, a non-toxic precursor of the phytotoxic compound L-phosphinothricin (referred to as phosphinothricin from here out). Bialaphos, once taken up by plant cells, is converted to phosphinothricin, which inhibits glutamine synthetase, an enzyme necessary for the production of glutamine (an essential amino acid) and ammonia detoxification. Reduced levels of glutamine and accumulation of ammonia in plant cells interferes with photosynthesis and other metabolic processes, resulting in plant death. Glufosinate is a synthetic form of phosphinothricin, and used as a non-selective broad spectrum herbicide. Glufosinate is the only commercial herbicide that targets glutamine synthetase.

Resistance to glufosinate is conferred by the enzyme phosphinothricin acetyltransferase (PAT), which inhibits the phytotoxic activity of glufosinate by acetylation, altering its chemical structure and function. Genes encoding PAT have been isolated from the bacterium *Streptomyces hygroscopicus* (e.g., the *bar* gene, which is short for bialaphos resistance) (Thompson et al. 1987) and *Streptomyces viridochromogenes* (*pat* gene) (Wohlleben et al. 1988). The *pat* gene, that introduced into DP23211 corn, is very similar to the *bar* gene with 87 % nucleotide sequence identity. Both *bar* and *pat* encode PAT proteins with similar substrate affinity and biochemical activity (Wehrmann et al. 1996).

Herbicide Resistant Weeds

Herbicide resistant (HR) weeds are more of an agronomic than ecological concern, however, HR weed populations can potentially contribute to environmental impacts when herbicide use or/and tillage is increased for their control. HR weeds relative to glufosinate use are discussed below.

Herbicides are highly effective in the control of crop weeds, however, they impart selection pressure on plants inherently resistant to the herbicide active ingredient (a.i.), or plants capable of developing resistance, resulting in survival of those particular plants. Inherently resistant plants occur naturally within weed populations. They differ slightly in genetic makeup from the rest of the population but remain reproductively compatible. HR plants are normally present in a weed population in extremely small numbers; about 1 in 100,000 to less than 1 in 1,000,000 (Campbell et al. 2015). The repeated use of one herbicide—a single mode of action (MOA)—allows these few resistant plants to survive and reproduce; thereby selecting for the naturally resistant weeds.[10] The number of resistant plants then increases in the population.

Weed populations can also “evolve” resistance to an herbicide a.i., where the weed adapts to an external chemical stressor. Evolved resistance is broadly classified into two types; target-site resistance (TSR) and

[10] The MOA is the unique biological mechanism at the cellular/molecular level by which an herbicide is lethal to a plant.

non-target-site resistance (NTSR). TSR mechanisms involve mutation(s) in the target site of action of an herbicide, resulting, for instance, in an insensitive or less sensitive target protein (Jugulam and Shyam 2019). A mutation in a gene can cause a minor change in a protein's structure resulting in an herbicide no longer being able bind and alter the protein's function, rendering the plant "resistant" to the herbicide (e.g., (Yang et al. 2016; Rey-Caballero et al. 2017)). Additionally, TSR can also evolve as a result of the over-expression of the target gene (Jugulam and Shyam 2019).

NTSR to herbicides in weeds can be conferred as a result of the alteration of one or more physiological processes, such as impeded herbicide absorption or translocation, sequestration of the herbicidal compound, and/or increased metabolism/breakdown of the herbicide. The mechanisms of NTSR are generally more complex to decipher than TSR. Development of NTSR can also impart cross-resistance to herbicides with different modes of action, and complicate resistance management strategies (Jugulam and Shyam 2019).

Over-reliance on herbicides for weed control in lieu of non-chemical methods, and continued issues with the development of HR weed populations, has generated considerable attention on how to best utilize herbicides so as to sustain their longevity and prevent the development of HR weed populations (e.g., (Duke 2015; Owen 2016; Heap and Duke 2018; Beckie et al. 2019; Korres et al. 2019), and others). Currently, 48 states report the presence of HR weed populations (Heap 2020). This is not a recent concern, nor is it unique to HR crops. Herbicide resistant weed populations have been occurring since the advent of chemical herbicides in the 1950s. As illustrated in Figure 4-7, significant increases in HR weed populations began to occur in the mid-1980s. Currently, there are approximately 165 unique cases of HR weeds in the United States (weed species by herbicide mode of action (MOA)) (Heap 2020).

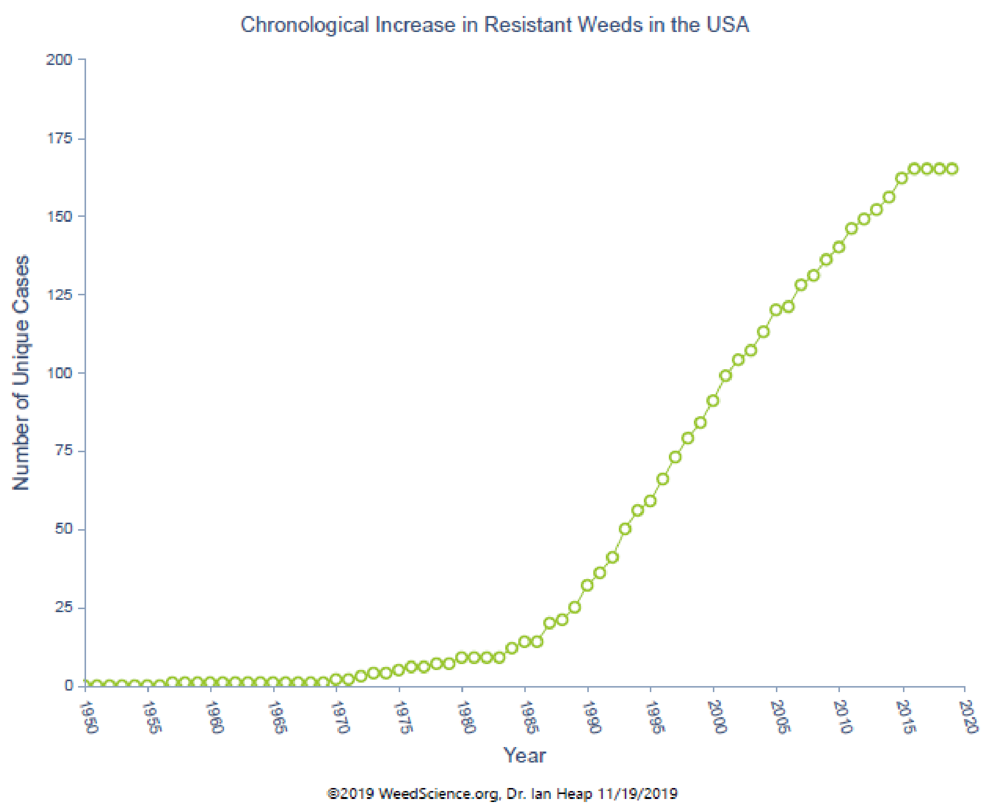

Figure 4-7. HR Weeds in the United States, 1950 – 2020

Source: (Heap 2020)

Globally, weeds have developed resistance to 23 of the 26 herbicide MOAs that are currently available, which reduces the number of herbicide options available to growers (Figure 4-8). Most corn growing states have from around 3 to 8 different species of weeds that are herbicide resistant (Heap 2020). There have been no herbicides with completely novel MOAs developed and commercialized over the last several decades. Consequently, there are no herbicides with new MOAs with which to control HR weeds.

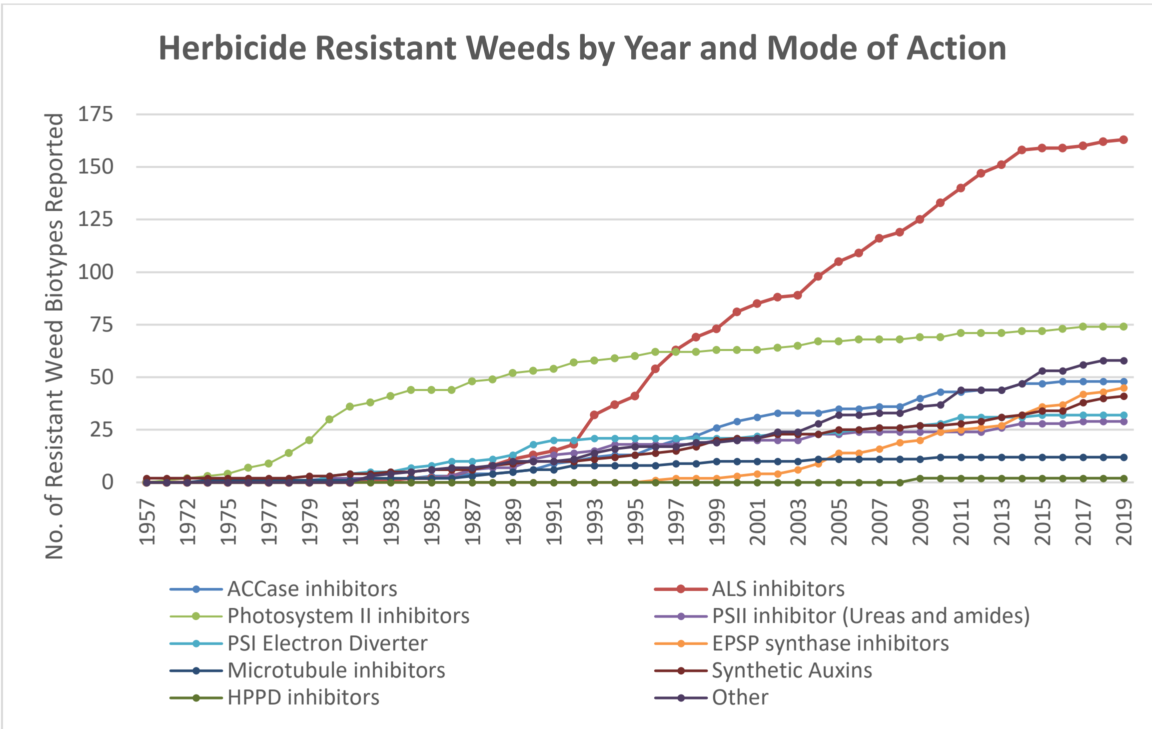

Figure 4-8. Increase in the Development of Herbicide Resistance: Herbicide Modes of Action

The herbicide groups with the most HR weeds are acetolactate synthase (ALS) inhibitors (i.e., imidazolinone and imazethapyr); ACCase inhibitors (i.e., phenylpyrazoline); triazine based photosynthesis II inhibitors (i.e., atrazine); synthetic auxins (i.e, dicamaba, 2,4-D); bipyridilium based photosynthesis I inhibitors such as paraquat; glycines, which include the EPSP synthase inhibitor glyphosate; various ureas and amides that inhibit the photosynthesis II process; and dinitroaniline based microtubule inhibitors such as trifluralin. Source: (Heap 2020)

Problematic is that fact that many HR weed populations continue to develop resistance to more than one herbicide MOA. For example, in U.S. corn crops (as of the end of 2020), there were 16 instances with a weed population developing resistance to 2 MOAs, 5 confirmed weed populations with resistance to 3 MOAs, 3 with confirmed resistance to 4 MOAs, and 1 species (tall waterhemp) with confirmed resistance to 5 herbicide MOAs (Heap 2020). Relative to DP23211 corn: To date there are only two weed species reported to be resistant to glufosinate-ammonium in the United States; Italian ryegrass (*Lolium perenne* ssp. *multiflorum*), with populations reported in orchards in California and Oregon, and Palmer Amaranth (*Amaranthus palmeri*), with a population reported in Arkansas' Mississippi County, this being discovered in 2020 (Heap 2020; AgWeb 2021).

Herbicide Resistant Weed Management

Strategies for managing weeds and avoiding the development of HR weed populations in U.S. agriculture are steadily being refined (e.g., (Duke 2015; Owen 2016; Heap and Duke 2018; Beckie et al. 2019; Korres et al. 2019), and others). A combination of preventive, cultural, mechanical, biological, and chemical methods are required for effective weed, and weed resistance management. The coordinated use of these is termed integrated weed management (IWM). Crop producers are advised to, and are implementing, IWM strategies to address development of HR weeds—strategies developed by the crop protection and seed industries, the USDA, university extension services, the EPA, and Weed Science Society of America (WSSA). In 2017, the EPA issued PR Notice 2017-2, *Guidance for Herbicide-Resistance Management, Labeling, Education, Training and Stewardship* (US-EPA 2017b), which provides registrants and growers detailed information on slowing the development and spread of HR weeds. The EPA focused on and provided the guidance for herbicide use because: (1) herbicides are the most widely used agricultural chemicals, (2) no new herbicide mechanisms MOAs have been developed in the last 30 years, (3) the number of HR weeds has rapidly increased over the last 30 years, and (4) the potential economic impact of HR weeds on U.S. crop production are significant (US-EPA 2017b).

4.3.1.2.2.4 Insect and Insect Resistance Management

Corn and other crop plants modified to express insecticidal Bt based Cry toxins can provide effective pest control as well as economic gains for farmers (James 2014; Brookes and Barfoot 2018b). Reductions in insecticide use with insect resistant (IR) crops has also resulted in environmental and worker health benefits (James 2014; Brookes and Barfoot 2018b). Due to the benefits provided by Bt crops there have been significant levels of adoption; Bt corn currently comprises around 80% of U.S. corn crops, and Bt cotton 89% (USDA-ERS 2022a).

Studies conducted by USDA-ERS (Fernandez-Cornejo et al. 2014a; Fernandez-Cornejo et al. 2014b), the National Academy of Sciences (NAS 2016), and others (Osteen and Fernandez-Cornejo 2016; Fleming et al. 2018) have found that soil-applied and foliar insecticide use—in terms of lbs a.i./acre—has declined in corn production due in part to the adoption of Bt corn (Figure 4-9). For example, insecticide use among U.S. corn farmers fell by over 80% from 1996 to 2018 (Fernandez-Cornejo et al. 2014c; USDA-NASS 2020a). A combination of use of integrated pest management (IPM) strategies, the success of the boll weevil eradication program, and introduction of Bt crops led to a significant reduction in the number of insecticide applications. Insecticide use with corn, which peaked in the late 1970s and 1980s at an average 0.35 – 0.45 pounds per acre, declined throughout the 1990s and 2000s to an average of under 0.03 pounds per planted acre in 2018 (latest data). As of 2018, 80% of U.S. corn acres were planted with IR varieties, only 13% of corn acres were treated with insecticides (USDA-NASS 2019a; USDA-ERS 2022b). Insecticide use on corn and cotton are at near all-time lows, averaging around 0.02 – 0.03 lbs a.i./acre, and 0.29 – 0.35 lbs a.i./acre, respectively (USDA-NASS 2020a). Soil and foliar applied insecticides currently use in U.S. corn production are provided in Table 4-5.

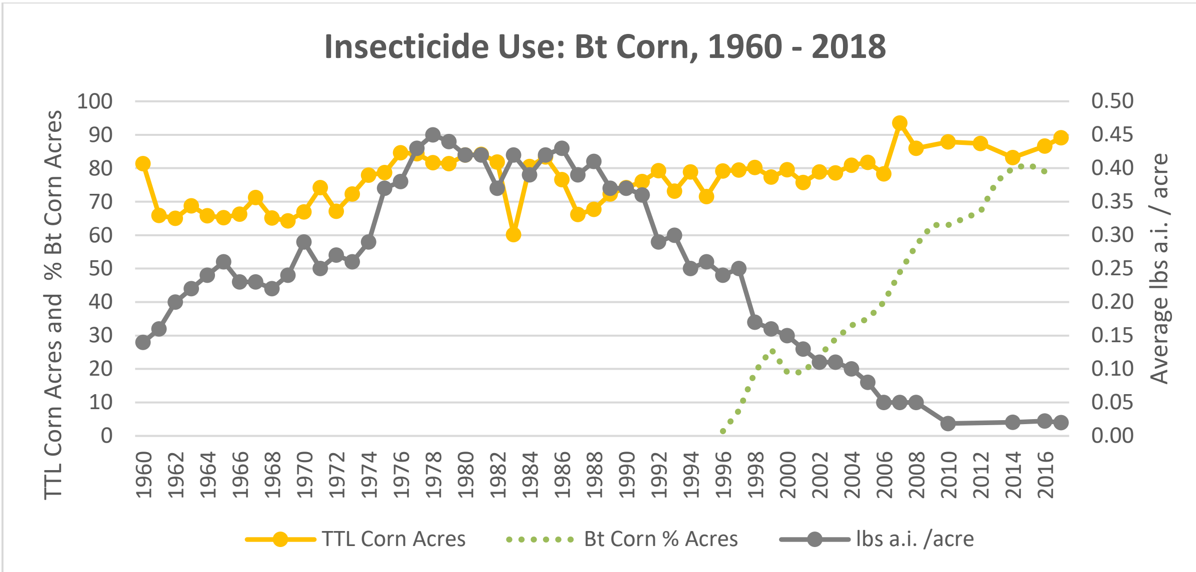

Figure 4-9. Insecticide Use in Bt Corn Production

Source: (Fernandez-Cornejo et al. 2014c; USDA-NASS 2020a)

Table 4-6. Soil and Foliar Applied Insecticide Use in Corn – 2018

Insecticide Active Ingredient	Class	Pounds a.i./yr	Application: lbs a.i./acre/Yr (Average)	Treated Acres, % of Area Planted	Portion of Total Insecticide Use
Bifenthrin	pyrethroid	402,000	0.089	5	19.3%
Chlorantraniliprole	ryanoid	6,000	0.062	< 1	0.3%
Chlorpyrifos	organophosphate	623,000	0.818	1	29.9%
Cyfluthrin	pyrethroid	10,000	0.015	1	0.5%
Lambda-cyhalothrin	pyrethroid	59,000	0.026	3	2.8%
Permethrin	pyrethroid	31,000	0.06	1	1.5%
Propargite	organosulfur	587,000	2.031	< 1	28.1%
Spiromesifen	butenolide	11,000	0.153	< 1	0.5%
Tebupirimphos	organothiophosphate	45,000	0.109	< 1	2.2%
Tefluthrin	pyrethroid	158,000	0.1	2	7.6%
Terbufos	organophosphate	7,000	0.073	< 1	0.3%
Zeta-Cypermethrin	pyrethroid	17,000	0.017	1	0.8%
TOTAL		**2,087,000**		**13%**	

* Does not include seed treatments

Source: (USDA-NASS 2020a)

In areas where cultivation of Bt corn and Bt cotton is high, the use of Bt crop varieties has also been associated with reduced insecticide use in adjacent cropping systems cultivating non-Bt varieties, a result of the area-wide suppression of insect pest populations (NAS 2016). For example, several studies have found that the use of Bt corn and Bt cotton are positively associated with the area-wide suppression of European corn borer and pink bollworm, respectively (e.g., see review by Dively et al. (2018)). In general, peer review literature and similar studies indicate that cultivation of Bt crops can potentially provide tangential benefits to adjacent farms by tempering the prevalence of certain insect pest populations, reducing the need for insecticide use in nearby cropping systems (NAS 2016)).

While IR crops can provide agronomic and economic benefits, they can also create selection pressure on insect populations inherently resistant to transgenic Bt trait proteins (discussed further below).

Insect Resistant Management in Insect Resistant Crops

Similar to HR weeds, continued exposure of insects to insecticides can result in the development of resistance in populations. The first documented case of insect resistance to an insecticide was in 1914, which described the resistance of the San Jose scale (*Quadraspidiotus perniciosus*) to lime-sulfur (Melander 1914). In 1916, resistance to hydrogen cyanide fumigant was identified in a citrus pest, the California red scale (*Aonidiella auruntii* (Maskell)), and in the black scale (*Saissetia oleae* (Olivier)) (Melander 1914; Quayle 1922). With the steady introduction and use of synthetic chemical insecticides in insect control programs, the number of documented cases of insecticide resistance, worldwide, had increased to 600 species by 2017 (Mota-Sanchez and Wise 2017).

The risk of resistance development is, as with synthetic chemical insecticides, an important issue for crop plants engineered to express insecticidal proteins—plant incorporated protectants (PIPs) (Fleming et al. 2018).[11] While there are agronomic, environmental, and economic benefits in the use of IR cropping systems, the potential for the development of insect populations resistant to PIPs is present and has occurred in some areas. For example, resistance of corn earworm (*Helicoverpa zea*) to Cry1Ab, Cry1Ac, and Cry1A.105+ Cry2Ab has emerged in the eastern and central Cotton Belt (Luttrell et al. 2004; Ali et al. 2006; Tabashnik et al. 2008; Bruce and Yves 2010; Dively et al. 2016). Evolved resistance of CRW to Cry3Bb1 corn, mCry3A corn, and eCry3.1Ab corn has also been documented, in multiple midwestern states (Gassmann et al. 2016; Jakka et al. 2016). Additionally, cross-resistance among Cry3Bb1, mCry3A, and eCry3.1Ab has been reported (Jakka et al. 2016).

While insects are capable of developing resistance to chemical based insecticides, for PIP based crops there can be other considerations:

- insecticidal proteins may be expressed at high levels in plant tissues;
- the proteins are produced by the plant continually during the growing season (i.e., throughout the lifespan of the plant); and
- some of the major target pests, such as European corn borer, corn rootworm, and pink bollworm, feed almost exclusively on corn or cotton.

These factors can increase insect exposure to an insecticidal protein and thereby increase selection pressure for development of resistant populations. The cases of pest resistance to Bt Cry proteins

[11] It should be noted, development of resistance is likewise possible with use of Bt sprays, one of the most widely used foliar applied insecticides used in conventional and organic crops (Tabashnik BE, Brevault T, and Carriere Y. 2013. *Insect resistance to Bt crops: lessons from the first billion acres*. Nat Biotech,Vol. 31(6), pp. 510-521. Retrieved from https://www.nature.com/articles/nbt.2597.pdf .)

produced by transgenic crops increased from 3 in 2005 to 16 in 2016 (Tabashnik and Carrière 2017). These 16 cases represent resistance of some populations of seven major pests in five countries to each of the nine Cry toxins produced by widely grown Bt crops: Cry1Ab, Cry1Ac, Cry1A.105, Cry1Fa, Cry2Ab, Cry3Bb, mCry3A, eCry3.1Ab, and Cry34/35Ab. Both corn earworm (Jakka et al. 2016) and CRW (Gassmann et al. 2016; Jakka et al. 2016) have developed resistance to multiple Cry toxins (e.g., Cry3Bb1, mCry3A, and eCry3.1Ab). For the 16 cases of resistance that have been documented, the average time for evolution of resistance was around 5.2 years (Tabashnik and Carrière 2017).

The use of Bt based crops has been effective in the control of crop pests, although the efficacy wanes when a pest population adapts to the mechanism of action of the particular introduced PIP, and becomes less susceptible. Thus, when not judiciously utilized, the PIPs in IR crops decline in efficacy over time, while also contributing to increasingly limited pest management options for IR, conventional, and organic cropping systems. For instance, Bt based insecticides (Cry toxins) are widely used in organic farming, either as a spray or ground application to help control insect pests, and one of the few insecticides permitted by USDA organic standards.

Effective insect resistance management (IRM) is a basic aspect of IR cropping systems (Tabashnik and Carrière 2017). Several strategies, such as the use of multiple Cry proteins (PIPs) in transgenic crops, spatial and temporal refuges, and high or ultrahigh doses of Cry protein are employed to prevent the development of insect resistance. The IRM strategy that has received the most attention involves a "high dose/refuge" (HDR) concept (Bates et al. 2005; Siegfried and Hellmich 2012). With this approach, insects that feed on Bt crops are exposed to a high dose of toxin. This is complemented with a refuge, a non-Bt crop variety or other plant hosts, which supports a population of unexposed insects, thereby eliminating selection pressure on those insect populations. Resistant insect pest populations that develop as a result of exposure to Cry toxins, instead of mating with each other, are able to mate with individuals among a large number of non-resistant pests from the refuge. This process essentially dilutes resistance genes in populations and sustains populations of susceptible insects (Bates et al. 2005; Siegfried and Hellmich 2012).

Other strategies include the use of combinations of different Cry proteins, proteins that have different cell surface receptors or different mechanisms of action (Reisig and Kurtz 2018), use of RNAi based PIPs (Ni et al. 2017; Vélez et al. 2020), and use of insecticidal non-Bt based proteins (Boeckman et al. 2019). DP23211 corn utilizes the latter two, RNAi and a non-Bt toxin.

To help counter the development of insect resistance the EPA has mandated the implementation of an IRM plan for each commercially registered Bt derived PIP (US-EPA 2020h). The goal of an IRM plan is to prevent or delay the development of resistant insect populations. In 2017, the EPA issued PRN 2017-1, *Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling* (US-EPA 2017a) for all conventional pesticides and resistance management. The EPA also requires that any incidents of pest resistance for a regulated pesticide product be reported to the EPA. This reporting requirement is in accordance with the FIFRA Adverse Effects Reporting Section 6(a)(2), which requires pesticide product registrants to submit adverse-effects information about their products.

In 2018, the USDA announced updated guidance to the National Road Map for Integrated Pest Management (USDA 2018). The update is the product of the Federal Integrated Pest Management

Coordinating Committee (FIPMCC), a joint effort that is coordinated by the Office of Pest Management Policy in the Office of USDA's Chief Economist with representatives of all federal agencies with responsibilities in IPM research, implementation, or education programs. These agencies include Environmental Protection Agency (EPA), Department of the Interior (DOI), and Department of Defense (DoD).

4.3.1.3 Potential Effects on U.S. Corn Production

Potential Effects on Land Use

Approval of the petition and subsequent production of DP23211 corn would have little to no effect on lands used for U.S. corn production. U.S. corn acreage is primarily determined by market demand for corn-based food, feed, fuel, and industrial commodities, independent of APHIS' regulatory status decision. Acreage could also be determined by the yields/acre achieved over time with given corn varieties, which in turn is determined by the inherent potential yield of a given corn cultivar, pest and weed pressures present, the potential contribution of HR and IR traits to effectively controlling weeds and insect pests (which also contributes to yield), weather, and agronomic production factors. DP23211 corn exhibited slightly lower yield per acre than the control corn in field trials, although is within the normal range for dent corn varieties (Pioneer 2020). DP23211 corn, if adopted by growers, would be expected to replace other HR/IR field corn varieties currently cultivated, as opposed to augmenting current corn crops.

Agronomic Practices and Inputs

Pioneer conducted agronomic and ecological evaluations to assess the comparability of DP23211 corn to conventional corn (Pioneer 2020). Agronomic characteristics evaluated included factors that influence reproduction, growth, and crop survival. In each field trial DP23211 corn was comparable to the conventionally bred, near-isoline control corn or conventional comparators, with the exception of the days to flowering and final population metrics. The mean values for days to flowering and final population are, however, within the reference range for *Zea mays* varieties (Anderson et al. 2020). Ecological evaluations included responses to biotic and abiotic stressors during multi-year and multi-site field trials. Observations included the presence of insect and disease stressors in the field and DP23211 corn responses. In each field trial DP23211 corn performed similarly to the conventionally bred, near-isoline control corn plants.

These studies demonstrated that the agronomic practices and inputs used for DP23211 corn would be similar to those of other corn varieties, except for, as an HR variety, facilitating the use of glufosinate, and as an IR variety, less reliance on use of synthetic chemical insecticides for insect pest control. As an IR corn variety comprised of plant incorporated protectants (PIPs) with specify for WCR, cultivation of DP23211 corn would likely entail less use of soil- and foliar-applied insecticides to control WCR.

As summarized in Table 4-4, glufosinate is one of around 48 herbicides used in corn production, comprising 0.23% of total herbicide use on corn in 2018. Of 19 HR corn varieties APHIS has previously deregulated, 11 are glufosinate resistant (USDA-APHIS 2022a). There are several glufosinate resistant corn products currently available to growers (e.g., LibertyLink®, Genuity™ SmartStax™). DP23211 corn would present growers another option among currently available HR/IR varieties. Corn growers will select a particular HR/IR variety based on insect and weed populations present; resistant insect and weed populations; the potential for high yield; efficacy of the PIP and herbicide(s) used with the crop; costs of pesticide inputs; and ease and flexibility in management of insect pests and weeds. At the national level, HR/IR corn varieties have emerged as the most favored by growers, currently comprising around 80% of U.S. corn acres (Figure 4-10). Note that adoption rates will vary at the state level on an annual basis due to weed and pest pressures, among other factors.

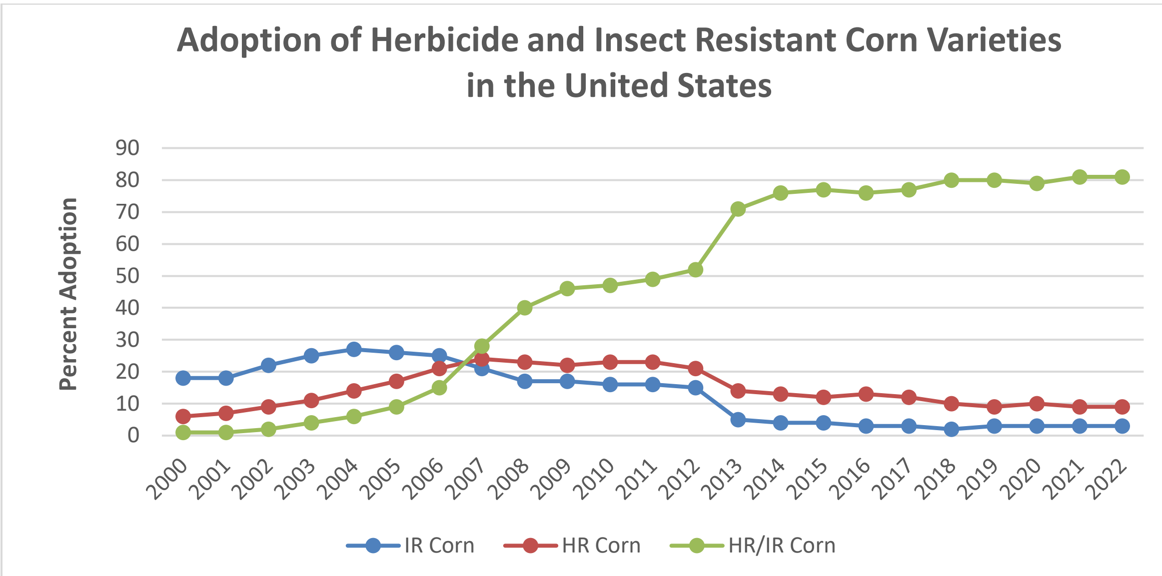

Figure 4-10. Share of Corn Acreage Planted with Stacked-Trait HR/IR Seed – 2022
Source: (USDA-ERS 2022a)

In the event DP23211 corn is available for use growers would have the option to (1) produce DP23211 corn in lieu of other glufosinate resistant varieties they are currently cultivating, or (2) produce DP23211 corn in lieu of HR varieties that are resistant to herbicides other than glufosinate. To the extent growers do the latter, an increase in glufosinate use and decrease/substitution in use of other herbicides with similar spectrums of weed control would occur in corn production. Thus, DP23211 corn production, to the extent grown, would influence the mix of herbicides used on U.S. corn acres. No significant increase in total herbicide use on corn would be expected in either case (e.g., Table 4-4). Annual fluctuations in total herbicide use are expected due to variances in the use rates in lbs a.i./acre/year among herbicides, herbicide rotation, weed and HR weed pressures, and annual acreage planted to corn.

Weed and HR Weed Management

The development of HR weeds continues in many areas of the United States and is problematic for growers (Heap 2020). Most corn growing states have from 3 to 8 different species of weeds that are herbicide resistant (e.g., Iowa has 8, with tall waterhemp and giant ragweed particularly problematic (Heap 2020)). Glufosinate is considered a valuable herbicide for grower's in the management of weeds, and weed resistance (US-EPA 2016a; Takano and Dayan 2020). This is attributed to its MOA (a glutamine synthetase inhibitor), and the fact that there are only two reported weed species in the United

States that have developed resistance; Italian ryegrass (*Lolium perenne* ssp. *multiflorum*), with populations in orchards in California and Oregon, and Palmer amaranth (*Amaranthus palmeri*), with a population in Arkansas' Mississippi County reported in 2020 (Heap 2020; AgWeb 2021).

Certain herbicide MOAs are more susceptible to development of resistance. For example, development of resistance to ALS-inhibitors is the most common. As of the end of 2020, 166 cases of weeds resistance to ALS-inhibitors had been reported worldwide (Heap 2020), a trend that is attributed in part to the relative ease with which plants can evolve resistance to its MOA. Several single amino acid substitutions that are sufficient to confer resistance to ALS-inhibitors have been identified in genes (Holt et al. 2013). In contrast, development of resistance to the PSII-inhibitor herbicide group is considered more difficult (29 reported cases worldwide) because in most cases reported resistance results from a specific base pair substitution in the *psbA* gene—from adenine in the susceptible to guanine in the resistant biotype—which is maternally inherited (Hirschber et al. 1984; Holt et al. 2013). Currently, there are only five reported weed species, worldwide, developing resistance to glufosinate—glutamine synthetase inhibition (Heap 2020; AgWeb 2021). While development of glufosinate resistance in weedy plants has occurred, it appears the target and non-target pathways by which plants can evolve resistance to glutamine synthetase inhibition are comparatively limited, relative to other herbicide MOAs (Takano and Dayan 2020).

Preventing the development of glufosinate resistance weed populations—sustaining the efficacy of the herbicide MOA—would be relative to implementation of EPA resistance management guidance (US-EPA 2017b), Pioneer product stewardship requirements (Pioneer 2019), and use of recommended IWM strategies in DP23211 corn cropping systems (e.g., (Heap and Duke 2018; Beckie et al. 2019; Gage et al. 2019)). Corteva Agriscience, the parent company of Pioneer, is a member of the global Herbicide Resistance Action Committee (HRAC 2020), an industry-based group administrated by CropLife International (CLI 2020). HRAC supports and equips regional offices to help educate farmers, agronomists, and industry members in the management of herbicide-resistant weeds. In 2017 the EPA issued PR Notice 2017-2, *Guidance for Herbicide-Resistance Management, Labeling, Education, Training and Stewardship* (US-EPA 2017b), which provides registrants and growers information on slowing the development and spread of HR weeds. In addition, the EPA has issued specific guidance for glufosinate resistance management (US-EPA 2016b).

Insect Pest Management

Insects are highly adaptable and, over time, can evolve resistance to any single control method; synthetic chemicals, Bt toxins, or RNAi based mechanisms (Mallet 1989; Tabashnik and Carrière 2017). Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is a major pest of corn in the United States and has adapted over time to many management tactics (Gray et al. 2009; Miller et al. 2009). Broadcast application of organochlorine insecticides starting in the late 1940's became rapidly ineffective with WCR control issues apparent by late 1950's (Ball and Weekman 1962). Aerial applications of carbamates and organophosphates selected for adult WCR resistance in the 1990's (Melander 1914). Resistance to transgenic corn producing rootworm-specific Cry toxins was initially reported for Cry3Bb1 in 2009, mCry3a in 2011, and Cry34/35Ab1 in 2016 (Gassmann et al. 2016; Jakka et al. 2016). The practice of corn rotation with a nonhost crop was circumvented in areas of the eastern Corn Belt by WCR oviposition in nonhost crops, indicating the evolution of behavioral resistance (Levine et al. 2002; Gray et al. 2009;

Meinke et al. 2009). These events collectively have made WCR management in U.S. cropping systems very difficult (Souza et al. 2019).

A basic element of insect resistance management (IRM) is the implementation of several different types of control measures (Karlsson Green et al. 2020). RNAi is utilized as an alternative, or complement, to Bt toxins in IR crops to help diversify control methods (MOAs), and thereby reduce selection pressure for resistance in insect populations (Kim et al. 2015; Head et al. 2017; Ni et al. 2017). Similarly, the IPD072Aa protein provides a MOA that differs from Cry toxins. Because the MOAs so markedly differ between Bt toxins (cell surface receptor recognition and binding) and RNAi (targeted mRNA cleavage and disruption of mRNA translation), development of cross-resistance is unlikely. Development of dual-resistance to both a Bt based toxin and RNAi is conceptually possible (Vogel et al. 2019), although the topic has not been well studied (i.e., controlled lab and field studies). In general, development of dual-resistance to a Cry toxin and RNAi mechanism would be considered an unlikely event. Based on current data, development of cross-resistance to Bt toxins and the IPD072Aa protein would not be expected (Schellenberger et al. 2016; Boeckman et al. 2019).

Current data indicate that RNAi can be highly species-specific if the dsRNA construct is well-designed (Agrawal et al. 2003; Vogel et al. 2019; Christiaens et al. 2020). The DvSSJ1 dsRNA expressed in DP23211 corn has been shown to primarily affect *Diabrotica* species within the Chrysomelidae family of Coleoptera—namely WCR and southern corn rootworm (SCR) (Hu et al. 2019). WCR is the most sensitive species, based on 14 day feeding bioassays, with a median LC50 of 0.036 ng/mg diet (parts per million (ppm)) (Table 4-7). Of the other Coleoptera and Lepidoptera species evaluated, SCR was observed to be the only species affected, with decreased survival at a dietary concentration of 100 ng/ml (ppm)—much less sensitive/susceptible than WCR. The remaining coleopteran and lepidopteran species evaluated were unaffected at diets containing 1 ng/mg DvSSJ1 dsRNA, and unlikely to be affected feeding on DP23211 corn under field conditions (Roper 2019). For example, expression of DvSSJ1 dsRNA is around 0.001 to 0.007 ng/mg in grain, 0.002 to 0.038 ng/mg in leaf, and 0.0002 - 0.009 ng/mg in root (Table 4-8).

Note that because expression of DvSSJ1 dsRNA in DP23211 corn is low, it was necessary to produce RNA microbially using in vitro techniques in order to obtain enough DvSSJ1 dsRNA to perform specificity analyses. Sequence analyses confirmed that the microbially produce dsRNA was equivalent to the dsRNA produced *in planta*, and suitable for use in specificity studies (Pioneer 2020).

Table 4-7. Effects of DvSSJ1 dsRNA on Coleoptera and Lepidoptera Species

Order	Family	Species	Common Name	Endpoints[a]	LC50 or NOEC
Coleoptera	Chrysomelidae	*Diabrotica virgifera vergifera*	Western Corn Rootworm	S, W	LC50 = 0.036 ng/mg
	Chrysomelidae	*Diabrotica undecimpunctata*	Southern Corn Rootworm	S, W	100 ng/ml
	Chrysomelidae	Leptinotarsa decemlineata	Colorado Potato Beetle	S, W	>1.0 ng/mg[b]
	Tenebrionidae	*Tenebrio molitor*	Mealworm	S, W	>1.0 ng/mg
	Tenebrionidae	*Zophobas morio*	Superworm	S, W	>1.0 ng/mg
	Tenebrionidae	*Tribolium castaneum*	Red Flour Beetle	S, W	>1.0 ng/mg

	Coccinellidae	*Epilachna varivestis*	Mexican Bean Beetle	S, W	>1.0 ng/mg
	Coccinellidae	*Hippodamia convergens*	Convergent Lady Beetle	S, W, AE	>1.0 ng/mg
	Coccinellidae	*Coleomegilla maculata*	Pink Spotted Lady Beetle	S, W, AE	>1.0 ng/mg
	Staphylinidae	*Dalotia coriaria*	Rove Beetle	S	>1.0 ng/mg
Lepidoptera	Crambidae	*Ostrinia nubilalis*	European Corn Borer	S, W	>1.0 ng/mg
	Noctuidae	*Helicoverpa zea*	Corn Ear Worm	S, W	>1.0 ng/mg
	Nymphalidae	*Vanessa cardui*	Painted Lady	S, W	>1.0 ng/mg
	Tortricidae	*Cydia pomonella*	Codling Moth	S, W	>1.0 ng/mg

a. S = Survival; W = Weight; AE = Adult Emergence

b. For all species studied except for western corn rootworm and southern corn rootworm, there were no adverse effect observed (NOEC) at 1ng DvSSJ1_210 dsRNA per mg diet

Source: (Pioneer 2020)

Table 4-8. DvSSJ1 dsRNA Tissue Expression Levels in DP23211 Corn

	ng/mg Tissue Fresh Weight
	(parts per million)
Root	0.00019 - 0.00877
Leaf	0.00177 - 0.0379
Grain	0.00102 - 0.0073
Pollen	0.000330 – 0.00096

Boeckman et al. (2019) evaluated the spectrum of activity of IPD072Aa in diet bioassays. The IPD072Aa protein used in these assays was microbially produced, versus *in planta*, although the activity of microbially produced and *in planta* IPD072Aa is considered equivalent (Pioneer 2020). IPD072Aa was fed at high concentrations in sub-chronic and chronic feeding studies to 11 different Coleoptera species representing four families, and an additional four species representing four families of Lepidoptera (Table 4-9). No adverse effects were noted in the Lepidoptera species. A range of responses was observed within each of the four families of Coleoptera evaluated, which included either no-observed effects, or reduced growth, developmental delays, and/or reduced survival. The range of expression levels of IPD072Aa protein in DP23211 corn tissue, over the course the DP23211 corn life cycle, are provided in Table 4-10.

Table 4-9. IPD072Aa Protein Spectrum of Activity Bioassay Evaluation

Order	Family	Species	Common Name	Endpoints[a]	LC50, Effects Concentration, or NOEC
Coleoptera	Chrysomelidae	*Diabrotica virgifera vergifera*	Western Corn Rootworm	S	LC50 = 26 ng/mg diet
Coleoptera	Chrysomelidae	*Diabrotica undecimpunctata*	Southern Corn Rootworm	S* W*	1000 ng/mg diet 100 ng/mg diet

Coleoptera	Chrysomelidae	*Leptinotarsa decemlineata*	Colorado Potato Beetle	S W	1000 ng/mg diet 1000 ng/mg diet
Coleoptera	Tenebrionidae	*Tenebrio molitor*	Mealworm	S* W*	1000 ng/mg diet 500 ng/mg diet
Coleoptera	Tenebrionidae	*Zophobas morio*	Superworm	S W*	1000 ng/mg diet[b] 100 ng/mg diet
Coleoptera	Tenebrionidae	*Tribolium castaneum*	Red Flour Beetle	S, W	No adverse effect[c]
Coleoptera	Coccinellidae	*Epilachna varivestis*	Mexican Bean Beetle	S* W*	500 ng/mg diet 100 ng/mg diet
Coleoptera	Coccinellidae	*Hippodamia convergens*	Convergent Lady Beetle	S* W* AE*	1000 ng/mg diet 100 ng/mg diet 500 ng/mg diet
Coleoptera	Coccinellidae	*Coleomegilla maculata*	Pink Spotted Lady Beetle	S* W* AE*	500 ng/mg diet 500 ng/mg diet 500 ng/mg diet
Coleoptera	Coccinellidae	*Cryptolaemus montrouzieri*	Mealybug Destroyer	S W	No adverse effect[c] 100 ng/mg diet[d]
Coleoptera	Staphylinidae	*Dalotia coriaria*	Rove Beetle	S	No adverse effect[c]
Lepidoptera	Crambidae	*Ostrinia nubilalis*	European Corn Borer	S, W	No adverse effect[c]
Lepidoptera	Noctuidae	*Helicoverpa zea*	Corn Ear Worm	S, W	No adverse effect[c]
Lepidoptera	Nymphalidae	*Vanessa cardui*	Painted Lady	S, W	No adverse effect[c]
Lepidoptera	Tortricidae	*Cydia pomonella*	Codling Moth	S, W	No adverse effect[c]

a. S = Survival, W = Weight, AE = Adult Emergence. Those with an asterisk (*) were statistically significant at the concentrations reported

b. 20% mortality was reported at 1000 ng/mg diet, although not statistically significant relative to the control

c. No adverse effects on survival or weight at 1000ng/mg diet

d. Weight gain was statistically lower for MBD in the 100 ng/mg concentration (P = 0.025); however, no effects were observed at 500 (P = 0.167) and 1,000 ng/mg (P = 0.468) IPD072Aa

Source: (Boeckman et al. 2019; Pioneer 2020)

Table 4-10. IPD072Aa Tissue Expression Levels in DP23211 Corn

	ng/mg Tissue Dry Weight (parts per million)
Root	0.93 - 84
Leaf	0.054 - 39
Grain	0.51 – 4.8
Pollen	0.14 – 1.3

The bioassay data indicate that IPD072Aa protein was shown to primarily affect WCR. This is due to binding specificity for mid-gut epithelial cell surface receptors in western corn rootworm—specifically for the brush border membrane of enterocytes—although the exact mechanism of binding has not yet been clearly elucidated (Pioneer 2020). Once in the midgut, the IPD072Aa protein dimer disassociates into a monomeric form which binds to receptors on the brush border membrane. After binding, disruption of gut function caused by death of enterocytes leads to WCR death. Lethal concentrations ranged from 12 to 39 ng/mg IPD072Aa, with a mean of 26 ng/mg (Boeckman et al. 2019).

The closely related southern corn rootworm also exhibited sensitivity to IPD072Aa, although at a much higher dose, where 17% of treated insects exhibited mortality at 500 ng/mg IPD072Aa. Reductions in weight were observed and statistically significant at dietary concentrations of 100 ng/mg and higher. No effects were observed on the survival or growth of Colorado potato beetle (*Leptinotarsa*) at concentrations up to 1,000 ng/mg.

Within Tenebrionidae, survival of super worm (*Zophobas morio*) was unaffected when fed 1,000 ng/mg IPD072Aa; however, mean weight was lower at concentrations 100 ng/mg and higher (Boeckman et al. 2019). Survival of yellow mealworm (*Tenebrio molitor*) was affected at 1,000 ng/mg, and weight affected at 500 ng/mg IPD072Aa. IPD072Aa had no effects on red flour beetle (*Tribolium castaneum*) at the concentrations tested.

Species within the Coccinellidae showed varying levels of sensitivity to IPD072Aa. Mexican bean beetle (*Epilachna varivestis*) was the most sensitive Coccinellidae insect tested—statistically significant effects on survival at 500 ng/mg IPD072Aa were observed. Reduced growth was observed in Mexican bean beetle fed 100 ng/mg IPD072Aa. The Mexican bean beetle is one of the few North American lady beetles that feed on plants rather than other insects and can be a significant agricultural pest in legume crops.

Pink spotted lady beetles (*Coleomegilla maculata* De Geer) and convergent lady beetle (*Hippodamia convergens*) were both less sensitive to IPD072Aa than WCR, but affected at high concentrations. Convergent lady beetle exhibited reduced growth at 100 ng/mg, and adverse effects on survival were statistically significant at 1,000 ng/mg. For Pink spotted lady beetles, statistically significant reduced survival, weight, and time to emergence was observed at 500 ng/mg. No effects of IPD072Aa were observed on mealybug beetle (*Cryptolaemus montrouzieri*) at the concentrations evaluated. One representative of the Staphilinidae family, greenhouse rove beetle (*Dalotia coriaria*) was evaluated and no effects were observed up to a concentration of 1,000 ng/mg IPD072Aa.

Pink spotted lady beetles and convergent lady beetle are beneficial predators and commonly found in agroecosystems feeding upon aphids and other prey species; thus, they provide a beneficial service in pest control. Potential IPD072Aa effects on non-target organisms are discussed below in Section 4.3.3.2–Animal Communities.

Schellenberger et al. (2016) also tested IPD072Aa protein, at concentrations up to 875 µg/ml (ppm) in diet assays with several lepidopteran and hemipteran species, including black cutworm (*Agrotis ipsilon* Hufnagel), corn earworm (*Helicoverpa zea* Boddie), European corn borer (*Ostrinia nubilalis* Hübner), fall armyworm (*Spodoptera frugiperda* J. E. Smith), soybean looper (*Pseudoplusia includens* Walker), and western tarnished plant bug (*Lygus hesperus* Knight). Tissue expression in DP23211 corn range from 0.054 to 84 ppm across roots, leaf, grain, and pollen (Table 4-10). No mortality or inhibitory effects on any of these species were reported.

Current studies indicate the activity of IPD072Aa has been shown to be limited to the order Coleoptera. Western corn rootworm was the most sensitive species tested, followed by Mexican bean beetle, for mortality was affected at concentrations approximately 10 times greater than those required for western corn rootworm. Other representatives of the Coccinellidae showed varying levels of sensitivity to IPD072Aa. Statistically significant effects on survival for Convergent lady beetle (*Hippodamia convergens*) and pink spotted lade beetles (*Coleomegilla maculata*) were observed at concentrations > 500 ng/mg diet. Southern corn rootworm (*Diabrotica undecimpunctata*) and yellow mealworm (*Tenebrio molitor*) likewise exhibited sensitivity, with survival affect at concentrations 1,000 ng/mg diet. Where effects on survival in these species were observed at > 500 ng/mg diet, these are dietary concentrations that are unlikely to be achieved in the field, considering the tissue expression levels summarized in Table 4-10.

While microbially produced IPD072Aa protein was used in the spectrum of activity studies, functional equivalency data shows that the activity of IPD072Aa is equivalent between *in planta* and microbially produced IPD072Aa protein (Pioneer 2020).

Based on these data, DP23211 corn is effective in control of WCR, with limited potential to affect other pest species. DP23211 corn was found to be comparable to control corn lines in respect to disease response (Pioneer 2020). APHIS has not identified any significant changes to agronomic practices or inputs, nor the DP23211 corn phenotype (other than the intended insecticidal activity) that would have adverse 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. Tillage, cover crops, crop rotation, irrigation, and pesticide and fertilizer inputs can influence the biological, physical, and chemical properties of soil and have a substantial impact on soil fertility and erosion (Baumhardt et al. 2015). Soil quality loss occurs through declines in soil organic matter (SOM), minerals (e.g., magnesium, calcium), essential nutrients (e.g., 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, on the order of millimeters per year, soil is considered a nonrenewable resource that requires conservation and stewardship for sustainable crop production. Soil erosion not only increases fertilizer requirements and production costs, it leads to impaired air and water quality. Soil erosion occurs in all areas of the United States but is more concentrated in those regions where the percentage of total area in cropland is highest and a larger proportion of the land is highly erodible (Magleby et al. 1995; USDA-NRCS 2010; Baumhardt et al. 2015). Excessively eroding cropland soils are concentrated in the Midwest and Northern Plain States, to include the Corn Belt (Figure 4-11).

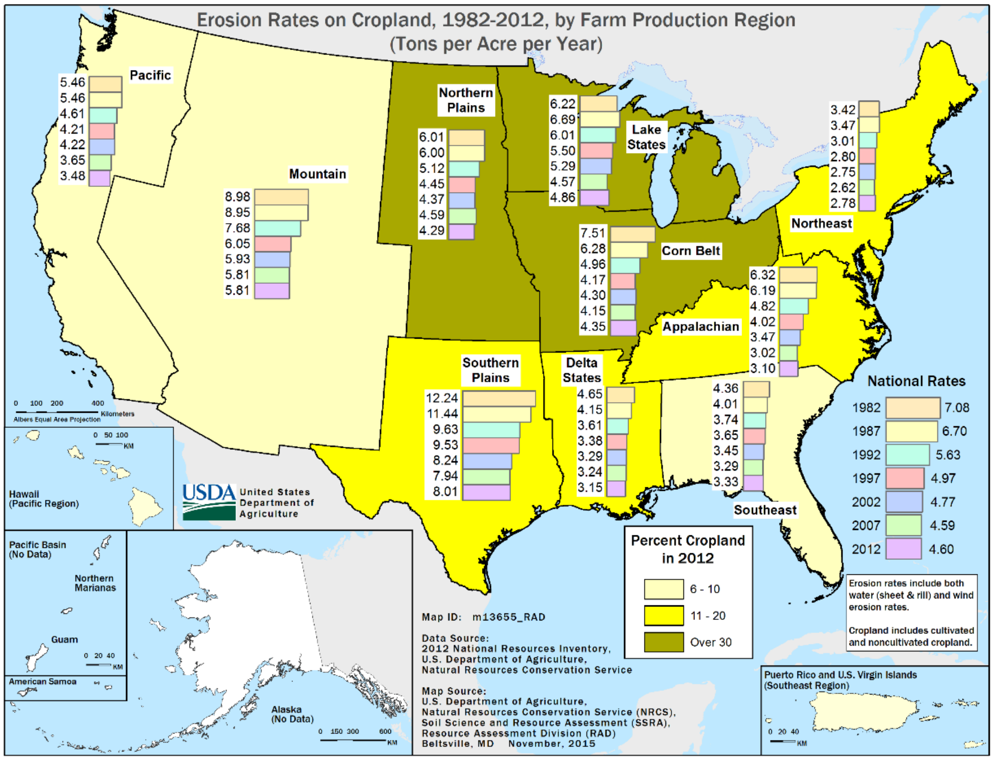

Figure 4-11. Locations and Status of U.S. Croplands Subject to Erosion

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 significantly 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. For wind erosion, erosion rates reduced from 3.21 to 1.91 tons per acre over the same time period (USDA-NRCS 2018a).

As of 2017 (latest survey), 41% of farmers applied continuous no-till practices, 14% rotational no-till, 27% reduced tillage, and 4% vertical tillage (a type of conservation tillage), with only 14% using conventional tillage (SARE/CTIC 2017). Overall, surveyed farmers applied reduced tillage on 97.7 million acres, conventional tillage on 80 million acres, and no-till on 104.4 million acres (Table 4-11). In addition, they are adopting the use of cover crops to conserve soils, SOM, and soil quality (Ghabbour et al. 2017; 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 use of conservation tillage is also attributed, in part, to cultivation 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. 2014b; Claassen et al. 2018).

Table 4-11. 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)

4.3.2.1.1 Potential Effects on Soils

The agronomic characteristics of DP23211 corn are similar to conventionally bred varieties (Pioneer 2020), and practices and inputs used for DP23211 corn production that can impact soil quality, namely tillage, chemical inputs, and cover cropping, are expected to be similar to/same as those currently used. Any potential impacts on soils resulting from DP23211 corn cultivation would be similar to that of other corn varieties. HR crops are correlated with use of conservation tillage practices, which help sustain soil health and water retention, and reduce runoff (NRC 2010; NAS 2016; Claassen et al. 2018). As discussed below in Section 4.3.3.1, soil biota communities, which are primary determinants of soil quality, and in turn erosional capacity (Ghabbour et al. 2017), are unlikely to be affected by the PIPs in DP23211 corn.

All farmers producing crops on highly erodible land are required to maintain and implement a soil conservation plan that is approved by the USDA National Resources Conservation Service (USDA-NRCS 2019a). 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 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.

4.3.2.2 Water Resources

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

While pollutants come from various sources, the EPA's 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 2020l). The most common NPS contaminants in agricultural runoff are sediment, nutrients such as nitrogen and phosphorus, and pesticides (Table 4-12), all of which can adversely affect aquatic ecosystems.

Table 4-12. Causes of Impairment in Assessed Rivers and Streams, 2020

	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 2020l)

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 contributes to eutrophication of surface waters. Sixty five percent of U.S. estuaries have moderate to high levels of eutrophication (NOAA 2020). Eutrophic conditions cause impairments to human uses and living resources as a result of harmful algal blooms and hypoxic/anoxic conditions,[12] which lead to fish kills, fish consumption warnings, declines in tourism, and impacts on fisheries (Bricker et al. 2008; CENR 2010). Based on a USGS study by Munn et al. (2018) some the most impaired streams, as assessed by 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 4-12). Watersheds with a high potential to discharge nitrogen and phosphorus from agriculture sites to estuaries are located primarily in the midwestern Unites States—Mississippi river basin—and Southern Seaboard regions (Wiebe and Gollehon 2006; CENR 2010; US-EPA 2020f).

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

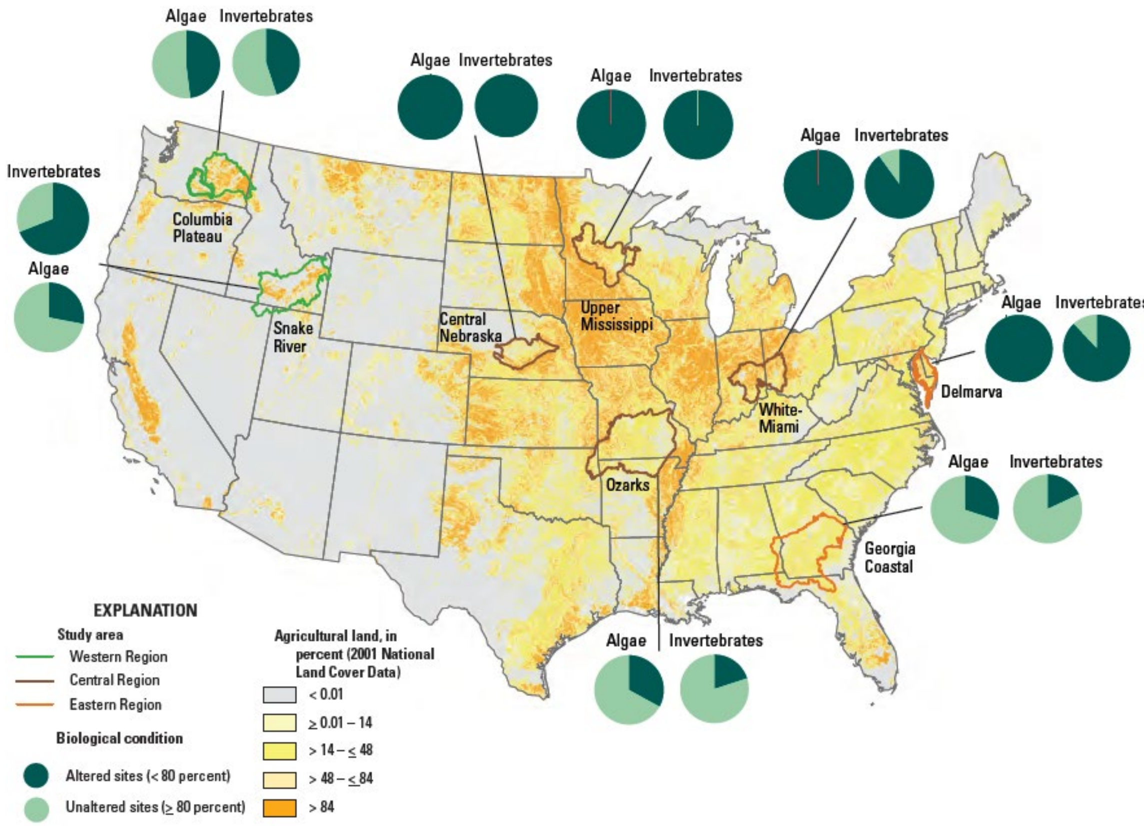

Figure 4-12. Impaired Rivers and Streams in the United States

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 impaired 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). Nutrient run-off has a major economic impact—causing an estimated $2.2 billion per year in damages related to recreational water usage, waterfront real estate, and drinking water treatment (Dodds et al. 2009). In all regions where crops are produced controlling non-point sources remain a primary focus (US-EPA 2020l, e).

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 2019b, 2020l). Agricultural sources contribute around 70% of the nitrogen and phosphorus delivered to the Gulf of

Mexico, versus 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 4-13). 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).

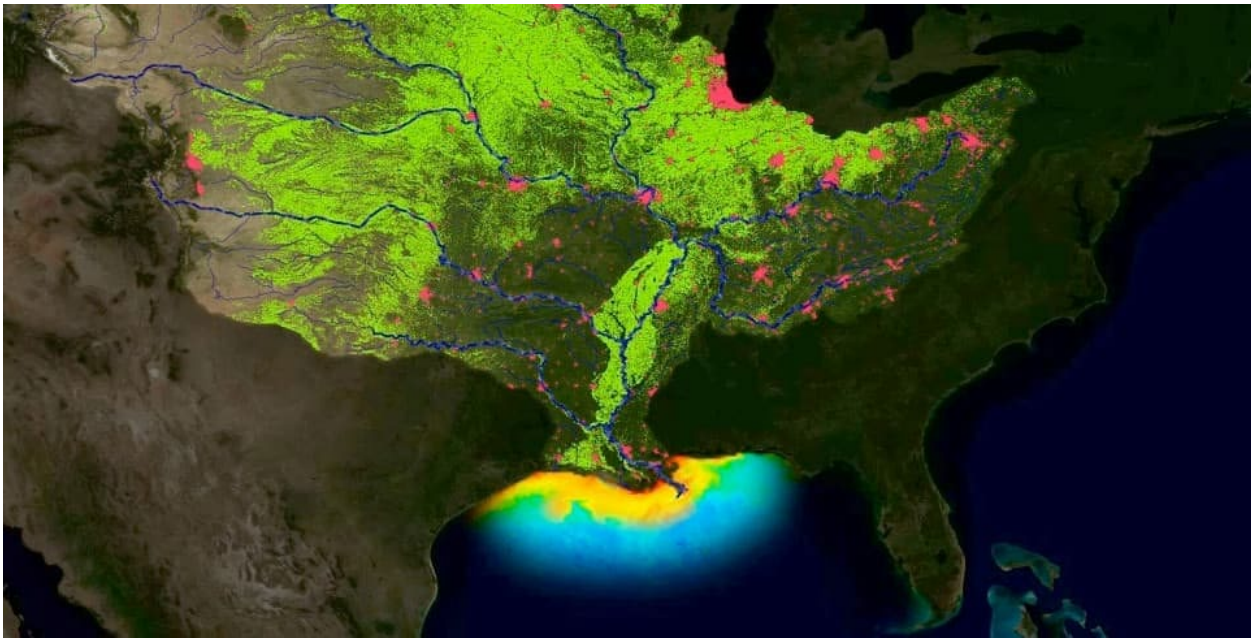

Figure 4-13. Agricultural Run-Off: Mississippi River Watershed

This image from a NOAA Environmental Visualization Lab animation illustrates how run-off from farms (green areas) and cities (red areas) drains into the Gulf of Mexico (GOM). This run-off contains nutrients from fertilizers, wastewater treatment plants, and other sources, which leads to hypoxic "dead zones" on an annual basis; areas in the GOM where the oxygen concentration is so low that aquatic biota can suffocate and die. The largest hypoxic zone in the United States, and the second largest hypoxic zone worldwide, forms in the northern Gulf of Mexico near New Orleans. Source: (NOAA 2019)

4.3.2.2.1 Water Quality Regulation

Point and Non-Point Source Discharges

Pollutant sources are classified by the EPA and state agencies as NPS and point source. NPS pollution is the most significant source of pollution, overall (US-EPA 2020d). NPS contaminants in runoff originate from sources such as construction sites (e.g., residential and commercial development, construction of roads/highways), impervious surfaces (parking lots, roads/highways, rooftops), and crop fields and livestock rearing facilities. NPS pollutants include fertilizers and pesticides applied to residential, commercial, and agricultural sites, and sediments from the built environment and croplands, as well as unmanaged landscapes. The most common NPS contaminants in agricultural run-off are sediment, nitrogen and phosphorus, and pesticides.

Point source pollutants are discharged from any identifiable, singular source, such as a pipe, drain, or vessel. Factories and sewage treatment plants are examples of point sources. Factories, such as oil refineries, pulp/paper mills, and chemical manufacturers typically discharge one or more pollutants in EPA regulated effluents. Livestock rearing facilities (e.g., dairy and beef cows, hogs, chickens) are other sources of point source pollution (e.g., nutrients, microbial pathogens, pharmaceuticals) (Burkholder et al. 2007).

The Clean Water Act (CWA) established the National Pollutant Discharge Elimination System (NPDES) for regulation of point sources (US-EPA 2019g). Under the NPDES program, factories, certain livestock rearing facilities (concentrated animal feeding operations (CAFOs)), sewage treatment plants, and other point sources must obtain a permit from the state and EPA before they can discharge their waste or effluents into any body of water. Prior to discharge, the point source must use the latest technologies available to treat its effluents and reduce the level of pollutants.

NPS pollution, which is the primary type of discharge from cropping systems, is not regulated under the CWA/NPDES permit program, rather, it is left largely to voluntary controls implemented by states and local authorities. Thus, most crop production activities do not require a Section 404 permit. To be exempt, the farming activity must be part of an ongoing farming operation, cannot be associated with bringing a wetland into agricultural production, or converting an agricultural wetland to a non-wetland area. While the CWA does not provide for direct regulation of nonpoint sources, Section 319 of the CWA created a federal grant program that provides money to states, tribes, and territories for developing and implementing NPS management programs.

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 2019h, d). The EPA provides label use restrictions and guidance for product handling 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 surface waters. Certain pesticides—depending on mobility and persistence characteristics—can leach into groundwater at sites where the pesticide is mixed or applied. Total U.S. corn acreage comprises round 90 million acres annually (USDA-NASS 2019b). Collectively, runoff of nutrients, pesticides, and topsoil from croplands can have adverse impacts on surface waters and nearshore coastal waters. Because the agronomic practices and inputs utilized for DP23211 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).

DP23211 corn would facilitate the use of glufosinate in lieu of other herbicides. No significant increase in total herbicide use (lbs a.i./year) on corn would be expected. Minor annual fluctuations in total herbicide use would occur due to variances in the use rates in lbs a.i./acre/year among herbicides, herbicide rotation, and annual acreage planted to corn. Glufosinate use would be subject to EPA label and other use requirements, which are in part established to be protective of water quality (US-EPA 2019d). Any reduced insecticide use with DP23211 corn—relative to the IR PIP traits—would reduce the risk of insecticides in run-off. Adopters of IR corn crops have been found to use around 11.2% (0.013 kg/ha) less insecticide than nonadopters—growers of non-Bt crops (Perry et al. 2016).

4.3.2.3 Air Quality

National Ambient Air Quality Standards

Air pollution is inherently a problem resulting from the collective emissions of various sources. To protect environmental and public health the EPA, pursuant to the Clean Air Act, establishes National Ambient Air Quality Standards (NAAQS) that aim to limit atmospheric emissions (US-EPA 2019f). 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 (SIPs), which are designed to achieve the 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 from corn production include smoke from agricultural burning (PM); fossil fuel combustion 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) and ammonia (NH_3) emissions from the use of fertilizers/manure; and atmospheric emissions through the volatilization of pesticides, and gases from manure (Aneja et al. 2009; Hill et al. 2019; US-EPA 2019k).

Prescribed burning is a land treatment used under controlled conditions to accomplish resource management objectives. Open combustion produces particles of widely ranging sizes, depending to some extent on the rate of energy release of the fire (US-EPA 2019k). The extent to which agricultural and other prescribed burning may occur is regulated by individual SIPs to achieve/maintain compliance with NAAQS. Prescribed burning of fields would likely occur only as a pre-planting option based on individual farm characteristics. There is no known association between prescribe burning and GM HR/IR crops that is unique or differs from conventional corn crops.

While the EPA establishes NAAQS, the standards do not set emission control requirements for any particular industry, including agriculture.[13] The USDA and 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 situations/conditions and desired purposes. 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 risks to human health and wildlife, non-crop plants, and 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, soil wetness, and temperature (US-EPA 2019j). Drift is more likely to occur with fumigants (gasses), dusts, or when liquid pesticides are applied as a very fine mist (US-EPA 2019i). Certain pesticide ingredients stay in the atmosphere for only a short period of time, while others can persist longer (NPIC 2020). Some pesticides can give off chemicals called volatile organic compounds, which can react with other chemicals and form a pollutant called tropospheric ozone. Pesticide use accounts for about 6% of total tropospheric ozone levels (UC-IPM 2006; Zeinali et al. 2011).

The EPA, in addition to label use requirements, introduced initiatives to help pesticide applicators minimize off-target pesticide drift through voluntary Drift Reduction Technology Program, which encourages the manufacture, marketing, and use of spray technologies that reduce pesticide drift (US-EPA 2019i). The EPA also, through the National Emission Standards for Hazardous Air Pollutants (NESHAP), established standards to reduce emissions of hazardous air pollutants (HAP) from existing and new facilities that manufacture organic pesticide active ingredients used in herbicides, insecticides, and fungicides (US-EPA 2020c).

4.3.2.3.1 Potential Effects on Air Quality

Because the agronomic practices and inputs for DP23211 corn production would be the same as/similar to other corn varieties, and there would be no increase/decrease in acreage resulting from DP23211 corn production, no changes to emission sources (i.e., tillage, fossil fuel burning equipment, the application of fertilizers and pesticides), or volume of emissions from U.S. corn production, would be expected. As

[13] Many types of stationary engines exist and are found on farms, including diesel engines, spark ignited engines, and reciprocating internal combustion engines. Air quality requirements vary for stationary engines, depending on whether the engine is new or existing, where the engine is located, and what type of ignition system is used. The National Emission Standards for Hazardous Air Pollutants (NESHAP) for Reciprocating Internal Combustion Engines (RICE) are outlined in the Code of Federal Regulations under 40 CFR 63 Subpart ZZZZ.

compared to production of non-IR corn varieties, due to fewer insecticide applications (approx. 11% reduction (Perry et al. 2016)) and associated fuel use, there would be, to some extent, fewer NAAQS pollutant emissions with DP23211 corn production.

4.3.3 Biological Resources

4.3.3.1 Soil Biota

Soil biota determine soil health, which in turn determines the efficacy by which crops can be produced and soil erosional capacity (FAO 2017). 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) that live all or part of their lives in or on the soil (Fortuna 2012). Soil biota play a key role in the formation and cycling 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 root zone (rhizosphere). Millions of species of soil organisms exist but only a fraction of them have been cultured and identified (Fortuna 2012).

Some soil borne microorganisms can cause plant diseases that can result in substantial economic losses in crop production. Soil borne corn diseases include fungal corn rusts, corn leaf blights, ear smuts, ear and kernel rot fungi, and maize mosaic viruses. Soils are commonly treated to control plant pathogens.

Relative to crop production, the main factors affecting soil biota populations and diversity are soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), plant type (providing specific root exudates and 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 moisture and heat content of soil, is a principal determinant of soil biological activity.

4.3.3.1.1 Potential Effects on Soil Biota

HR and IR Crops

Potential changes to the soil microbial community as a result of cultivating HR and IR crops has been of much research interest since their introduction in the late 1990s (e.g., (Motavalli et al. 2004; Locke et al. 2008; Kremer and Means 2009)). Potential impacts considered include changes to the structure and function of microbial and insect communities near the roots of transgenic plants due to altered root exudation, transfer of novel proteins into the soil, or a change in microbial populations due to changes in agronomic practices used to produce transgenic crops (e.g., pesticide use). The majority of these studies have focused on Bt crops due to their insecticidal activity. Most studies to date have found no significant effect of Bt crop traits on soil community structures (Kowalchuk et al. 2003; Hannula et al. 2014; Zaman et al. 2015; Xie et al. 2016; Yasin et al. 2016).

As summarized below, the HR and IR traits in DP23211 corn are not expected to have any significant long-term effects on soil biota or community structures in DP23211 corn fields. Apart from the dsRNA, the introduced genes/gene products in DP23211 corn naturally occur in soils; they are derived from soil-borne bacteria.

Pat Gene and the Enzyme Phosphinothricin N-Acetyltransferase

Phosphinothricin N-acetyltransferase (PAT) is an enzyme that confers glufosinate resistance in transgenic plants. PAT acetylates glufosinate—changes its structure—which inhibits its herbicidal activity. The gene encoding the PAT protein in DP23211 corn was isolated from *Streptomyces viridochromogenes,* which occurs predominantly in soils (Liu et al. 2013; GCM 2019). The EPA conducted an ecological risk assessment for PAT and concluded that no unreasonable adverse effects on non-target organisms, to include soil biota, are expected from exposure to the PAT protein (US-EPA 2005). There are no identifiable risks to soil biota associated with the PAT expressed in DP23211 corn.

Phosphomannose Isomerase

Phosphomannose isomerase (PMI) is an enzyme involved in carbohydrate metabolism and it, or homologous enzymatic proteins, are expressed in various taxa including bacteria, fungi, insects, some species of plants and nematodes, and mammals—including humans (de Lonlay and Seta 2009; Hu et al. 2016a). The PMI protein produced in DP23211 corn is encoded by the native *pmi* gene from *E. coli* (Pioneer 2020), which commonly occurs soils. The EPA conducted an ecological risk assessment for *E. coli* derived PMI and concluded that no unreasonable adverse effects on non-target organisms, to include soil biota, are expected from exposure to the PMI protein (US-EPA 2005). There are no identifiable risks to soil biota associated with the PMI expressed in DP23211 corn.

DvSSJ1 dsRNA and IPD072Aa: Soil Degradation and Potential Risks Posed

The risks DvSSJ1 dsRNA and IPD072Aa may present to soil biota is a function of the potential hazard they may pose, and exposure. The transport and fate of dsRNA and IPD072Aa in soils determines the potential routes and duration of exposure. DvSSJ1 dsRNA and IPD072Aa will be produced continually in DP23211 corn, in all tissues. The primary route of direct exposure to the dsRNA and IPD072Aa trait protein for soil-dwelling invertebrates would be from feeding on root tissue. It is expected that potential exposure of soil biota to DvSSJ1 dsRNA and IPD072Aa via root tissues would be continuous, throughout the life cycle of DP23211 corn. It is not known whether DvSSJ1 dsRNA and IPD072Aa would be present in root exudates, though upon root cell lysis, small amounts of DvSSJ1 dsRNA and IPD072Aa could be released into the soil. Soil biota could also be exposed to dsRNA and IPD072Aa via decaying post-harvest plant material left on the field, or plant material plowed into the soil.

DNA/RNA, to include dsRNA and other small RNAs (sRNA), are continuously released into soils through the decomposition of plants, animals, fungi, bacteria—all life forms—and common components of soils. Some soil microbes and macrofauna can potentially uptake exogenous (environmental) dsRNA and sRNA. Bacteria (prokaryotes) do not have a homologous RNAi process found in eukaryotes, and targeted modification of gene expression via RNAi is thus not possible (Shabalina and Koonin 2008; Rusk 2012). In general, environmental DNA/RNA is ubiquitous in most soil environments. Utilization of environmental/exogenous DNA/RNA by bacteria is for (1) horizontal gene transfer among bacteria, and (s) as a nutrient source. Many species of bacteria actively take up DNA from their environment through a genetically programmed process called transformation. Natural transformation is a common mode of horizontal gene transfer in bacteria. It results from the intrinsic capacity of bacteria to import exogenous

DNA and integrate it by recombination into their chromosome (Attaiech et al. 2016). For bacteria to take up extracellular genetic material via transformation, it must be what is termed “competent”. Natural competence is the genetic ability of a bacterium to uptake environmental DNA under natural or in vitro conditions. Bacteria can also be made competent artificially by chemical treatment and heat shock to make them transiently permeable to DNA. When bacteria uptake DNA and incorporate it into their genome, changing the cell's genotype, the bacteria is said to be transformed (Lorenz and Wackernagel 1994; Sinha and Redfield 2012; Mell and Redfield 2014). Bacterial transformation is presumed to allow for bacteria populations to adapt to harsh/extreme environmental changes.

Apart from the utilization of extracellular DNA for transformation, environmental DNA/RNA provide a source of nucleotides—used as a nutrient source for soil microbes (Lorenz and Wackernagel 1994; Mell and Redfield 2014). All bacteria cells take up preformed nucleotides where possible (as opposed to *de novo* synthesis), and many species produce nucleases that degrade DNA/RNA and allow them to use the derived nucleic acids as nutrients (Mell and Redfield 2014). One major fate of extracellular DNA/RNA in in the soil environment is degradation by indigenous soil microbial extracellular deoxyribonucleases (exDNases) into smaller fragments (Kamino and Gulden 2021). Most intact DNA/RNA that is taken up by bacteria is degraded upon uptake and used as a nutrient source, even when it has sequence homology to that of the bacterial chromosome (Mell and Redfield 2014).

Environmental RNA uptake by eukaryotes was initially discovered in the nematode *Caenorhabditis elegans*, which uses transmembrane channel proteins to assimilate environmental RNAs, namely long dsRNAs (Fire et al. 1998; Winston et al. 2007; Whangbo and Hunter 2008). Some of the exogenous dsRNAs assimilated by *C. elegans* have been shown to induce gene silencing—RNAi (Wang et al. 2016). RNAi triggered by environmental exposure to dsRNA has also been documented in planaria and parasitic nematodes (Newmark et al. 2003; Orii et al. 2003; Bakhetia et al. 2005). In *C. elegans*, RNA uptake from the environment requires dsRNAs that are longer than 50 bp; shorter dsRNAs cannot be effectively taken up by *C. elegans* (Wang et al. 2016). Similarly, some fungal plant pathogens can take up long dsRNAs, as well as sRNAs, from the environment, though the precise mechanism for uptake remains unknown (Wang et al. 2016). The DvSSJ1 dsRNA in DP23211 corn is 210 bp (Pioneer 2020), thus, it is conceptually feasible that uptake by micro- and macro-fauna could occur.

Bidirectional cross-kingdom RNAi via transfer/uptake of sRNA among plants and plant pathogens is also known to occur (Wang et al. 2016). For example, plants have been found to transport sRNAs into fungal pathogens, using extracellular vesicles, to suppress virulence-related genes (Huang et al. 2019). Arabidopsis plants have similarly been shown to deliver sRNAs into an oomycete pathogen to induce gene silencing (Huang et al. 2019). Endogenous plant sRNAs are known to play a regulatory role in symbiotic plant-microbe interactions (Song et al. 2019).

The decay rate, which determines the environmental availability of DNA/RNA in soils, is affected by microbial activity, pH, temperature, soil nuclease activity, the valence and concentration of cations, water content, and size and characteristics of the DNA/RNA (Greaves and Wilson 1970; Antheunisse 1972; Keown et al. 2004). As previously summarized, the majority of species of soil microbiota, such as bacilli, non-coryneform rods, streptomycetes, and fungi produce nucleases that degrade environmental DNA and RNA (Antheunisse 1972). Both DNA and RNA have been found to be fully degraded in soils, mineralized to nitrogen, within about 30 days (Greaves and Wilson 1970; Keown et al. 2004; Levy-Booth et al. 2008). The dissipation of the DvSSJ1 dsRNA was observed to be around 80% within 24 hours, and 90% or more at 7 days, in three soil types (loam, sandy clay loam, and silt loam) (Pioneer 2020). In 2015, the EPA registered a similar plant incorporated protectant in Monsanto MON87411 corn; an insecticidal DvSNf7 dsRNA that also targets corn rootworm. This dsRNA is of similar size and molecular characteristics; DvSNf7 dsRNA is 240 base pairs and DvSSJ1 dsRNA 210 base pairs. DvSnf7 dsRNA was undetectable after 48 hrs (Dubelman et al. 2014). Other studies found DT90 values (time to 90% degradation) for DvSNf7 dsRNA to be 39 and 50 hours for sandy clay and sandy soil, respectively (Joaquim et al. 2019). These dissipation rate estimates are comparable to those observed when DvSnf7 dsRNA is exposed to temperate agricultural soils (DT50 < 30 hours) and suggest that the dissipation rate of dsRNA is largely independent of soil physical and chemical characteristics (Joaquim et al. 2019). DvSSJ1 dsRNA was, however, observed to be biologically active after 54 days in one study on terrestrial degradation.

Given the structural and size similarities, and common processes by which DNA/RNA are degraded by soil microbiota (e.g., nucleases), the environmental fate and degradation of DvSSJ1 dsRNA, its typical residence time in soil, would be expected to be around 2 weeks or less, depending on soil type and environmental conditions. Generally, studies have shown 90% degradation within 7 days, with only trace quantities of DvSSJ1 dsRNA persisting after that (Pioneer 2020).

The DvSSJ1 dsRNA targets an mRNA sequence encoding for translation of the DvSSJ1 protein, which is unique to WCR. Previous work with WCR (*Diabrotica virgifera virgifera*; Coleoptera: Chrysomelidae) demonstrated that at least one ≥ 21 nucleotide (nt) match must be present in DvSnf7 dsRNA of approximately ≥ 60 base-pairs (bp) for activity. Neither 19 nor 20 nt sequence matches were active, supporting that a ≥ 21 nt sequence match is required for activity (Bachman et al. 2020). Using bioinformatics analyses, the closest sequence match (percent identity to the 210-bp *dvssj1* sequence) was the *ssj1* homologous gene from WCR, which as intended had a 100% sequence match, and 190 21-nt matches. The *ssj1* homologous gene from the closely related species, northern corn rootworm, shared 97.1% sequence identity with the 210-bp dvssj1, with 135 21-nt matches. The *ssj1* homologous gene from southern corn rootworm shared 92.9% sequence identity with 79 21-nt matches. The *ssj1* homologous genes from the other Coleoptera within the family Chrysomelidae as well as species within the family Tenebrionidae, the family Coccinellidae, and the family Staphylinidae had decreasing percent identity with the *dvssj1* mRNA sequence, ranging from 77.6% to 61.9% similarity. All Lepidoptera species tested, as well as the honeybee and the insidious flower bug also had lower percent identity with the 210-bp dvssj1 sequence, ranging from 68.1% to 60% similarity. There were zero 21-nt matches observed across all of the non-Diabrotica species analyzed (Pioneer 2020).

To date, there is limited data on the potential effects of DvSSJ1 dsRNA on soil biota, specifically. At 1 ng DvSSJ1 dsRNA/mg diet no effects on springtails (*Folsomia candida*) were observed—this is the only data on soil biota APHIS is aware of. This dietary exposure was at 33 times the worst-case estimated environmental concentration (Pioneer 2020). Studies evaluating a similar DvSnf7 dsRNA targeting corn rootworm found no effects on earthworm (*Eisenia andrei*), Collembola (*Folsomia candida*), or microbially-mediated soil processes, nor were any adverse effects on a battery of non-target arthropods observed (Bachman et al. 2016). This was attributed to the limited spectrum of activity of DvSnf7 dsRNA—its nucleotide sequence specificity for DvSnf7 mRNA (*Snf7* gene) and protein translation in corn rootworm (Bachman et al. 2016).

Based on these data, exposure of eukaryotic soil biota would be limited to entry of DvSSJ1 dsRNA into soils upon decaying plant matter, with approximately 90% of DvSSJ1 dsRNA decaying within about 7 days. Were eukaryotic soil biota exposed to DvSSJ1 dsRNA, and assuming cell uptake, dsRNA recognition, and processing of sRNA into a RNA-induced silencing complex (RISC), absent a matching mRNA target sequence in the organism—the *dvssj1* mRNA sequence— it is unlikely any potential hazard, alteration of physiology via RNAi, would be presented to eukaryotic soil biota. Considering all of the factors discussed, DvSSJ1 dsRNA would be present in soils for a short period of time and present negligible risk to eukaryotic soil communities, nor any risk to communities of soil bacteria. Rather, DvSSJ1 dsRNA would likely serve as a nucleotide (nutrient) source for soil bacteria.

The *ipd072Aa* gene extant in DP23211 corn was derived from an *Escherichia coli* protein expression system. It is a cloned form of the naturally occurring *ipd072Aa* expressed by the common soil bacterium *Pseudomonas chlororaphis* (Schellenberger et al. 2016; Boeckman et al. 2019). Most Pseudomonas species, including *P. chlororaphis*, are ubiquitous in the environment—have widespread distribution in soil and water (Anderson et al. 2018). Considering *P. chlororaphis* naturally produces the IPD072Aa protein (Schellenberger et al. 2016), IPD072Aa protein commonly occurs in soils. Interestingly, *P. chlororaphis* has also been reported to promote plant growth, and protect plants, by producing various other compounds (e.g., phenazine-type antibiotics, hydrogen cyanide, chitinases, and proteases) that inhibit fungal growth (EFSA 2015), as well as insects and nematodes (Anderson and Kim 2018). For this reason, *Pseudomonas chlororaphis* is widely used as a soil inoculant in agriculture and horticulture on various cereal and vegetable crops; it can act as a biocontrol agent against certain fungal plant pathogens via production of phenazine-type antibiotics (Chin-A-Woeng et al. 2000; Anderson et al. 2018). Studies presented by Pioneer indicate that the dissipation of IPD072Aa protein in diverse soil types occurs in less than 7 days; the protein is therefore unlikely to persist or accumulate in soils (Pioneer 2020).

The only soil dwelling organism evaluated to date, that APHIS is aware of, is springtail (*Folsomia candida*). There were no biologically relevant adverse effects on springtail reproduction and survival at 500 ng IPD072Aa protein/mg diet (tissue expression levels in DP23211 corn range from about 1 ng/mg to 84 ng/mg). As discussed later in 4.3.3.2.3.1–Non-Target Organisms, the IPD072Aa protein has insecticidal activity limited to species among the order Coleoptera, primarily *Diabrotica* species (Schellenberger et al. 2016; Boeckman et al. 2019; Carlson et al. 2019). APHIS is unaware of any data describing IPD072Aa bioactivity outside the order of Coleoptera.

Based on the data reviewed, there are no identifiable risks to non-target soil biota posed by the IPD072Aa protein expressed in DP23211 corn.

Glufosinate Use with DP23211 Corn

DP23211 corn would facilitate use of glufosinate-ammonium based herbicides. Herbicides can differ from each other with regard to their potential effects on soil biota relative to their biological activity (mode of action) and environmental behavior (Wolmarans and Swart 2014; Dennis et al. 2018). Herbicides used at normal field application rates—in part because their modes of action target plant specific physiological processes—generally have no major long-term effect on soil biota (Busse et al. 2001; Zabaloy et al. 2008; Rose et al. 2016; Nguyen et al. 2018). Most herbicides are degraded by and serve as sources of carbon, nitrogen, and phosphorus for soil microorganisms. A global review by the

Food and Agriculture Organization (FAO) likewise found that there is limited evidence that the observed effects of herbicides on soil organisms have led to significant and long-lasting decreases in soil functions (FAO 2017). Some herbicides can however transiently effect soil organisms, namely shifts in community structure, and associated soil biochemical and enzymatic processes (Bünemann et al. 2006; FAO 2017).

Studies on the effects of glufosinate based herbicides on soil communities are limited. Among those that have been conducted, no significant adverse effects on the composition of soil bacterial and archaeal communities, or nematode communities, have been identified (Tothova et al. 2010; Dennis et al. 2018). Studies by Dennis et al. (2018) found that recommended treatments of glufosinate in the form of Basta® and Liberty® have been shown to have negligible effects on microbial community composition, or microbial enzyme activity. Using microbe sequencing technologies, a study by Tang et al. (2019) concluded that glufosinate (Basta®) application had no adverse effects on the rhizosphere bacterial community composed of a wide variety of phyla, including proteobacteria, bacteroidetes, acidobacteria, gemmatimonadetes, and actinobacteria. Mandl et al. (2018) found shifts in community structures with the use of glufosinate. Composition of soil bacteria and archeobacteria communities in vineyard rows after mechanical weeding and three herbicide treatments (glyphosate, glufosinate, and flazasulfuron) were evaluated. Interestingly, next-generation sequencing analyses showed that abundances of soil bacteria under all herbicide treatments were on average 264% higher than under mechanical weeding (Mandl et al. 2018); this was presumed to be due to soil biota use of the herbicide active ingredient, or other ingredients, or both, as carbon/energy sources. However, due to high data variability this increase in microbial biomass was not statistically significant. Glufosinate, as other herbicides, can stimulate or suppress certain fungi, in particular, which suggests that herbicide-specific active ingredients, adjuvants, or other herbicide ingredients might be responsible for this effect (Mandl et al. 2018). Whether such shifts in soil fungal species composition has adverse effects on soil processes and health is unknown—it has not been well studied.

While the application of glufosinate may lead to temporary variations in species composition—and thereby biomass, enzymatic activity, and soil respiration—the resiliency of soil organisms, and functional redundancy across various taxa, serve to limit the long-term effects of herbicides on soil ecosystem processes (Locke and Zablotowicz 2004; FAO 2017). Any exposure of soil biota to glufosinate would be transient and short-lived. For glufosinate, and most all other herbicides, soil microbial degradation is the primary process by which it is degraded in the environment. The aerobic half-life for glufosinate in soil is typically 3-11 days with an anaerobic half-life of 5-10 days. Field dissipation half-lives of 6-20 days (avg. 13 days) are typical (TOXNET 2020). All pesticide use on DP23211 corn would be subject to EPA label use requirements.

4.3.3.2 Animal Communities

4.3.3.2.1 Birds and Mammals

Commercial cornfields, which are intensively cultivated, provide less 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 may forage on corn at various stages of plant growth. Large- to medium-sized mammals that are common foragers of cornfields include those in Table 4-13. The most notable of these is the white-tailed deer that inhabit woodlots adjacent to cornfields and frequent corn 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). 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 mammals may use cornfields for shelter and forage (USDA-NRCS 1999; U-Illinois-Ext 2000; Sterner et al. 2003).

Table 4-13. 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*

Source: (Fleharty and Navo 1983; ODNR 2001)

4.3.3.2.2 Invertebrates

Although certain invertebrates in corn fields 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 prey on other insects and mites that are considered plant pests (Landis et al. 2005). Some of these beneficial species include the convergent lady beetle (*Hippodamia convergens*), carabid beetles, caterpillar parasitoids (e.g., *Macrocentrus cingulum*), and 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 Animal Communities

DP23211 corn cropping systems would not be expected to affect vertebrate animal communities any differently from that of current corn cropping systems. As summarized in Section 4.3.3.1–Soil Biota, and

Section 4.3.4.2–Safety of the Herbicide Resistance and Insect Resistance Traits, it is unlikely PAT nor PMI present any hazard to wildlife. DvSSJ1 dsRNA expressed in DP23211 corn has been shown to primarily affect a subset of arthropods—*Diabrotica* species within the Chrysomelidae family of Coleoptera (Hu et al. 2019). The IPD072Aa protein expressed in DP23211 corn has likewise been shown to primarily affect *Diabrotica* spp. (Boeckman et al. 2019). A review of the potential risks of DvSSJ1 dsRNA and IPD072Aa protein to non-target arthropods is provided below.

The potential impacts of glufosinate use on wildlife have been assessed by the EPA as part of the pesticide registration process (US-EPA 2016a). Glufosinate use with DP23211 corn would be subject to the EPA label and other use requirements.

4.3.3.2.3.1 Non-Target Organisms

The potential risk to non-target organisms, as a matter of hazard assessment, would be from exposure to the trait genes and gene products via consumption of the kernel, leaf, pollen, or root tissues. Invertebrate kernel and leaf feeders are primarily members of four insect orders: Lepidoptera (larval butterflies and moths), Coleoptera (beetles), Hymenoptera (larval wasps) and Orthoptera (grasshoppers). Many invertebrates such as honeybees, wasps, and beetles feed upon pollen. Invertebrate root feeders are primarily among the order Coleoptera. The most ecologically relevant route of exposure for soil-dwelling organisms, such as earthworms and Collembola, is considered primarily to be from root tissue or root exudates, with some addition of post-harvest decaying plant tissue that enters the soil (Bachman et al. 2016).

DP23211 produces the insecticidal protein IPD072Aa, insecticidal dsRNA targeting Dvssj1, the PAT protein for tolerance to glufosinate herbicides, and the PMI protein that was used as a selectable marker during the plant transformation process. PAT and PMI are not known to be toxic to any nontarget organisms beneficial to agriculture. PAT and PMI are present in numerous deregulated crop plants that are commercially available to growers (ISAAA 2022). Therefore, this part of the assessment focuses on the novel insecticidal compounds produced by DP23211 corn.

When rats and broiler chickens were fed grain from DP23211 corn, which expresses both the IPD072Aa protein and dsRNA of DvSSJ1, no adverse effects were observed (Smith et al. 2021). In summary, these studies suggest no adverse impacts to humans and vertebrate non-target organisms due to the insecticidal compounds produced by DP23211; therefore, subsequent analyses below focus primarily on invertebrate non-target organisms.

DvSSJ1 dsRNA: Terrestrial Biota

RNA interference (RNAi) is a conserved gene-silencing mechanism—across the plant and animal kingdoms—initiated by double-stranded RNA (dsRNA) (Fire et al. 1998; Shukla et al. 2020). One of the primary functions of RNAi is the protection of the genome against invasion by mobile genetic elements such as transposons and viruses, which can produce aberrant RNA or dsRNA in the host cell (Jensen et al. 1999; Ratcliff et al. 1999; Elbashir et al. 2001). In general, mRNA silencing provides protection against diverse RNA viruses in many eukaryotic organisms (Ding 2010; Llave 2010; Qu 2010), to include mammals (Schuster et al. 2019). Specific mRNA degradation prevents transposon and virus replication—although it is noted that some viruses are able to overcome or prevent this process by expressing proteins that suppress posttranscriptional gene-silencing (Lucy et al. 2000; Voinnet et al. 2000).

The presence of DsRNA in a cell can trigger the specific degradation of mRNAs with homologous nucleotide sequences after being recognized and processed into smaller 21–23 nucleotide (nt) RNA fragments (Zamore et al. 2000). Different types of small RNAs (sRNA) have been identified over the years, of which microRNAs (miRNAs) and small-interfering RNAs (siRNAs) are the main types (Knip et al. 2014). sRNAs are typically 21–23 nt long (although can range from 19–25 nt) and produced from larger dsRNA or hairpin RNA (hpRNA) by Dicer or Dicer-like proteins. sRNAs then associate with Argonaute proteins to from what is called an RNA-Induced Silencing Complex (RISC). Post-transcriptional gene silencing (RNAi) in cells via a RISC involves mRNA cleavage and inhibition of protein synthesis (translation). This process of RNAi via RISC is widely conserved in eukaryotes (plants and animals); the RNAi process is also highly specific, based on the particular 21–23 nt sequence (Castel and Martienssen 2013; Martínez de Alba et al. 2013; Knip et al. 2014).

The DvSSJ1 dsRNA in DP23211 corn was developed to target and inhibit *dvssj1* mRNA expression in western corn rootworm (*Diabrotica virgifera virgifera*). The *dvssj1* and *dvssj2* genes in corn rootworm encode cell membrane proteins associated with smooth septate junctions, which are cell-to-cell structures that are required for gut cell integrity and function in arthropods. When ingested by corn rootworm, plant-derived DvSSJ1 dsRNA is processed into 21-nt siRNAs, which decreases synthesis of DvSSJ1 protein in WCR via RNAi. Loss of DvSSJ1 protein production in the gut cells of WCR disrupts the formation and maintenance of the smooth septate junction complex, leading to loss of cell barrier integrity and larval mortality (Hu et al. 2019). Expression of DvSSJ1 dsRNA in DP23211 corn tissues is provided in Table 4-14.

Table 4-14. DvSSJ1 dsRNA: Tissue Expression Levels in DP23211 corn*

	ng/mg tissue Fresh Weight (parts per million; ppm)	**ng/mg tissue Dry Weight** (parts per million; ppm)
Root	0.00019 - 0.00877	0.00150 - 0.0944
Leaf	0.00177 - 0.0379	0.00240 - 0.0985
Grain	0.00102 - 0.0073	0.00122 – 0.0109
Pollen	0.000330 – 0.00096	0.000561 – 0.00202
Whole Plant	0.00153 - 0.0120	0.00459 - 0.0359
Forage	0.00264 – 0.0177	0.00977 – 0.0565

*Range of tissue expression levels from V6 to R6 growth stages

For non-target organisms (NTOs) to be adversely affected by dsRNA, NTOs must possess a genetic sequence matching the dsRNA, and ingest it in sufficient quantities to elicit an RNAi response. Exposure can occur when NTOs feed directly on plant material, consume prey that ingested the dsRNA, or are exposed through root exudates. Evaluation of DvSSJ1 dsRNA activity in representative NTOs, including pollinators and pollen feeders, soil-dwelling organisms, and predators and parasitoids was conducted using diet-based bioassays. The DvSSJ1 dsRNA median lethal concentration (LC50), no observed effect dose (NOED), no observed dietary dose (NOEDD), or no observed effect concentrations (NOEC) were determined for the NTOs listed in Table 4-15. Note that lyophilized DP23211 corn tissue incorporated into insect diets was used for the DvSSJ1 dsRNA bioassays (Pioneer 2020). This methodology is considered to provide more consistent data as the DvSSJ1 dsRNA protein concentrations in fresh corn tissue can add variability to the data due to varying levels of moisture and range of DvSSJ1 dsRNA expression among corn tissues.

Table 4-15. DvSSJ1 dsRNA: Spectrum of Activity in Representative Non-Target Organisms

Class	Order	Family	Species	Common Name	Bioassay Duration (days)	Endpoints[a]	Test Dose/ Dietary Concentration
Insecta	Hymenoptera	Apidae	*Apis mellifera*	Honeybee (larvae)	22	LPS, AE, AW	4.0 ng/larva[b]
Insecta	Hymenoptera	Apidae	*Apis mellifera*	Honeybee (adult)	14	W, AS	26 ng/bee/day[c]
Entomo-bryomorpha	Collembola	Isotomidae	*Folsomia candida*	Springtail	28	R, AS	1 ng/mg diet[d]
Insecta	Neuroptera	Chrysopidae	*Chrysoperla rufilabris*	Green Lacewing	21	AS, P	1 ng/mg diet[d]
Insecta	Coleoptera	Coccinellidae	*Coleomegill a maculata*	Pink spotted Lady Beetle	28	AS, W, DE	1 ng/mg diet[d]
Insecta	Coleoptera	Coccinellidae	*Hippodamia convergens*	Ladybird Beetle	28	AS, W, DE	1 ng/mg diet[d]
Insecta	Hymenoptera	Eulophidae	*Pediobius foveolatus*	Parasitic Hymenoptera	14	AS	1 µg/ml diet[d]
Aves	Galliformes	Odontophoridae	*Colinus virginianus*	Northern bobwhite quail	14	AS, AB, T	>105 mg/kg body weight[e]

a. LPS = Larval and Pupal Survival, AE = Adult Emergence, W = Adult Weight AW = Adult Weight at emergence, AS = Adult Survival, R = Reproduction, P = Pupation, DE = Days to Emergence, AB = Abnormal Behavior, T = Toxicity

b. NOED – No Observed Effect Dose

c. NOEDD – No Observed Effect Diet Dose

d. NOEC – No Observed Effect Concentration. For pink spotted lady beetle 10.3% mortality was observed at 1 ng/mg diet, although not statistically significant (P = 0.1124)

e. LD50 – Median Lethal Dose

Source: (Pioneer 2020)

Similar to that reviewed for corn insect pests in 4.3.1.3 – Potential Effects on U.S. Corn Production, the specificity of DvSSJ1 dsRNA, observed effects on NTOs, was limited to *Diabrotica* species. No other Coleoptera or Lepidoptera species evaluated were affected by exposure to DvSSJ1 dsRNA. Thus, the spectrum of activity of DvSSJ1 dsRNA—its specificity—appears to be primarily limited to western corn rootworm, namely *dvssj* mRNA. Based on the data summarized in Table 4-15, adverse effects on NTOs via exposure to DvSSJ1 dsRNA would not be expected.

Four species representing the predator and parasitoid group were assessed: two Cocinellidae (Ladybugs; *C. maculata and H. convergens*), one Neuroptera (net-winged insects; *C. rufilabris),* and one Hymenoptera (parasitic wasp; *P. foveolatus).* Additionally, one surrogate non-target soil dweller (springtail; *F. candida*) was assessed. No effects on survival were observed for any of these species.

Honeybees consume both pollen and nectar from flowers. Nectar provides carbohydrates and small amounts of other nutrients, while pollen provides the bulk of the honeybee's protein and lipid requirements (Carroll et al. 2017). Individual bees consume no more than a few mg during a feeding event (Carroll et al. 2017). Average pollen consumption by bees is generally reported to be around 3.4 to 3.9 mg/bee/day (see review by Rodney and Purdy (2020)). For DP23211 corn, the maximum concentration of DvSSJ1 dsRNA in pollen is very low relative to expression in other tissues. The maximum concentration of DvSSJ1 dsRNA in DP23211 corn pollen is 0.00096 ng/mg. For honeybee, there were no observed effects at 4.0 ng/larva, or 26 ng/bee/day. Assuming larva consume 2 mg of pollen (= 0.002 ng DvSSJ1 dsRNA), the observed no effect dose of 4.0 ng/larva is 2000 times the potential exposure level to DvSSJ1 dsRNA. For adult honeybees, assuming consumption of 10 mg pollen (=0.0096 ng DvSSJ1 dsRNA), or around twice the reported consumption rates, the observed no effect dose of 26 ng/bee/day is 2,700 times the potential exposure to DvSSJ1 dsRNA. Considering these data—DvSSJ1 dsRNA specificity for *Diabrotica* species, and no observed effects on *Apis mellifera* or other species reviewed—the risks of DP23211 corn pollen presenting any hazard to species of Apidae (bees) is considered unlikely.

The molecular target of DvSSJ1 dsRNA, smooth septate junction protein 1 (*dvssj1* mRNA), is arthropod-specific; it has not been identified in vertebrates (Hu et al. 2016b). Bioinformatic sequence comparisons of DvSSJ1 dsRNA 21 nucleotide (21-nt) small interfering RNA (siRNA) with mammalian, avian, and fish transcriptomes identified no 21-nt siRNA exact matches (Mirsky 2019). Sequence comparison of the 210-bp *dvssj1* (dsRNA) sequence to *ssj1* homologous genes among Coleoptera, Lepidoptera, Hymentoptera, Hemiptera found 100%, 97.1%, and 92.9% identity for western, northern, and southern corn rootworm, respectively. The next closest matches were crucifer flea beetle (77.6%), striped flea beetle (76.2%), and Colorado potato beetle (73.3%). All other sequence matches among Coleoptera, Lepidoptera, Hymentoptera, and Hemiptera species evaluated were below 70% (Pioneer 2020).

Based on these data, summarized in Table 4-15, the DvSSJ1 dsRNA expressed in DP23211 corn would not be expected to present any hazard to NTO populations— adverse effects on NTOs via exposure to DvSSJ1 dsRNA would not be expected. The DvSSJ1 dsRNA appears to be highly specific for *Diabrotica virgifera vergifera*. The only other species showing sensitivity to DvSSJ1 dsRNA was southern corn rootworm, which was observed to have decreased survival at a concentration of 100 ng DvSSJ1 dsRNA/ml diet.

A similar RNAi PIP introduced into a commercial corn variety, SmartStax® corn, was evaluated for potential off-target effects. SmartStax® corn expresses a DvSnf7 RNA that likewise targets corn rootworm using a similar MOA—disruption of protein synthesis in the gut epithelium of Coleoptera species. The MOA of DvSnf7 RNA has been well characterized and shown to, likewise, have a narrow spectrum of activity within *Diabrotica* species (Bolognesi et al. 2012). The potential effects of DvSnf7 RNA on NTOs included a pollinator (honey bee, *Apis mellifera*), six beneficial insect species that function as biocontrols (parasitic wasp (*Pediobius foveolatus*), ladybird beetle (*Coleomegilla maculata*), carabid beetle (*Poecilus chalcites*), rove beetle (*Aleochara bilineata*), green lacewing (*Chrysoperla carnea*), and insidious flower bug (*Orius insidiosus*)), soil biota (earthworm (*Eisenia andrei*) and Collembola (*Folsomia candida*)), and microbially-mediated soil processes (Bachman et al. 2016). Laboratory tests evaluated ecologically relevant endpoints such as survival, growth, development, and reproduction and were of sufficient duration to assess the potential for adverse effects. Survival, growth and/or developmental observations were examined in the ladybird beetle, carabid beetle, insidious flower bug, honey bee, and vertebrate studies. Survival and reproduction were evaluated with Collembola, rove beetle and green lacewing, and survival and biomass with earthworm. All NTO bioassays were conducted with diet-incorporation methodology and the organisms fed as much as much as desired (ad libitum). No adverse effects were observed with any species tested at, or above, the maximum expected environmental concentration (MEEC). All margins of exposure for NTOs were >10-fold the MEEC (Bachman et al. 2016).

The EPA reviewed NTO hazard data for DvSnf7 (SmartStax® corn) on two species of birds, two mammal species, a freshwater fish, seven species of non-target arthropods, an earthworm, and honey bees. The EPA also included assumptions about common barriers to dsRNA uptake in vertebrates and bioinformatics analyses. Based on these data, the EPA concluded that adverse effects to NTOs would not be expected to result from cultivation of corn comprised of DvSnf7 dsRNA (US-EPA 2016d).

The studies summarized for SmartStax® corn, examining the potential effects of DvSSJ1 dsRNA and DvSnf7 dsRNA on corn rootworm and NTO species support the concept that dsRNA constructs can be highly specific with targeted effects on a limited spectrum of insect pest species.

IPD072Aa Protein: Terrestrial Biota

The ranges of IPD072Aa protein expression in DP23211 corn leaf, root, grain, and pollen tissues over the course of the growing season are summarized in Table 4-16.

Table 4-16. IPD072Aa Tissue Expression Levels in DP23211 Corn*

	ng/mg Tissue Dry Weight (parts per million; ppm)
Root	0.93 - 84
Leaf	0.054 - 39
Grain	0.51 – 4.8
Pollen	0.14 – 1.3
Whole Plant	1.7 - 24
Forage	0.51 - 28

*Range of tissue expression levels from V6 to R6 growth stages.
Source: (Pioneer 2020)

Evaluation of the potential effects of IPD072Aa protein on NTOs across several orders/families, including pollinators and pollen feeders, soil-dwelling organisms, and predators and parasitoids was conducted using diet-based bioassays (Table 4-17). The IPD072Aa protein used in the bioassay studies was extracted from lyophilized DP23211 corn tissue and reconstituted in an artificial diet. The targeted IPD072Aa protein concentrations in treatment diets used in the bioassays were based on ng/mg IPD072Aa in the diet wet weight (Pioneer 2020). As with the DvSSJ1 dsRNA bioassays, this methodology is intended provide more accurate data than use of fresh DP23211 corn tissue-based diets. The NTO bioassay studies indicate that IPD072Aa primarily affects species within the order Coleoptera, with no activity observed within tested species among the orders Lepidoptera, Hymenoptera, Collembola, Neuroptera, Hemiptera, or Galliformes (Schellenberger et al. 2016; Boeckman et al. 2019).

Table 4-17. IPD072Aa Protein: Spectrum of Activity in Representative Non-Target Organisms

Class	Order	Family	Species	Common Name	Study (days)	Endpoint[a]	Test Dose/ Dietary Concentration

Insecta	Hymenoptera	Apidae	*Apis mellifera*	Honeybee (larvae)	22	LPS, AE, AW	200 ng/larva[b]
Insecta	Hymenoptera	Apidae	*Apis mellifera*	Honeybee (adult)	10	AS, W	1300 ng/bee/day[c]
Entomobryo-morpha	Collembola	Isotomidae	*Folsomia candida*	Springtail	28	R, AS	500 ng/mg diet[e]
Insecta	Neuroptera	Chrysopidae	*Chrysoperla rufilabris*	Green Lacewing	21	AS, P	500 ng/mg diet[e]
Insecta	Coleoptera	Coccinellidae	*Coleomegilla maculata*	Pink spotted lady beetle	28	AS,W,AE	100 ng/mg diet[e]
Insecta	Coleoptera	Coccinellidae	*Hippodamia convergens*	Convergent ladybird beetle	28	AS, W, AE	500 ng/mg diet[e] 100 ng/mg diet[f]
Insecta	Hymenoptera	Eulophidae	*Pediobius foveolatus*	Parasitic wasp	7	AS	1000 µg/ml diet[e]
Aves	Galliformes	Odontophoridae	*Colinus virginianus*	Northern bobwhite quail	14	AS, AB, T	>2000 mg/kg body weight[e]
Mammalia	Rodentia	Muridae	*Mus musculus*	Mouse	14	AS, T	>2000mg/kg body weight

a LPS = Larval and Pupal Survival, AE = Adult Emergence, AW = Adult Weight at emergence, W = Adult Weight, AS = Adult Survival, R = Reproduction, P = Pupation, DE = Days to Emergence, AB = Abnormal Behavior, T = Toxicity
b NOED – No Observed Effect Dose
c NOEDD – No Observed Effect Diet Dose
d NOED – No Observed Effect Dose
e NOEC – No Observed Effect Concentration. For pink spotted lady beetle 26.7% mortality was observed at 500 ng/mg IPD072Aa diet, and statistically significant (P=0.045). Weight was affected at 500 ng/mg diet (P=0.007). Adult emergence was affected at 500 ng/mg (P=0.0015). A statistically significant difference was observed in mortality between convergent ladybird beetle fed the test diet containing 1000 ng IPD072Aa protein per mg diet. Convergent ladybird beetle fed 500 ng/mg and 1000 ng/mg (median 17 and 22 days, respectively) had significantly greater probability to take longer to emerge.
f For convergent lady beetle, statistically significant effects on weight were observe at all doses.
Source: (Boeckman et al. 2019; Pioneer 2020)

Based on the expression level of the IPD072Aa protein in DP23211 corn pollen (0.14 – 1.3 ng/mg), no observed effects doses summarized in Table 4-17, and pollen consumption rate of honeybees, summarized following, the IPD072Aa protein would not be expected to present any hazard to honeybees. Bees have been reported to consume on average around 3.4 to 3.9 mg pollen/bee/day (see review by (Rodney and Purdy 2020)). Worker honeybee larvae under experimental conditions have been observed to consume a total amount of 1.5 mg to 2 mg corn pollen during development (Babendreier et al. 2004). Maximum consumption by larva—during development, which lasts about six days—would be about 2.6 ng IPD072Aa protein /larva. Maximum consumption by adult bees would be about 5 ng IPD072Aa protein/bee/day. The NOED are 200 ng IPD072Aa/larva and 1,300 ng IPD072Aa/bee/day, which are 77 and 260 times potential dietary exposure, respectively.

Two surrogate species representing predator and parasitoid groups were assessed: Green lacewing (*C. rufilabris*: Neuroptera) and parasitic wasp (*P. foveolatus*: Hymenoptera). No effects on survival or pupation were observed for green lacewing at 500 ng/mg IPD072Aa diet. Similarly, no effects on survival were observed for the parasitic wasp at 1000 ng/mg diet.

IPD072Aa has been found to affect the survival, weight, and emergence time of pink spotted lady beetles (*Coleomegilla maculata* De Geer; Coleoptera) and convergent lady beetles (*Hippodamia convergens*: Coleoptera), albeit at dietary concentrations that would be difficult to achieve in the field. Both are beneficial predators that feed upon aphids and other insect pest species. Convergent lady beetles will also feed on pollen and nectar from flowers when prey is scarce. Statistically significant effects on convergent lady beetle weight were observed at 100 ng/mg and above (Table 4-17). For pink spotted lady beetles, there was no significant effect of IPD072Aa at 100 ng/mg on survival, growth, or days to adult emergence, although statistically reduced survival was observed in pink spotted lady beetles at the 500 and 1,000 ng/mg concentrations (Pioneer 2020). No effects on survival were observed in experiments with convergent lady beetle exposed to 100 and 500 ng/mg IPD072Aa; however, survival was reduced at 1,000 ng/mg. Convergent lady beetle exposed to 500 and 1,000 ng/mg IPD072Aa showed reduced growth and required longer to emerge as adults (Pioneer 2020).

Lundgren et al. (2005) estimated that spotted lady beetle (*Coleomegilla maculata*) consumed 0.66, 1.67 and 3.30 mg of corn pollen per day (over a 24 hour period) during the second, third, and fourth stadia, respectively. Adults consumed an estimated 13.15 mg/day during flowering (anthesis), which for corn can last for approximately 8 days. The observed IPD072Aa expression levels in pollen were 1.3 ng/mg dw or less. Adults consuming 13 mg/day of DP23211 corn pollen would intake approximately 17 ng IPD072Aa total. Based on a No Observed Effect Diet Dose of 100 ng/mg IPD072Aa for *Coleomegilla maculata*, there are no adverse effects on *Coleomegilla maculata* populations that would be expected to arise from feeding on DP23211 corn pollen. For convergent lady beetle it is noted that weight was affected at all experimental exposure concentrations of 100 ng/mg IPD072Aa and above (Pioneer 2020). However, assuming similar pollen consumption rates (13.15 mg/day), it is improbable that convergent lady beetles would be exposed to this level of IPD072Aa via pollen consumption. APHIS is unaware of any specific data as to the amount of pollen consumed by convergent lady beetles (*Hippodamia convergens*), although it is surmised pollen intake would be similar to that of the pink spotted lady beetle given the overall similarity of their dietary preferences (e.g., aphids and other soft-bodied insects, pollen when prey is scarce).

Lady beetles may also be exposed to IPD072Aa by feeding on prey that have consumed DP23211 corn leaf or kernel tissue (maximum expression of 39 ng/mg IPD072Aa in leaf (dw)). Much of the lady beetle diet is comprised of sucking insects (e.g., aphids, spider mites, scale insects, thrips) that feed on plant sap or other fluids from the phloem sieve tube or xylem of the plant, respectively. Aphididae feed exclusively upon sap in the plant sieve tube (or sieve element; SE) where they ingest sugars, nitrogen compounds, and other nutrients. The xylem in plants is the main conduit for water, minerals, and other compounds from roots to shoots. Other insects, such as the leaf hopper, feed from the xylem.

There is currently no data on the amount of IPD072Aa that may be present in corn phloem sap or xylem fluids. Hence, an accurate assessment of potential lady beetle exposure to IPD072Aa via consumption of prey that have fed upon DP23211 corn phloem sap or xylem is not possible. Bearing this limitation in mind a review of potential lady beetle exposure to IPD072Aa via consumption of prey is provided.

The phloem sap and xylem fluid both play key roles in long and short distance transport of compounds vital to plant development, maintenance, and defense. Sugars, potassium, and amino acids are the main nutritional components for aphids that are commonly found in phloem sap (Sjolund 1997). The xylem transports water, minerals, metabolic products, and signaling compounds from the root to the shoot (Peuke 2009). These plant fluids also contain/transport proteins which are vital to plant physiology and health, protecting against local and systemic pathogen infection (Dinant et al. 2010). A comparison of the proteomes of the these fluids indicates that although functional categories are somewhat similar, proteins are likely to be fluid-specific, except for a small group of proteins present in both fluids, which may have a universal role, such as in cell wall maintenance and defense (Rodríguez-Celma et al. 2016).

The phloem sap of corn has not been well studied, although various studies have quantified the phloem sap protein content of rice, melon, cucumber, pumpkin, canola, and castor bean plant (e.g., see (Sjolund 1997; Dinant et al. 2010)). Several hundred phloem sap proteins have been identified among these species (Turgeon and Wolf 2009), to include transcripts encoding proteins related to metal homeostasis, stress response, protein degradation or turnover, and phloem structure and metabolism (Turgeon and Wolf 2009). Current studies have provided accumulating evidence that proteins and other macromolecules may sporadically appear in phloem sap (e.g. in response to stress of infection), while a large number of RNAs and soluble proteins are constantly present in phloem sap (Hayashi et al. 2000; Walz et al. 2004). Most of the identified phloem sap proteins repeatedly occur in more than one plant species; this indicates a high degree of conservation of the phloem sap protein composition in higher plants (Kehr 2006).

Because mature sieve element (SE) cells, which form the phloem sieve tube, lack the capability for protein synthesis (Sjolund 1997; Kehr 2006), phloem sap proteins are believed to be synthesized and imported through specialized, adjacent companion cells (CCs) (Kehr 2006). In general, there are studies that indicate that protein movement in and out of the phloem, protein occurrence in the phloem, is an active transport, highly regulated event (Turgeon and Wolf 2009; Yesbergenova et al. 2016).

There is a high degree of conservation of the phloem sap and xylem fluid proteins in higher plants—constitutively expressed proteins, signaling compounds, and plant nutrients are actively and selectively secreted into and out the plant phloem and xylem (Alvarez et al. 2006; Kehr 2006; Djordjevic et al. 2007; Turgeon and Wolf 2009; Neumann et al. 2010). While IPD072Aa is not a normal component of constitutively expressed proteins in corn, present in the sap of the phloem sieve tube or xylem fluid, whether it may occur in phloem sap and xylem fluid is unknown as IPD072Aa content in the phloem sap and xylem fluid of DP23211 corn has not been quantified. In the event IPD072Aa were to occur in phloem sap or xylem fluid, a general estimate of potential exposure is provided below. Because there is no quantitative data on the individual protein content of corn phloem sap or xylem fluid, a putative IPD072Aa protein concentration in phloem sap and xylem fluid can only be roughly estimated based on the concentrations of other proteins identified in the phloem and xylem of other plants.

Phloem sap proteomes have been analyzed in rice, melon, cucumber, pumpkin, pumpkin, canola, and castor bean plant. Proteins serving various functions have been observed to include plant defense, signal transduction, RNA trafficking, In general, the phloem sap in most plant species studied have total protein concentrations in the range of 0.1–2.0 μg/μl (Schobert et al. 1998; Thompson and Schulz 1999; Dinant et al. 2010). However, cucurbits are known to have significantly higher protein concentrations in phloem sap, up to 60 μg/μl in cucumber and 35 μg/μl in pumpkin. Most proteins identified range in size from 5 to

100 kDa. Individual plant species appear to exhibit a characteristic set of soluble proteins within their sieve-tube exudate, with total protein concentrations of 0.1 µg/µl for wheat (*Triticum aestivum*), 0.2 µg/µl for Asian rice (*Oryza sativa*), 0.1 -0.3 µg/µl for linden tree (*Tilia platyphyllos*), 0.2 -0.4 µg/µl for black locust (*Robinia pseudoacacia*), 0.2 - 0.5 µg/µl for castor bean plant (*Ricinus communis*), 0.5 - 1.0 µg/µl for yucca (*Yucca filamentosa*), and 19 – 30 µg/µl for winter squash (*Cucurbita maxima*) (Schobert et al. 1998). Studies have established that there is a set of constitutive proteins present in the sieve-tube system of monocot and dicot plants, which likely reflects some basic function in the highly specialized phloem long-distance transport system. In addition, for certain sieve-tube exudate proteins, there is wide species-to species variation in the respective abundance of individual proteins. Furthermore, some phloem sap proteins appear to be species specific (Schobert et al. 1998).

Up to 200 soluble polypeptides with a wide range of molecular masses and isoelectric points have been identified in the plant species studied (Thompson and Schulz 1999; Dinant et al. 2010). The number of proteins identified in phloem vary widely across species, from 140 in canola, over 300 in *Cucurbita maxima* (winter squash), and 107 in *Oryza sativa* (Asian rice), with other studies identifying 50 or fewer proteins in *Cucumis* species (melon), *Lupinus* species (flowering plants), and *Ricinis communis* (castor bean plant) (Carella et al. 2016). In tomato, (Ogden et al. 2020) observed 169 proteins whose abundance changed significantly within the phloem sap, either during drought or recovery from drought. Proteomics analyses of xylem sap collected during host interactions with *Fusarium oxysporum*, a plant pathogen, were performed using tomato and *Brassica oleracea* (Gawehns et al. 2015; Pu et al. 2016). Both studies identified substantially more total proteins (∼150–285) with a relatively high proportion of those proteins showing differential abundance during infection.

In summary, most plants exhibit total protein in phloem around 0.1–2 µg/µL, while the phloem sap of cucurbits contains around 10–60 µg/µL. Studies on *Cucurbita maxima* have identified from 11 to 320 proteins in phloem sap (Carella et al. 2016). Based on available data, this would yield individual protein concentrations ranging from 30 ng/µL to 900 ng/µL. Cucurbit phloem sap-purified phloem protein II (PP2) has been found at a concentration of between 25 and 40 ng/µl (Balachandran et al. 1997). In *Cucurbita maxima* proteins in the 10 – 20 kDA range were found at a total concentration of 50 ng/µl, and in the 50 – 80 kDA range at a total concentration of 25 ng/µl; the number of individual proteins in these mass ranges, and their respective concentrations, was not quantified (Balachandran et al. 1997). For castor bean plant (*Ricinus communis*) individual protein concentrations in phloem sap, based on the data reviewed, would range from 10 - 20 ng/µl.

The extracted xylem fluid of corn has been shown to have a total protein concentration of 12 µg/mL (Alvarez et al. 2006). This concentration is comparable with xylem sap extracted from squash roots which contained 19 µg/mL total protein. A total of 154 proteins were identified in corn xylem fluid, although many were observed to have had the same molecular weight with a variable isoelectric point, indicating the presence of multiple forms of a protein (Alvarez et al. 2006). Among this total, 54 proteins were considered unique (Alvarez et al. 2006). Average protein concentration in the xylem fluid, for individual proteins, would be around 0.2 µg/mL (0.2 ng/µl). The proteins in the xylem fluid belong to three major categories: cell wall metabolism (58%), plant defense (26%), and proteins involved in proteolysis and peptidolysis (11%) (Alvarez et al. 2006).

Corn phloem sap composition has been analyzed in only a limited number of studies. The specific protein content of corn phloem sap has not been studied, to date. Profiling of corn phloem sap exudates identified 45 compounds including carbohydrates, amino acids, organic acids and a number of less abundant molecules such as sterols, vitamins, and polyamines (Yesbergenova et al. 2016). Potassium, chloride, and sucrose were the primary phloem sap constituents in corn, comprising 21%, 11.6%, and 38.3% respectively (Yesbergenova et al. 2016). Soluble sugars represent on average around 88% of the overall metabolite content of the phloem sap exudates. Sucrose was the most abundant carbohydrate, representing up to 76% (mean value) of the soluble sugars, depending on the line examined (Yesbergenova et al. 2016). Amino acids have been found to comprise 5% to 16%, and nucleotides 0.2% (Ohshima et al. 1990; Yesbergenova et al. 2016). Over 40% of the nucleotide pool has been found be comprised of ATP. ATP, ADP and AMP can comprise up to 80% of the total pool of nucleotides (Ohshima et al. 1990). Other compounds such as myo-inositol, putrescine, and ethanolamine, were also identified, although they represented less than 2% of the total (Yesbergenova et al. 2016). Smaller amounts of arginine, asparagine, alpha-aminobutyrate (GABA), glycine, histidine, isoleucine, lysine, phenylalanine, proline, threonine, tyrosine, and valine were also detected in the phloem exudates (Yesbergenova et al. 2016). The concentration of these compounds in corn phloem sap was not analyzed.

Douglas (2006) estimated that larvae of *Acyrthosiphon pisum* (pea aphid) ingested, on average, 1.92 µL phloem sap over 2 d (from days 6 to 8 after birth), during which time they increase in weight from 0.7 mg to 1.32 mg, equivalent to protein growth of 31 µg (31,000 ng). Assuming IPD072Aa protein could occur in the phloem sap in the range of protein concentrations found in other plants, summarized above, at around 30 ng/µL (ppm), pea aphid larvae of would intake 28 ng/day IPD072Aa protein. How much of this putative intake would be excreted or otherwise transformed via digestion is unknown. Adverse effects via direct consumption in diet studies on Coccinellidae were observed at 500 ng/mg (ppm) diet and higher (Table 4-16).

Potential exposure of Coccinellidae to any IPD072Aa via sap would likely be below an effects level of 100 ng/mg diet; consequently, it is unlikely that any IPD072Aa present in DP23211 corn phloem sap and xylem fluid presents a hazard to Coccinellidae that may feed on aphids or other species of sucking insects that have ingested DP23211 corn phloem or xylem fluids.

Based on the leaf, grain, and pollen expression levels of IPD072Aa, specificity for western corn rootworm with an LC50 of 26 ng/mg diet; absence of adverse effects on honeybee larvae at 200 ng/larva and adults 1300 ng/bee/day; absence of adverse effects on most NTOs studied at dietary concentrations below 100 ng/mg diet, and limited effects on coccinellids at 100 ng/mg diet or higher, dietary concentrations that would not be achieved in the field; the IPD072Aa protein in DP23211 corn is not expected to present a any hazard to NTO populations, to include coccinellids.

Aquatic Invertebrates

As previously discussed, the DvSSJ1 dsRNA nucleotide sequence is specific for smooth septate junction protein (DvSSJ1) mRNA in mid-gut epithelial cells of western corn rootworm (*Diabrotica virgifera vergifera;* Coleoptera), with no activity observed in other orders. The IPD072Aa proteins has targeted activity in Coleoptera. Hence, it is unlikely DvSSJ1 dsRNA and the IPD072Aa protein would have biological activity in aquatic invertebrate species (e.g., among the orders Plecoptera, Megaloptera, Diptera, Anostraca). Nevertheless, an overview of potential exposure is provided.

The worst-case EEC for aquatic organisms to DvSSJ1 dsRNA and the IPD072Aa protein in DP23211 corn tissues was estimated using the EPA standard agricultural field-farm pond model (also called the U.S. EPA standard pond model). Based on the assumptions in the EPA farm pond model, the worst-case EECs for aquatic organisms are 2.46 x 10^{-4} mg/L and 0.124 mg/L (ppm) for the DvSSJ1 dsRNA and the IPD072Aa protein, respectively. The LC50 of DvSSJ1 dsRNA for western corn rootworm is 0.036 ng/mg (ppm), which is 146X higher than the worst-case EEC for aquatic organisms. The LC50 of the IPD072Aa protein for WCR is 26 ng/mg (ppm), which is 210X higher than the worst-case EEC for aquatic organisms (Pioneer 2020).

Several factors would limit aquatic exposures to DP23211 corn plant material. It would generally take a very heavy rain event to transport corn plant material to nearby water bodies (i.e., a fallen leaf or kernel). Topsoil would be more readily transported. DvSSJ1 dsRNA and IPD072Aa would not be expected to commonly occur in soils; only as a matter of degradation of plant material that was left on the field post-harvest, plant material that may have fallen to the ground as a result of animals foraging on the crop, plant material that may have fallen to the ground during drying for silage—or decaying root material. As previously discussed for potential impacts on soil biota, environmental DNA/RNA is generally degraded within a week, most studies stating two or three days (Dubelman et al. 2014; Parker et al. 2019). The IPD072Aa protein is fully degraded after about a week (Pioneer 2020). Albright et al. (2017) examined dissipation of a 100 base pair dsRNA in three different microcosms (laboratory water over sterilized sediment, sterilized pond water over sterilized sediment, and active pond water over active sediment). This study concluded that the dsRNA degraded rapidly within all three microcosms and was undetectable by 96 hrs. Degradation kinetics estimated a half-life of less than 3 days and a time to 90% dissipation of approximately 4 days. Studies by Fischer et al. (2017) found DvSnf7 RNA to fully degrade in surface waters within 4 to 6 days. There is no data on the degradation rate of IPD072Aa in water.

Considering the slow rate of plant tissue decay, amount of dsRNA and IPD072Aa that would be deposited in soils, and residence times of dsRNA and IPD072Aa in soils, it is unlikely that intact dsRNA or IPD072Aa of sufficient quantity to elicit biological activity would enter surface waters via topsoil runoff. Hence, the risk of exposure of aquatic invertebrates to DvSSJ1 dsRNA or IPD072Aa via com detritus is expected to be negligible. While some exposure to DvSSJ1 dsRNA may conceptually occur in the water column, it would likely be short lived.

Based on these factors, the buildup of post-harvest plant debris or topsoil comprised of DvSSJ1 dsRNA and IPD072Aa protein in surface waters, particularly those waters with continual movement, is expected to be of negligible quantity and limited in occurrence.

Synthetic Chemical Insecticides: *Invertebrates and Aquatic Biota*

To some extent, there could be less reliance on use of insecticides with DP23211 corn to control WCR. As an IR corn variety comprised of PIPs (DvSSJ1 dsRNA and IPD072Aa protein) with specify for WCR, cultivation of DP23211 corn would likely entail less use of soil- and foliar-applied insecticides to control WCR. Adopters of transgenic PIP based IR corn have been found to use around 11.2% (0.005 kg/acre) less insecticide than nonadopters (Perry et al. 2016). These types of IR crops have also been found to facilitate reductions in use of insecticides in nearby non-IR grain and vegetable crops, owing to the area-wide suppression of insect populations targeted by the PIP. For example, Dively et al. (2018) found that insecticides (total active ingredient) applied in non-IR sweet corn declined from 1.1 kg/acre (2.64 kg/ha) in 1992 to 0.23 kg/acre (0.55 kg/ha) in 2016, a 79% decrease. In pepper crops, insecticides applied declined from 2.05 kg/acre (4.92 kg/ha) in 1992 to 0.31 kg/acre (0.75 kg/ha) in 2016, an 85% decrease. Insecticide usage in peppers significantly declined as a function of proximity of Bt corn, while in sweet corn, the negative association was marginally significant. These results indicate that a significant proportion of the decreased insecticide usage in Mid-Atlantic vegetable crops could be attributed to the regional pest suppression associated with widespread Bt field corn adoption (Dively et al. 2018).

Certain insecticides can be toxic to invertebrates and fish at relatively low concentrations. Thus, any reductions in insecticide use, or limitation of insecticide use, would be environmentally beneficial. The four main agricultural insecticides used are pyrethroids (PYs), organophosphates (OPs), carbamates (CBs), and biological insecticides (BIs) (Helfric 2013; US-EPA 2019a). Among these, PYs, OPs, and CBs are used for CRW control at planting and post-planting (Wright 2009).

PYs can be toxic to fish and aquatic invertebrates and use in proximity to waterways is cautioned (US-EPA 2016e). EPA risk assessments for freshwater, estuarine, and marine vertebrate and invertebrate species indicate pyrethroids can be highly toxic to aquatic organisms on an acute basis (US-EPA 2016e). Chronic effects are also seen in aquatic taxa. LC50 values for aquatic biota are in the parts per billion (ppb) and parts per trillion (ppt) range; thus, highly toxic (US-EPA 2016e).

Many OP and CB insecticides are also highly toxic to fish and wildlife. Fish kills involving these insecticides have been documented (US-EPA 2016e). OP insecticides can bioconcentrate in fish, frogs, tadpoles, and toads to levels that pose hazards to their predators (Hall and Kolbe 1980; Helfric). U.S. Geological Survey (USGS) studies found OP insecticides to be major contributors to toxicity in over half of the streams studied (Nowell et al. 2018). The CB insecticide carbofuran— used to control insects in a wide variety of field crops, including potatoes, corn, and soybeans—is highly toxic to wildlife and fish (Struger et al. 2016).

While the use of IR crops typically limits the use of broad-spectrum chemical insecticides (foliar sprays and soil insecticides), it is noted, however, that this benefit can to some extent be countered by the use of insecticidal seed treatments (to both IR and non-IR crop seed) for the management of early season pests, and as insurance against sporadic soil-borne pests (Allen et al. 2018; Sappington et al. 2018). For example, neonicotinoids, which are used on more than 90% of corn acres (USGS 2020b), can persist in the environment, accumulate in soils, enter waterways via runoff, and can pose a threat to a number of non-target species, especially bees and other pollinators, and soil and aquatic invertebrates (Perry and Moschini 2020; Schulz et al. 2021). Neonicotinoid use for seed treatment is related to their toxicity in broad array of insect species, and simultaneously low acute toxicity to mammals (Douglas and Tooker 2016). Pyrethroids are effective in controlling a broad range of insect pests, although are toxic to aquatic organisms (US-EPA 2016e). While this point is noted, use of neonicotinoid seed treatment, which may be applied to conventional and biotechnology based seeds, is unrelated to the introduced IR traits in DP23211 corn.

Summary

The DvSSJ1 dsRNA has been shown to have a narrow spectrum of activity, primarily affecting corn rootworm (*Diabrotica;* Coleoptera) (Boeckman et al. 2019; Hu et al. 2019). The IPD072Aa protein likewise has specific activity targeting western corn rootworm. The tissue expression levels of both in DP23211 corn are expected to effect western corn rootworm populations, with negligible effects on nontarget species.

No synergism among DvSSJ1 dsRNA and IPD072Aa has been observed, nor it is expected: Based on the separate mechanisms of action for IPD072Aa protein and DvSSJ1 dsRNA, it is unlikely that DvSSJ1 dsRNA and IPD072Aa can combine to elicit an increased synergistic effect on insects that ingest DP23211 corn (Pioneer 2020). IPD072Aa protein binds to brush border membrane receptors in the western corn rootworm gut causing disruption of the mid-gut epithelium function. The DvSSJ1 dsRNA acts to inhibit translation of the target mRNA, which, as a result, diminishes the level of an essential protein needed for the formation/maintenance of smooth septate junctions in WCR (Pioneer 2020).

Due to the species specificity of the insecticidal traits, and to some extent reduction/limitation in the use of synthetic chemical insecticides with DP23211 corn, as well as possible contribution to reductions/limitations in insecticide use in nearby corn crops owing to area-wide suppression of *Diabrotica virgifera virgifera*, DP23211 corn production would be expected to present fewer hazards to non-target insect populations, as compared to broad spectrum chemical insecticides. As discussed in Section 4.3.1.2.2–Agronomic Inputs, over the top insecticide use in corn production has significantly declined during the last 20 years due in part to the adoption of IR corn varieties. The use rate of insecticides applied to corn fell from an average of around 0.08 kg/acre in 1998 to about 0.02 kg/acre in 2011 (a 75% decrease), as the adoption of IR corn crops increased (Perry et al. 2016). In general, adopters of IR corn use around 11.2% less insecticide than nonadopters (Perry et al. 2016). Limits on chemical insecticide use with IR corn, and possible contributions to reductions in insecticide use in nearby cropping systems, is of environmental benefit to both terrestrial and aquatic biota. In this respect, production of IR crops, to include DP23211 corn, has the potential to be more environmentally benign—have less of an effect on non-target pest populations—as compared to conventional synthetic chemical-based pest management approaches.

Overall, studies to date have shown that the effects of IR crops on non-target populations are minimal to negligible in comparison to the effects of broad-spectrum chemical insecticides (e.g., see review by (NAS 2016; Romeis et al. 2019)). Because DP23211 corn crops would have more targeted control of a specific species of plant pest (*Diabrotica virgifera virgifera*) with less reliance on broad-spectrum synthetic chemistries, this could, in a comparative sense, be favorable to sustaining beneficial insect activity, and reduce the adverse effects of synthetic chemical insecticides on non-target insect populations (Gatehouse et al. 2011; Dively et al. 2018; Romeis et al. 2019).

Lastly, transgenic corn hybrids expressing Cry3Bb1, mCry3A, eCry3.1Ab as well as Cry34/35Ab1 were introduced into the market to manage WCR. Development of resistance in some WCR populations to Cry3 based PIPs has constrained the options for management of WCR (Zhao et al. 2019), ultimately resulting in greater reliance on broad-spectrum chemical insecticides to control resistant pest populations. Because the modes of action of both DvSSJ1 dsRNA and IPD072Aa MOAs differ from the Cry3A, Cry3Bb1, Cry 34/35Ab1, Cry3.1Ab toxins, this corn variety, to the extent grown, could help alleviate selection pressure for Cry resistant WCR populations, and thereby the potential for increased insecticide use to control Cry resistant WCR populations.

4.3.3.3 Plant Communities

Plant diversity in areas surrounding crop fields is an important component of a sustainable agricultural system (Scherr and McNeely 2008; CBD 2020a). Hedgerows, woodlands, and fields provide not only wildlife habitat but serve as important reservoirs for beneficial insects (although plant pests as well). 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). However, for corn production, pollinators would not be as valued from an agronomic perspective, as corn is primarily wind pollinated. Surrounding plant communities can also help regulate run-off, reduce soil erosion, and improve water quality. Hence, sustaining surrounding plant communities can provide benefits to corn crop production via control of insect pests and agricultural run-off (Altieri and Letourneau 1982), and provide pollinator services to other plants that benefit from insect pollination (Nichols and Altieri 2012).

Members of plant communities in and around cornfields that adversely affect corn cultivation are generally characterized as weeds, and the presence of these plants controlled to maximize crop yield and quality (Section 4.3.1.2.2.3). Most relevant to environmental review of transgenic cropping systems are those sexually compatible plant communities with which the transgenic crop plant can interbreed (discussed following in Section 4.3.3.4).

4.3.3.3.1 Potential Effects on Plant Communities

Cultivation of DP23211 corn would be expected to have similar impacts on vegetation proximate to DP23211 corn fields as currently cultivated corn varieties, relative to the particular herbicides used. When crops are sprayed, sublethal doses of herbicides may reach non-target plant species in adjacent habitats through drift, runoff, and/or volatilization. Sublethal effects on sensitive species could include negative impacts on leaves (photosynthesis), seed production, delays in flowering times, and reductions in flower production. Reviews of this topic suggest that after application typically less than 1% of herbicide applied is lost to groundwater leaching, approximately 1%–4% is carried away in surface runoff, with losses via drift and volatilization ranging between 5%–25% (Boutin et al. 2014; Prueger et al. 2017).

Glufosinate is a broad spectrum herbicide that affects monocotyledonous (monocot) and dicotyledonous (dicot) species. Glufosinate spray drift may inadvertently impact non-target plants proximate to DP23211 corn fields. Consequently, glufosinate herbicides must be used in accordance with the spray drift management precautions on the EPA label to minimize off-site exposures (US-EPA 2020b). Herbicides that contain glufosinate are often applied with a boom-mounted sprayer. However, they can be occasionally applied aerially on corn, cotton, potato, and soybean. Generally, less than 1% of the total amount of glufosinate-containing herbicides were applied from the air, with the largest part on potatoes (22% of potato crops are treated with glufosinate by air) (US-EPA 2016a). The EPA provides guidance and label use requirements for reducing the probability of herbicide spray drift and volatilization (US-EPA 2019i, j).

4.3.3.4 Gene Flow and Weediness of Corn

Gene flow among transgenic crops and conventional and organic cropping systems, as wells as other transgenic crops, is of particular interest to farmers, and federal and state regulators, as such gene flow can adversely affect farmers' net returns on crops, and domestic and international trade.[14] Gene flow from transgenic plants to sexually compatible 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 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 (i.e., herbicide resistance, insect resistance) 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. Gene flow itself does not necessitate the increased fitness of a hybrid. 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 that traits that impart increased fitness or are neutral 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.

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. The salient issue is what the resultant ecological consequences of such gene flow to a wild population may be (Ellstrand 2014). Current understanding

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[14] The term "gene flow" can be synonymous with "outcrossing" and the terms are used here interchangeably. Neither term implies the long-term persistence or introgression of gene(s) into a recipient population. A hybrid is the offspring of two genetically dissimilar but sexually compatible species, generally within the same genus, although hybrids between different genera are possible.

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 (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 ecological consequences.

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

Corn (*Zea mays* L. subsp. *mays*) is one of the oldest domesticated food 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 (Wilkes 1967; Iltis and Doebley 1980; 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 among the genus *Tripsacum*, with which corn does not readily hybridize (discussed further below).

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). In general, humans breed 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 2019d). Teosinte 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; MPAWG 2016), 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 2020); 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 of *Tripsacum* have been identified in the United States: *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 (OECD 2003; USDA-NRCS 2019b). *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). *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).

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 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 2019d; USDA-APHIS 2022b).

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, depending on the density of the volunteer corn (Nicolai et al. 2018; Jhala et al. 2019). 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. 2018). 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). 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. 2018).

4.3.3.4.4 Probability and Potential Effects of Gene Flow

DP23211 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. As APHIS concluded in its PPRA, the introduced trait genes in DP23211 corn are not expected to alter characteristics associated with reproductive biology—change the ability of the plant to interbreed with other plant species (USDA-APHIS 2022b). Accordingly, a determination of nonregulated status for DP23211 corn and subsequent commercial production 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. Thus, there is no plausible opportunity for interbreeding. Three species of *Tripsacum* have been identified in the United States: Eastern gamagrass, Mexican gamagrass, and Florida gamagrass. Eastern gamagrass is the only *Tripsacum* species of widespread occurrence

(USDA-NRCS 2002). Conceptually, the IR trait could confer a competitive advantage to wild *Tripsacum* species. The *pat* transgene would confer resistance to glufosinate-ammonium. In the event gene flow from DP23211 corn to *Tripsacum* species occurred, the HR trait would not be expected to confer any fitness advantage, or disadvantage, in areas where glufosinate was not regularly used. While conceptually possible, the likelihood of *Tripsacum* populations harboring the IR and HR trait genes is considered remote. First, in contrast with corn and teosinte (*Zea* spp.), which may hybridize relatively easily under certain conditions, the potential for 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).

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). In terms of pollination: The number of potential 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).

4.3.3.5 Biodiversity

Biological diversity in the context of agriculture means the variety of and interactions among the communities of animals, plants, and microorganisms that comprise the structure and functions of agroecosystem (FAO 1999). Agricultural biodiversity provides stability, adaptability, and resilience to cropping systems, and central to sustainable cropping systems and food security (FAO 2008; Mijatović et al. 2013).

Various taxa contribute to essential ecological functions upon which agriculture depends, such as pollinators, soil biota, and predators of crop pests (CBD 2020a). One invaluable function of sustaining diverse insect populations on farms is the natural control of plant pest populations via predation, parasitism, and feeding competition among herbivores. In one study of corn farms across the Northern Great Plains, Lundgren and Fergen (2014) found that farms with lower insect diversity had more plant pests, and that more bio-diverse cornfields had fewer plant pests. The results from their study show that designing cropping systems for high insect diversity— increasing vegetation diversity on farms by lengthening crop rotations, using cover crops in rotations, intercropping, managing field margins, and using minimal-till agriculture— can facilitate control of plant pests, requires fewer insecticide inputs, and can save farmers time and money.

Relative to HR/IR crops, by facilitating conservation tillage, limiting insecticide use, and helping sustain maximum yield—which alleviates pressure to convert additional land into agricultural use—HR/IR crops can contribute to reducing the impacts of agriculture on biodiversity (Carpenter 2011; Raman 2017). A U.S. National Research Council assessment of the relationship between crop production and farm sustainability in the United States concluded that, generally, HR and IR crops have had fewer adverse effects on the environment than crop varieties produced conventionally (NRC 2010). During the 1996 – 2016 timeframe the adoption of HR and IR technology reduced pesticide spraying in the United States by 361 million kg (796 million pounds), and facilitated reductions in tillage and fossil fuel use (Brookes and Barfoot 2017).

Studies in sweet corn, which is routinely treated with foliar insecticides during production, have documented that the conservation of natural enemies of plant pests with Bt based crops can facilitate biological control in cropping systems. Musser and Shelton (2003) found that Bt sweet corn (lepidopteran resistant) was less toxic to the major predators in the cropping system (ladybeetles *C. maculata* and *H. axyridis* and the minute pirate bug, *Orius insidiosus*), than the commonly used pyrethroid insecticide lambda cyhalothrin, and the insecticides spinosad and indoxacarb. This study demonstrated that Bt corn provided effective control of lepidopteran pests, and did not negatively affect the predation rates of egg masses of the European corn borer (a significant plant pest), as did lambda cyhalothrin and indoxacarb (Musser and Shelton 2003; Romeis et al. 2019). A follow-up study proposed a model that integrates biological and chemical control with transgenic IR crops for suppressing not only the target pests, but secondary pests such as aphids that affect marketability (Musser et al. 2006).

HR and IR crops have also made important contributions to increasing global production levels. As of 2018, these traits have facilitated—since the introduction of the technology in the mid-1990s—the addition of 278 million metric tons and 498 million metric tons to the global production of soybeans and corn, respectively (Brookes and Barfoot 2018a). The average yield impact across the total area planted to HR and IR corn during the 1996 – 2018 timeframe has been +16.5% (Brookes and Barfoot 2018a). To maintain global production levels at 2018 levels, without crops with HR and IR traits, would have required farmers to plant an additional 4.9 million acres (12.3 million hectares) of soybeans, 3.3 million acres (8.1 million hectares) of corn, 1.25 million acres (3.1 million hectares) of cotton, and 0.2 million acres (0.7 million hectares) of canola, an area equivalent to the combined agricultural area of the Philippines and Vietnam (Brookes and Barfoot 2018a).

4.3.3.5.1 Potential Effects on Biodiversity

Commercial production of DP23211 corn would not be expected to affect biodiversity in and around DP23211 corn crops any differently than other corn cropping systems. As previously reviewed, the DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI trait proteins are unlikely to present any hazards to plant, animal, fungal, or bacterial communities—apart from the intended impacts of the DvSSJ1 dsRNA and IPD072Aa traits on targeted *Diabrotica virgifera virgifera*. While there would be reductions in *Diabrotica virgifera virgifera* with perhaps some cascading effects on predator/parasitoid populations in terms of availability of prey species (Chaplin-Kramer et al. 2011; Letourneau et al. 2011; González et al. 2016), this would not be expected to have a significant effect on insect populations and community dynamics within DP23211 corn crops, or areas adjacent to DP23211 corn crops (e.g., predators, pollinators) (Strand and Obrycki 1996; Obrycki et al. 2021). While some predatory species are highly specific and feed on only one species of prey, most others feed on either a limited range of related taxa (are stenophagous or oligophagous), or a wide variety of prey (are polyphagous—general feeders). Predators of *Diabrotica virgifera virgifera* include beetles (Carabidae, Staphylinidae, Dermestidae), mites

(*Hypoaspis aculeifer*), ants (*Lasius neoniger*, *Myrmica americana*, *Pheidole bicarinata*), and spiders (Araneae) (Kuhlmann and Burgt 1998; CABI 2020), all of which also feed on range of other species (Adalbert et al. 2008; Carvalho et al. 2017; Gathalkar and Sen 2018; Raupp et al. 2021).

In general, IR crops such as DP23211 corn, for the most part, have the potential to be more environmentally benign than chemical insecticide-based pest management approaches (Gatehouse et al. 2011; Romeis et al. 2019). While biodiversity will be inherently limited in commercial corn crops due to frequent disturbance of the landscape, tillage, mechanized planting, planting of a monoculture crop, and application of pesticides, HR/IR crops have generally reduced the environmental impacts of crop production, relative to conventional broad-spectrum chemical approaches to pest management (NRC 2010; NAS 2016; Raman 2017; Brookes and Barfoot 2018c; Romeis et al. 2019). Glufosinate use for weed control would be subject to EPA label requirements, no different than for other corn crops.

Growers and federal and state agencies well recognize the need for maintenance of cropland biodiversity. A variety of federally supported programs, such as the USDA funded Sustainable Agriculture Research and Education Program (SARE), and partnership programs among the EPA and the agricultural community support agricultural practices that are intended to protect the environment, conserve natural resources, and promote cropland biodiversity (i.e., (US-EPA 2019c; USDA-NIFA 2020)). The USDA Natural Resources Conservation Service, through its Conservation Stewardship Program, Landscape Initiatives, Environmental Quality Incentives Program, Landscape Planning, and other services provides technical and financial support to growers to assist in managing the complex interaction of cropping systems and the natural environment (USDA-NRCS 2019a). Tools are also developed by the industry. For example, *Field to Market: The Alliance for Sustainable Agriculture* supports various programs that helps farmers and the food supply chain benchmark sustainability performance, to included biodiversity (Field-to-Market 2019).

4.3.3.6 Threatened and Endangered Species

Section 7(a)(2) of the ESA requires that federal agencies, in consultation with the U.S. Fish and Wildlife Services (USFWS) and/or the National Marine Fisheries Services (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 on threatened and endangered species (TES) listed under the ESA, 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).

4.3.3.6.1 Potential Effects on TES

After reviewing the possible effects of a determination of nonregulated status for DP23211 corn, discussed in Appendix 1, APHIS has not identified any stressor that could affect listed TES or species proposed for listing. APHIS also considered the potential effect of DP23211 corn on designated critical habitat and habitat proposed for designation, and could identify no differences from effects that would occur from the production of other corn varieties. Corn is not sexually compatible with, nor serves as a host species for, any listed species or species proposed for listing. A discussed previously in 4.3.3.2–Animal Communities, consumption of DP23211 corn by any listed species or species proposed for listing would pose negligible physiological risks.

Based on our evaluation provided in Appendix 1, APHIS has concluded that a determination of nonregulated status of DP23211 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

Human health considerations are those related to (1) the safety and nutritional value of foods derived from biotechnology-derived crops, and (2) the potential health effects of pesticides that may be used in crop production. As for food safety, consumer health concerns are in regard to the potential toxicity or allergenicity of the introduced genes/proteins, the potential for altered levels of existing allergens in modified plants, or the expression of new antigenic proteins (substances capable of stimulating an immune response). Some consumers may be concerned about the potential consumption of pesticide residues on/in foods derived from biotech crops. Occupational exposure to pesticides is also considered.

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 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 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 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 created a voluntary premarket food safety consultation process in the 1990's. This consultation process enables developers of new crop varieties to engage with the FDA to ensure the safety of food and feed products derived from the biotechnology-derived crop (US-FDA 1992a, 2006). Pioneer completed a food/feed safety consultation with the FDA in 2022; the FDA had no questions concerning human or animal food derived from DP23211 corn (US-FDA 2022).

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.

4.3.4.2 Safety of the Herbicide Resistance and Insect Resistance Traits

PAT Safety Evaluations: Glufosinate-Ammonium Resistance

PAT, as an introduced trait in crop plants, can be encoded by the *bar* gene derived from the naturally occurring soil bacterium *Streptomyces hygroscopicus*, and by the *pat* gene derived from *S. viridochromogenes*. *Streptomyces* are gram-positive bacteria of the actinomycetal order with more than 600 species described in soils, sediments, and seawater (Labeda et al. 2012; Bontemps et al. 2013). The gene encoding the PAT protein in DP23211 corn was isolated from *Streptomyces viridochromogenes,* which occurs predominantly in soils (Liu et al. 2013; GCM 2019). Because the PAT enzyme occurs naturally in soils, it stands to reason that wildlife and humans, globally, have been and are potentially exposed, incidentally, to the *pat* gene and PAT enzyme through environmental sources on a daily basis (e.g., incidental ingestion, inhalation).

APHIS has evaluated and deregulated 11 varieties of corn comprised of either the *pat* or *bar* genes, as well glufosinate resistant varieties of soybean, cotton, canola, beet (USDA-APHIS 2022a). There are no human health or ecological risks associated with exposure to PAT; the enzyme has a long history of safe use in commercially produced corn, soybean, and cotton varieties. The FDA has previously consulted on PAT, and the *pat* and *bar* genes encoding for expression of PAT, for over 30 glufosinate resistant crop varieties (US-FDA 2020); these include canola, corn, cotton, soybean, and sugar beet. None of these modified crop varieties have been identified as presenting any risk to human or animal health. Previous evaluations of PAT have shown that it does not share amino acid sequence similarity to known toxins, nor does it possess characteristics associated with food allergens (Herouet et al. 2005; ILSI-CERA 2011).

Due to the negligible human health risks associated with PAT, the EPA granted an exemption from the requirement of a tolerance for PAT when used as plant-pesticide inert ingredients in all food commodities (40 CFR § 174.522).

Safety of DvSSJ1 dsRNA

There are no health hazards associated with consumption of DNA/RNA in foods. The mediators of RNAi, such as dsRNA, small interfering RNAs (siRNA), and microRNAs (miRNA), are present in all commonly consumed plant and animal based foods (Ivashuta et al. 2009; Petrick et al. 2013; Frizzi et al. 2014; Wagner et al. 2015). It follows, dietary nucleic acids, to include dsRNAs, are safely consumed on a daily basis worldwide—this includes plant dsRNAs with nucleic acid sequences complementary to human genes/transcripts (Ivashuta et al. 2009; Jensen et al. 2013; Dever et al. 2015). The FDA concluded that nucleic acids introduced into crop plants, in and of themselves, present no safety concerns (US-FDA 1992b).

IPD072Aa Protein Safety Evaluations

Bioinformatic assessment of the IPD072Aa protein sequence for potential allergenicity was conducted according to Codex guidelines (CODEX 2003; Pioneer 2020). The comparisons of the IPD072Aa protein sequence to the allergen sequences found no association between the IPD072Aa protein and potential allergenicity.

The potential toxicity of the IPD072Aa protein was assessed by comparison of its nucleic acid sequence to the sequences in the Pioneer toxin database (Pioneer 2020). No alignments were returned between the IPD072Aa protein sequence and sequences in the toxin database that suggested an association between the IPD072Aa protein and potential toxicity.

Carlson et al. (2019) conducted a 14-day acute oral toxicity study in mice using IPD072Aa protein. The IPD072Aa protein produced no evidence of adverse effects following administration of 2000 mg/kg body weight/day. There were no instances of clinical abnormalities, changes in body weight, or mortality observed in any of the animals. The concentration of IPD072Aa protein in DP23211 corn is 0.51 to 4.8 ng/mg tissue dry weight (dw) in grain and 0.054 to 39 ng/mg tissue dw in leaf (depending on growth stage). At these levels, humans or other animals could not consume enough grain or leaf tissue via diet to obtain an exposure of 2000 mg/kg body weight/day.

Phosphomannose Isomerase Safety Evaluations

Phosphomannose isomerase (PMI) is an evolutionary conserved enzyme found in various taxa including bacteria, fungi, insects, some species of plants and nematodes, and mammals—including humans (de Lonlay and Seta 2009; Hu et al. 2016a). In eukaryotes it is involved in carbohydrate metabolism, in prokaryotes it is involved in a variety of metabolic pathways including polysaccharide biosynthesis.

The PMI protein produced in DP23211 corn is encoded by the native *pmi* gene from *E. coli* (Pioneer 2020). In DP23211 corn, the *pmi* gene was used as a selectable marker in modification of this corn variety (Pioneer 2020). 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 *pmi* gene/PMI are capable of growth in a mannose medium, which utilize mannose as a carbon source. PMI as a selectable marker has been previously used in corn and rice, crops that have been evaluated for food safety by the FDA (see FDA Biotechnology Consultations 113 and 128, and 158, respectively (US-FDA 2019c)). As described further below, due to its inherent safety (endogenously expressed in prokaryotes and eukaryotes), PMI is exempt from the requirement of a tolerance in food and feed.

4.3.4.3 Pesticides, PIPs, 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 a tolerance limit, which is the amount of pesticide residue allowed to remain in or on each 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 food and feed for human and animal consumption. 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 the 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 potential 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.

Both the FDA and USDA monitor foods for pesticide residues to enforce tolerance limits and ensure protection of human health. 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 commonly consumed by infants and children (USDA-AMS 2020). 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 has established tolerance limits for glufosinate at 40 CFR §180.473, for a wide variety of commodities, including field corn for grain and forage (US-EPA 2019e).

Due to the negligible risk PAT and PMI pose to human health, summarized above, the EPA has issued permanent exemptions for the requirement of a tolerance limit in all food commodities in the United States (40 CFR §174.522 and 40 CFR § 174.527, respectively). Nucleic acids, because they are normal components of human and animal diets and have a history of safe consumption in food and feed, are also exempt from the requirement of a tolerance (40 CFR §174.507). Pioneer has submitted a registration application to the EPA Biopesticides and Pollution Prevention Division (BPPD) under FIFRA Section 3 for the DvSSJ1 dsRNA and the IPD072Aa protein. This submission included a petition for exemption from the requirement of a tolerance for the IPD072Aa protein under the FFDCA.[15] The DvSSJ1 dsRNA is exempt from the requirement of a tolerance under 40 CFR 174.507.

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.

4.3.4.4 Worker Safety

Agricultural worker hazards are primarily those associated with potential pesticide exposure, and the operation of farm machinery and other equipment. Agricultural operations are covered by several

[15] Federal Register / Vol. 85, No. 73 / Wednesday, April 15, 2020 / p. 20910: EPA, 40 CFR Part 180, Receipt of a Pesticide Petition Filed for Residues of Pesticide Chemicals in or on Various Commodities (February 2020). https://www.govinfo.gov/content/pkg/FR-2020-04-15/pdf/FR-2020-04-15.pdf

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. OSHA also requires employers to protect their employees from hazards associated with pesticides.

In November 2015, the 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 October 30, 2020, the EPA finalized narrow updates to the Application Exclusion Zone (AEZ) provisions under the Worker Protection Standard regulation.

4.3.4.5 Potential Effects on Human Health

As discussed below there are no food safety issues associated with the consumption of DP23211 corn products. Production of DP23211 corn seed/hybrids would present the same potential risks to public health and worker safety as production of other corn varieties. Risks to public health would be those associated with the potential contribution of crop production to impacts on air and water quality (e.g., runoff of pesticides and fertilizers into surface waters, leaching of pesticides into groundwater, drift of pesticides offsite). Risks to worker safety would be in relation to pesticide mixing and application.

DP23211 Corn Food Products and Consumption

Direct consumption of corn in the United States is primarily that of corn on the cob (sweet corn; *Zea mays* convar. *saccharata* var. *rugosa*) and popcorn (flint corn; *Zea mays* var. *everta*), and in certain areas grits or hominy (dent corn; *Zea mays* var. *indentata*). Humans also consume corn products such as corn meal, corn oil, and corn syrup in food items such as cereals, salad dressings, and snack foods. While most dent corn is grown for animal feed and production of fuel ethanol, certain dent corn hybrids with specific starch properties are used for food purposes—generally referred to as food grade corn. There are various dent corn varieties that have been developed—bred—for specific purposes/markets and given designations to distinguish which markets the grain is suited for. For example, HAE – High Available Energy (Pork & Poultry Feed); HTF – High Total Fermentables (Dry-Grind Ethanol); HES – High Extractable Starch (Wet Milling); WX – Waxy (starch for industrial and food uses); WH – White food corn; and YFC – Yellow food corn. Hence, varieties of dent corn designated WX, HES, WH, and YFC are used for food purposes. These are typically contracted and sold to wet-millers and dry-millers for processing into tortilla chips, corn starch, grits, corn syrup, corn oil, and other corn products.

DP23211 corn could potentially be used for production of food grade hybrids and processed into starch, grits, meal, flour, oil, and sweetener products. As discussed previously (4.3.4.2), there are no risks to human health associated with the DvSSJ1 dsRNA, and the IPD072Aa, PAT, and PMI proteins. Compositional analyses of DP23211 corn grain demonstrate that it is nutritionally equivalent to other dent corn varieties (Pioneer 2020). A voluntary safety and nutritional assessment for DP23211 corn was submitted to the FDA's Center for Food Safety and Applied Nutrition in 2019 (Pioneer 2020). Pioneer completed a food safety consultation with the FDA in 2022; the FDA had no questions concerning human food derived from DP23211 corn (US-FDA 2022).

The National Bioengineered Food Disclosure Law (NBFDL), passed by Congress in July of 2016, directed USDA to establish a national mandatory standard for disclosing foods that are or may be bioengineered. The mandatory compliance date was January 1, 2022. The Standard requires food manufacturers, importers, and certain retailers to ensure bioengineered foods are appropriately disclosed. Any food products derived from DP23211 corn would require labeling subject to NBFDL standards, and consumers would choose to consume such foods based on preference.

The EPA WPS regulations provide protections to agricultural workers, pesticide handlers, and other persons via training, pesticide safety and hazard communication requirements, personal protective equipment requirements, and provision of supplies for routine washing and emergency decontamination. Agricultural workers and handlers, owners/managers of agricultural establishments, commercial (for-hire) pesticide handling establishments, and crop production consultants are provided guidance for compliance with WPS regulations (US-EPA 2016c).

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 accounts for around 90% to 95% of feed grain in the United States on an annual basis; it is 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 Potential Effects on Livestock Health and Welfare

If used for animal feed, DP23211 corn/progeny would be expected to be of benefit to animal health and welfare. As discussed for human health, there are no risks to food animal health and welfare that are associated with the DvSSJ1 dsRNA, IPD072Aa, PAT, and PMI traits present in DP23211 corn. The nutrient composition of grain and forage samples derived from DP23211 corn and a near-isogenic control corn was evaluated. The compositional analyses of grain included crude protein, crude fat, crude fiber, ash, carbohydrates, fatty acids, total amino acids, key anti-nutrients, and key secondary metabolites. Compositional analyses of forage included crude protein, crude fat, crude fiber, ash, carbohydrates, calcium, and phosphorus. No statistical differences were observed in any of the analytes measured in DP23211 corn and as compared to other dent corn varieties. Based on these analyses, the grain and forage of DP23211 corn are comparable to conventional corn with respect to nutrient composition (Pioneer 2020). Pioneer completed an animal feed safety consultation with the FDA in 2022; the FDA had no questions concerning feed derived from DP23211 corn (US-FDA 2022).

4.3.6 Socioeconomics

4.3.6.1 Domestic and International Markets

U.S. Dent Corn Commodities

The production of corn and the various corn derived commodities utilized by the food, feed, fuel and industrial sectors is major component of the U.S. economy. In 2019, the United States produced 13.7 billion bushels of corn for grain (USDA-NASS 2020b) with a market value of $51.5 billion (USDA-NASS 2019c). The primary dent corn commodities are animal feed and fuel ethanol, which account for around 38% – 48%, and 25% – 35% of use, respectively. The remainder is processed for food and industrial products. During processing, corn is either wet or dry milled depending on the desired end products:

- Wet millers process corn into high fructose corn syrup (HFCS), glucose and dextrose, starch, corn oil, beverage alcohol, industrial alcohol, and fuel ethanol.

- Dry millers process corn into flakes for cereal, corn flour, corn grits, corn meal, and brewers grits for beer production.

Both the dry-milling and wet-milling methods of corn processing generate economically valuable co-products, the most prominent of which are distillers' dried grains with solubles (DDGS), which can be used as a feed ingredient for livestock (USDA-ERS 2019). In the United States, feed for both dairy and beef cattle 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 (USDA-ERS 2019).

Dent/field corn accounts for around 70% to 90% of total U.S feed grain production and use on an annual basis (DIS 2017). The other three major feed grains are sorghum, barley, and oats. Across the United States, more than 9.6 billion food-producing animals are raised annually. These include broilers, turkeys, egg-laying hens (layers), hogs, dairy cows, cattle, fish (aquaculture), and sheep, all of which are raised on corn-based feeds (DIS 2017). Around 120 to 140 million tons of corn grain per year are used in feeds for these animals (DIS 2017).

Ethanol

The Renewable Fuel Standard (RFS) is a federal program that requires transportation fuel sold in the United States to contain a minimum volume of renewable fuels. It originated with the Energy Policy Act of 2005 and was expanded by the Energy Independence and Security Act of 2007. Congress created the RFS program to reduce greenhouse gas emissions and expand the nation's renewable fuels sector while reducing reliance on imported oil. The EPA implements the program in consultation with the USDA and the Department of Energy (US-EPA 2020g).

The RFS requires renewable fuel to be blended into transportation fuel in increasing amounts each year, escalating to 36 billion gallons by 2022. The four renewable fuel categories under the RFS are: biomass-based diesel, cellulosic biofuel, advanced biofuel, and total renewable (conventional) fuel, the latter of which typically refers to ethanol derived from corn starch. The RFS began mandating the use of corn ethanol in U.S. fuels in 2006.

U.S. corn processing capabilities allow for production of fuel ethanol and DDGS on a level currently unmatched by any other country. The ethanol industry was comprised of approximately 205 plants in 27 states with a production capacity of 17.1 billion gallons as of 2019. The United States produced 15.8 billion gallons of corn ethanol in 2019, this comprised 54% of global corn ethanol production (RFA 2019). The industry created $23.3 billion in household income and contributed $43 billion to the national Gross Domestic Product (RFA 2020).

Industrial products

Corn wet-mill and dry-mill are also used to produce a variety of industrial products, such as soaps, paints, corks, linoleum, polish, adhesives, rubber substitutes, wallboard, dry-cell batteries, textile finishings, cosmetics, candles, dyes, pharmaceuticals, lubricants, insulation, and wallpaper.

HR Crops

HR crops have proven economically beneficial (Brookes and Barfoot 2018b). Globally, the adoption of HR corn has mainly resulted in lower costs of production, although yield gains from improved weed control have arisen in Argentina, Brazil, the Philippines, and Vietnam (Brookes and Barfoot 2018b). In the United States, the average gross farm income benefit from using HR corn (after deduction of cost of technology) has been around $12.2/acre (Brookes and Barfoot 2018b). During the 1996-2018 timeframe, the aggregate income benefit from using HR corn in the United States has been estimated to be around $10.8 billion (Brookes and Barfoot 2018a).

Globally, the total aggregate farm income gain from using HR corn during the 1996–2018 time frame was $17 billion (Brookes and Barfoot 2018a). Within this, $6.1 billion (36%) was due to yield gains and the rest derived from lower costs of production (Brookes and Barfoot 2018a).

Herbicide Resistant Weed Costs

Currently, HR weeds may be costing U.S. growers as much as $2 billion a year in decreased yields, increased input costs, and decreased land values, according to University of Wisconsin extension weed scientist Vince Davis (Van Deynze et al. 2020). Herbicide resistance can increases growers' chemical costs by 30%-40% as they try to maintain weed control (Weaver 2019). Reported costs to farmers for control of HR weeds range from $14 to $150 per acre, dependent on the weed species present (e.g., once Palmer amaranth gets above 6 inches tall—it can reach 6 ft in height at maturity—there's no herbicide that will control it), prevalence of HR weed populations, and number of herbicide MOAs to which it is resistant (Hurley et al. 2010; Hembree 2011; Livingston et al. 2015; Bayer-CropSci 2018). HR weeds are not unique to HR crops, development of HR weeds has been an issue since the widescale use of chemical herbicides in the 19050s.

IR crops

IR crops have proven effective in control of insect pests, and economically beneficial, thus the extent of their adoption in the United States (USDA-ERS 2022b). From 1996 to 2018, IR corn targeting corn boring pests provided a 7% increase in yield, and increased farm income by $81/ha ($32/acre). IR corn targeting CRW provided a 5% increase in yield, and increased farm income by $78/ha ($31.6/acre) (Brookes and Barfoot 2018a). In 2018, the global gross farm income gains from using IR corn in 2018 were $4.53 USD billion (Brookes and Barfoot 2018a). At the aggregate level, the global gross farm income gains from using IR corn was $4.5 billion (Brookes and Barfoot 2018a).

Another economic benefit derived from IR crop varieties is the area wide suppression of insect pest populations. In areas where cultivation of Bt corn and Bt cotton is high there has been observed a reduction in insecticide use and associated costs in adjacent cropping systems cultivating non-Bt varieties—a result of the area-wide suppression of insect pest populations (NAS 2016). For example, several studies have found that the use of Bt corn and Bt cotton are positively associated with the area-wide suppression European corn borer and pink bollworm, respectively (e.g., see review by (Fernandez-Cornejo et al. 2014b)). In general, current peer review literature and other reports indicate that cultivation of Bt crops can potentially provide tangential benefits to adjacent farms by tempering the prevalence of certain insect pest populations, reducing the need for insecticide use in nearby cropping systems, and the associated costs (Dively et al. 2018; Frisvold 2019).

International Trade

The United States is the world's largest corn producer providing over a third of the total supply of corn in the world market. Field (dent) corn is the largest component of global coarse grain trade—other grains include sorghum, barley, oats, rye, millet, and mixed grains—generally accounting for about two-thirds of the volume over the past decade (USDA-ERS 2019). Field corn grain exports represent a principal source of demand for U.S. producers and make the largest net contribution to U.S. agricultural trade for all agricultural commodities—reflective of the importance of field corn exports to the U.S. economy. The United States currently exports between 10% and 20% of its annual production (USDA-ERS 2019). In 2019, around 14.3% of production (13.7 billion bushels) was exported to more than 73 different countries (USGC 2020b), at an estimated value of around $7.6 billion (USDA-FAS 2020). The United States produces more ethanol and DDGS than consumers and industry can use, providing an ample export supply. As a result, the United States dominates trade in these two corn-based commodities. In 2018/2019, 1.55 billion gallons of U.S. ethanol—548 million bushels in corn equivalent—were exported to 69 countries (USGC 2020a). U.S. corn processed into fuel ethanol and DDGS generates around $4 to $5 billion in trade annually (USDA-FAS 2019).

U.S. corn exports are expected to remain steady over the next decade, largely due to demand for feed grains in support of meat production, and fuel ethanol (WAOB-IAPC 2020).

Identity Preservation

As food, feed, fuel, and industrial crop commodities and production systems have diversified to meet market demands, the need for segregation and identity preservation of agricultural commodities has increased. Farmers who grow corn that is used for different purposes in the same general area need to communicate and plan with their neighboring growers to ensure their crop commodity identities are preserved and price premiums can be realized (e.g., specialty starch corn, waxy corn, high lysine corn, blue corn). Identity preservation (IP) refers to a system of production, handling, and marketing practices that maintains the integrity and purity of various agricultural commodities (Sundstrom et al. 2002). IP typically involves independent, third-party verification of the identification, segregation, and traceability of a product's unique, value-added characteristic (USDA-AMS 2019). Verification is provided at every stage, including seed, production, processing, and distribution. 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). Similarly, commodity traders, marketing organizations, and food processors have established purity and quality standards for specific end-product uses.

IP is important to international trade. The low-level presence (LLP) or adventitious presence (AP) of biotechnology-derived trait material in internationally traded conventional, organic, or other biotechnology-derived crop commodities can disrupt trade and incur economic losses. 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. 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.

Asynchronous approvals—some countries may lag approval for import of biotechnology-derived corn varieties— and zero tolerance policies can result in the diversion of trade by some exporters, and rejection or market withdrawals by importers of corn. 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). The challenges associated with maintaining product identity in international trade can also increase costs, as well as the premiums paid, for certain biotechnology-derived crops.

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

Crop Protection and Yield

DP23211 corn could be cultivated to produce corn-based food, feed, fuel, and industrial products. DP23211 corn would provide protection against corn rootworm (yield), an economically significant pest in corn, and as a glufosinate resistant variety, may prove useful in the management of weeds, and HR weeds and their development. To the extent this HR/IR varietal facilitates insect pest and weed management, and obtaining optimal yields, it would be expected to support growers achieving optimal net-returns on crop production and limiting commodities costs.

These factors considered, the potential impacts of DP23211 corn on domestic markets would be largely beneficial. There are no adverse economic impacts associated with the introduction of DP23211 corn to commercial markets, crop production, or crop protection. Corn rootworm is a significant crop pest in the United States that can cause economic losses exceeding $1 billion annually, resulting from both management costs and yield losses (Shrestha et al. 2018). Corn rootworm damage has historically been managed with crop rotation, broad-spectrum soil insecticides, and transgenic crops expressing Bt Cry proteins.

As with synthetic chemical pesticides, insects are capable of developing resistance to Cry toxins—including those targeting corn rootworm. As adoption of Cry based IR corn increased over the last 20 years, without fully implemented insect resistance management (IRM) planning, the selection pressure on insects resistant to Cry toxins, or evolving resistance, became greater (Cullen et al. 2013). Field-evolved resistance by corn rootworm to Cry3Bb1 corn, mCry3A corn, and eCry3.1Ab corn has been documented in multiple Midwestern states (Gassmann et al. 2016; Jakka et al. 2016). Insect resistance to transgenic Cry traits can pose a threat to the long-term viability of the trait (US-EPA 2020h). New modes of action in PIPs targeting corn rootworm would be beneficial in maintaining sustainable corn rootworm management strategies in U.S. crops (Gassmann et al. 2016; Niu et al. 2017). DP23211 corn would diversify the currently available Cry protein-based MOA for corn rootworm control through the combination of an RNAi mediated MOA and new IPD072Aa protein MOA. Because DP23211 corn would provide farmers with an additional control option for management of corn rootworm—diversify PIP MOAs for control of corn rootworm—it would be expected to provide economic benefits to growers and corn markets by protection of corn grain yields, and helping sustain the efficacy of Cry3 based corn varieties.

Beneficial Insect Populations

Biological control of plant pests provided by populations of predator and parasitoid species is an invaluable ecosystem service. It is an underlying pillar of IPM, and likely provides one of the highest returns on investment in IPM, yet its economic value has rarely been estimated (Naranjo et al. 2015). One seminal estimate valuing biological control, as an ecosystem service, arrived at a value of around $400 billion per year worldwide (Costanza et al. 1997). Studies providing sufficient data to estimate a value for arthropod natural enemies alone are rare and generally have valuated the avoided-costs of insecticides, which, for an array of cropping systems have ranged from $0/acre to $918/acre ($2,202/ha).

Conservation biological control involves changes to the crop environment, such as the landscape in which the crop is embedded, to favor the abundance and pest-suppression activity of native or introduced natural enemies.[16] This involves minimizing factors that can harm natural enemies and/or providing them additional food and shelter (Naranjo et al. 2015). Cropland estimates for conservation biological control have suggested a value of around $14/acre ($33/ha) to $45/acre, although for cotton crops the value can be substantially higher (Pimentel et al. 1997; Naranjo et al. 2015). Losey and Vaughan (2006) estimated a value of $4.5 billion annually for the natural biological control of crop pests in the United States (Losey and Vaughan 2006), but this estimate may be very conservative (Landis et al. 2009).

In addition to functioning as biological controls for crop pests through predation and parasitism, beneficial insects provide pollination for more than two-thirds of the world´s cultivated plant species (Costanza et al. 1997). In the United States, the value of the pollination services has been estimated to be between $5 and $14 billion per year (Southwick and Southwick Jr 1992; Morse and Calderone 2000),

[16] There are several approaches to biological control: natural, classical, augmentative, and conservation. Natural means the control of insect pests by predators and parasitoids as an ecosystem service. Classical means the intentional introduction of an exotic biological control agent for permanent establishment and long-term pest management. Augmentative means the release of additional numbers of a natural enemy when too few are present to control a pest effectively. Conservation means the intentional management of a landscape to support beneficial species.

from which a large proportion is attributed to insect pollinators (Losey and Vaughan 2006; González et al. 2016).

Insecticides are one of the more important and widely used tactics in IPM, but they can also become barriers to effective biological control. IR crops incorporating PIPs targeting specific species of insects, and which are less reliant on chemical insecticides, generally have fewer impacts on arthropod biodiversity in and around crop fields, relative to broad-spectrum chemical insecticide based cropping systems (Gatehouse et al. 2011; Romeis et al. 2019). While DP23211 corn would reduce populations of *Diabrotica virgifera virgifera*, which could have some indirect cascading/trophic effects on beneficial predator/parasitoid populations (e.g., loss of prey) (Chaplin-Kramer et al. 2011; Letourneau et al. 2011; González et al. 2016), this would not be expected to result in deleterious effects on beneficial insect populations in DP23211 corn crops, or adjacent crops. Predators of *Diabrotica virgifera virgifera* include beetles (Carabidae, Staphylinidae, Dermestidae), mites (*Hypoaspis aculeifer*), ants (*Lasius neoniger*, *Myrmica americana*, *Pheidole bicarinata*), and spiders (Araneae) (Kuhlmann and Burgt 1998; CABI 2020), all of which feed on range of species (Adalbert et al. 2008; Carvalho et al. 2017; Gathalkar and Sen 2018; Raupp et al. 2021). For example, beetles *(Pterostichus s*pecies, *Poecilus* species, *Scarites quadriceps*), spiders, and mites are common predators of rootworm larvae and eggs (Lundgren 2010; Spagnol et al. 2020). Ground beetle feeding preferences are generally not fixed; most carabids will opportunistically feed on whatever food is available (Hodgson and Patterson 2007). Pterostichine species, including members of the genera *Pterostichus* and *Poecilus*, are the most diverse consumers, known to feed upon slugs, aphids, moth larvae, beetle larvae, and weed seeds (Jones et al. 2013). Predatory mites are increasingly used in biocontrol and consume large numbers of other pest mites (Xu and Enkegaard 2010; Schausberger et al. 2018). Spiders are opportunistic hunters and feed on a variety of insect prey. Thus, any cascading/trophic effects on *Diabrotica virgifera virgifera* predatory species would most likely be adaptive in nature, were predators fed on other prey available—prey other than corn rootworm.

In general, PIP based insecticidal crops such as DP23211 corn, for the most part, have the potential to be more environmentally benign than chemical insecticide-based pest management approaches (Gatehouse et al. 2011). While biodiversity will be inherently limited in commercial corn crops due to frequent disturbance, tillage, mechanized planting, planting of a monoculture crop, and application of fertilizers and pesticides, HR/IR crops have generally reduced the environmental impacts of crop production (NRC 2010; Carpenter 2011; NAS 2016; Romeis et al. 2019). Glufosinate use would be subject to EPA label requirements, no different than for other corn crops.

International Trade

U.S. corn exports—grain, ethanol, DDGS, corn oil, high fructose corn syrup, corn gluten feed and meal, corn starch, and corn groats/flour—averaged around 9.5 billion annually from 2015 to 2019 (USDA-FAS 2019). By facilitating achieving maximum yield and thereby domestic production, the potential impacts of DP23211 corn on the commodities pricing and U.S. trade of corn commodities would likely be beneficial.

As with all biotechnology-derived crop commodities there exists the potential for LLP occurring in countries importing U.S. agricultural commodities that have not yet been approved. The issue of asynchronous approval (AA), and resulting LLP situations, can lead to trade delays, shipment rejection, and costs to traders (FAO 2014). International trade is facilitated by the World Trade Organization (WTO) and the Organization for Economic Cooperation and Development (OECD). 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 FAO International Plant Protection Convention (FAO 2020), WTO Sanitary and Phytosanitary Measures (WTO 2020a), WTO Technical Barriers to Trade Agreement (WTO 2020b), and the Cartagena Protocol on Biosafety (CBD 2020b).

DP23211 corn would be subject to the same international standards and requirements, discussed above, as currently traded corn varieties. In general, developers have various legal, reputational, and marketing motivations to implement rigorous stewardship measures to ensure IP, prevent commingling, and avoid AP and LLP. By necessity, all international and industry standards and requirements must be met for the marketing of DP23211 corn commodities. Pioneer is a member of Excellence Through Stewardship®, a global organization that promotes product stewardship programs and quality management systems for the full life cycle of agricultural biotechnology products (ETS 2020). Pioneer products are commercialized in accordance with Pioneer policies regarding stewardship of those products and with Excellence Through Stewardship policy. This stewardship program in part helps growers and marketers understand and meet their grain and grain byproduct marketing responsibilities and export approvals (Pioneer 2019).

4.3.7 Climate Change and Greenhouse Gas Emissions

The primary sources of greenhouse gas (GHG) emissions in the United States are: Transportation (around 28% - 29%), electricity production (25% - 27%), industry (22% - 23%), commercial and residential properties (12% - 13%), and agriculture (10% - 11%). In 2019, U.S. greenhouse gas emissions totaled 6,558 million metric tons of CO_2-eq, or 5,788 million metric tons of CO_2-eq after accounting for sequestration from the land sector (Figure 4-14).

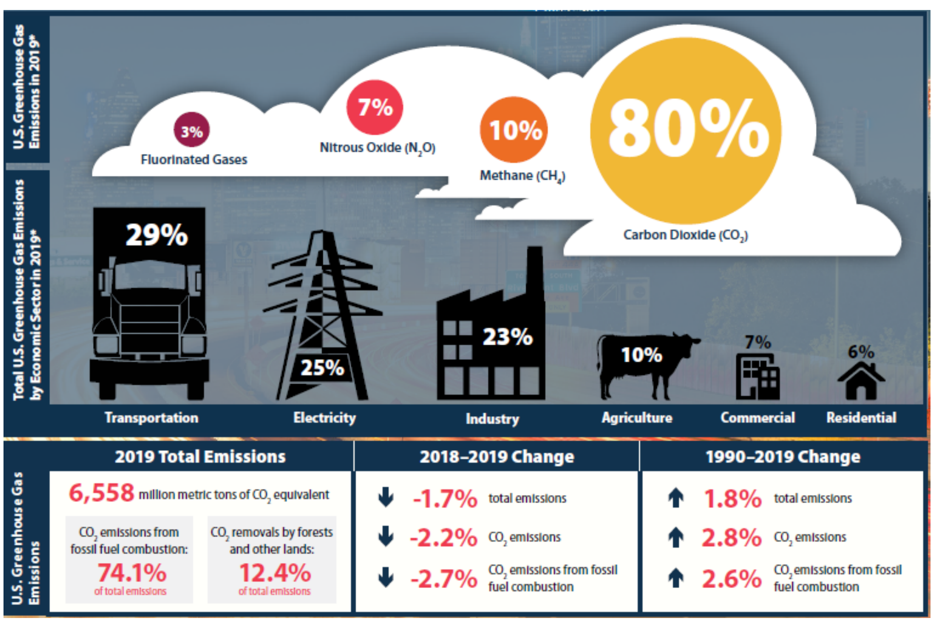

Figure 4-14. Sources of U.S. Greenhouse Gas Emissions

Source: (US-EPA 2020m)

GHGs associated with agriculture are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). N_2O emissions derive from cropped and grazed soils, CH_4 emissions from ruminant livestock production and rice cultivation, CH_4 and N_2O emissions from managed livestock waste, and CO_2 emissions from on-farm energy use. The management of cropped, grazed, and forestland can help offset GHG emissions by promoting the biological uptake of CO_2 through the incorporation of carbon into biomass, wood products, and soils—termed carbon sequestration. Net emissions equate to total GHG emissions minus CO_2 sequestration or removal of CO_2 from the atmosphere, including the net forest sink, as well as the net soil sink from grazed lands and croplands.

Agricultural emission sources can be grouped into mechanical and non-mechanical (Table 4-18). Mechanical sources are equipment or machinery operated on farms, such as mobile machinery (e.g., harvesters), stationary equipment (e.g., boilers), and refrigeration and air-conditioning equipment. These sources emit CO_2, CH_4, and N_2O, or HFCs and PFCs, and their emissions are determined by the properties of the source equipment and material inputs (e.g., fuel composition). Emissions from non-mechanical sources are larger than mechanical sources, with enteric fermentation (CH_4) and soils (N_2O) being the largest sources. Roughly 60% of all N_2O emissions and 50% of all CH_4 emissions derive from non-mechanical agricultural activities, namely agricultural soil management practices and enteric fermentation livestock, respectively (US-EPA 2020m). The exact contribution of agriculture to global CO_2 emissions is difficult to quantify, because the biomass and soil C pools not only emit large amounts of CO_2, but also take up CO_2 (sequester). Nevertheless, C sequestration offers most of the global emissions mitigation potential in agriculture (~89%).

Table 4-18. Agricultural Emissions Sources

	CO_2	CH_4	N_2O	HFCs and PFCs
Mechanical				
Purchased electricity[1]	x	x	x	
Pesticide Production and Use (use under mobile machinery below)	x			
Mobile machinery, fossil fuel combustion (e.g., tilling, seeding, harvesting, and transport)	x	x	x	
Stationary machinery (e.g., milling and irrigation equipment)	x	x	x	
Refrigeration and air-conditioning equipment:				x
Non-mechanical				
Tillage of soils	x	x	x	
Addition of synthetic fertilizers, livestock waste, and crop residues to soils	x	x	x	
Addition of urea and lime to soils	x			
Enteric fermentation		x		
Rice cultivation		x		
Manure management		x	x	
Land-use change	x	x	x	
Open burning of crop residues left on fields	x	x	x	
Managed woodland (e.g., tree strips, timberbelts)	x			
Composting of organic wastes		x		
Oxidation of horticultural growing media (e.g., peat)	x			

1. These gases are released during the combustion of fossil fuels, such as coal, oil, and natural gas, to produce electricity. N_2O emissions from stationary combustion sources result predominantly from the burning of coal at electric power plants (8 $MMTCO_2e$, or 60 percent of all nitrous oxide emissions from stationary combustion).

At the farm scale, the magnitude of different emission sources and GHGs will vary widely depending on the type of farm, management practices, and natural factors at play. These factors include land cover; farm topography and hydrology; soil microbial density and ecology; soil temperature, moisture, organic content and composition; crop or livestock type; cover cropping practices; and land and waste management practices (CLI 2012). It can be difficult to accurately predict the relative magnitude of different sources for a given farm, although general sources and patterns of emissions can be expected (CLI 2012).

Together, agricultural sources contributed about 10% of total anthropogenic emissions in 2019—around 628.6 Million Metric Tons (MMt) of CO_2 equivalent (CO_2–eq) (Table 4-19). Cropland agriculture is responsible for almost half (46%) of all emissions from the agricultural sector (USDA 2016). Methane emissions from enteric fermentation and manure management represent around 27% and 9% of total CH_4 emissions from anthropogenic activities, respectively. Of all domestic animal types, beef and dairy cattle are the largest emitters of CH_4. Rice cultivation and field burning of agricultural residues were minor sources of CH_4. Emissions of N_2O by agricultural soil management through activities such as fertilizer application and other agricultural practices that increased nitrogen availability in the soil was the largest source of U.S. N_2O emissions, accounting for around 75% percent. Manure management and field burning of agricultural residues are smaller sources of N_2O emissions. Urea fertilization and liming accounts for around 0.10% and 0.05% of total CO_2 emissions from anthropogenic activities, respectively (US-EPA 2020j).

Table 4-19. Emissions from Agriculture (MMT CO_2–Eq)

Gas/Source	1990	2005	2015	2016	2017	2018	2019
CO_2	**7.1**	**7.9**	**8.5**	**8.0**	**8.1**	**7.4**	**7.8**
Urea Fertilization	2.4	3.5	4.7	4.9	5.1	5.2	5.3
Liming	4.7	4.3	3.7	3.1	3.1	2.2	2.4
CH_4	**218.2**	**239.3**	**241.4**	**248.1**	**251.0**	**255.7**	**256.4**
Enteric Fermentation	164.7	169.3	166.9	172.2	175.8	178.0	178.6
Manure Management	37.1	51.6	57.9	59.6	59.9	61.7	62.4
Rice Cultivation	16.0	18.0	16.2	15.8	14.9	15.6	15.1
Field Burning of Agricultural Residues	0.4	0.4	0.4	0.4	0.4	0.4	0.4
N_2O	**330.1**	**329.9**	**366.2**	**348.4**	**346.4**	**357.9**	**364.4**
Agricultural Soil Management	315.9	313.4	348.5	330.1	327.6	338.2	344.6
Manure Management	14.0	16.4	17.5	18.1	18.7	19.4	19.6
Field Burning of Agricultural Residues	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total	**555.3**	**577.1**	**616.1**	**604.4**	**605.5**	**621.0**	**628.6**

Source: (US-EPA 2020j)

Note the above figures do not fully account for carbon sequestration by crops, and soil management practices. Crop production systems can be both sources and sinks of GHGs, with the balance depending on a complex relationship of management practices, geographic region, and site-specific factors (e.g., weather conditions, soil type, proximity to surface and ground water bodies, topography). At the farm level, the following practices have overlapping and interacting effects on GHG emissions, particularly CO_2 and N_2O: tillage system, the timing of tillage and other field operations, residue management, crop selection and rotation, cover cropping practices, and the amount and timing of nutrient applications and other soil amendments. Cropland sources of CH_4 emissions include rice cultivation and the burning of agricultural residues.

Isolated areas with high rates of C accumulation occur throughout the agricultural land base in the United States (US-EPA 2020j). In particular, higher rates of net C accumulation in mineral soils occur in the Corn Belt region, which is the region with the largest amounts of conservation tillage, along with moderate rates of Conservation Reserve Program (CRP) enrollment. The regions with the highest rates of emissions from drainage of organic soils occur in the Southeastern Coastal Region (particularly Florida), upper Midwest and Northeast surrounding the Great Lakes, and isolated areas along the Pacific Coast (particularly California), which coincides with the largest concentrations of organic soils in the United States that are used for agricultural production (US-EPA 2020j).

Calculation of GHG emissions from crops involves evaluation of the life cycle of an agricultural product starting from the processes of extracting raw materials, through crop production, to the use of crop products and waste management (Vermeulen et al. 2012). Life-cycle analysis (LCA) of emissions can be assessed using the carbon footprint (CF) method (Pandey et al. 2011; Holka and Bieńkowski 2020). In identifying the most significant sources of GHG emissions in the corn production process, LCA is a useful tool to work towards solutions aimed at reducing emissions in crop production. The total energy required to grow a crop (LCA) can be calculated by accounting for the energy associated with the inputs required for production. Energy and GHG emissions from agricultural inputs can be divided into *primary* (e.g., fuel for machinery operations), *secondary* (e.g., production and transportation of inputs), and *tertiary* (e.g., raw materials to produce items such as machinery and buildings) sources.

Holka and Bieńkowski (2020) performed LCAs and used life-cycle costing methodologies to assess the GHG emissions and costs associated with corn grain production in the stages from "cradle-to-farm gate", i.e., through the processes of corn cultivation to grain harvesting (e.g., seed production, fertilizer and pesticide production and use). The calculated values of the carbon footprint indicator for corn production in conventional, reduced, and no-tillage systems were 2,347.4, 2,353.4, and 1,868.7 CO_2 eq. ha^{-1}, respectively. The largest source of GHG emissions was the use of nitrogen fertilizers. Non-inversion tillage with cover crops and leaving a large amount of crop residues in the field increased the sequestration of organic carbon and contributed to a significant reduction of the carbon footprint in maize production. The conventional tillage system demonstrated the highest overall life-cycle costs per hectare.(Holka and Bieńkowski 2020). These studies showed that the CF per functional unit of one ton of grain amounted to 184.8 kg CO_2–eq. in CT, 189.8 kg CO_2–eq. in RT and 178.0 kg CO_2–eq. in NT. Other studies have found the CF value for corn ranges from 2440 to 4200 kg CO_2–eq/ha (Camargo et al. 2013)-

Including the sequestration of organic carbon (C) into the total LCA GHG emissions from corn production provides a net carbon footprint (CF net) value which was reduced compared to the baseline CF value by 42.9% in CT, 72.1% in RT, and 78.3% in NT (Holka and Bieńkowski 2020). Relating GHG emissions to the functional unit of one ton of corn grain shows that the inclusion of C sequestration contributes most effectively to lowering the total GHG emissions in NT and RT systems (by 78.3% and 72.1%, respectively). Leaving large amounts of crop residues in the field contributed to the prevention of C losses and its sequestration. Moreover, the application of organic fertilizers also influenced the accumulation of C in the soil of the fields with CT and RT. The importance of the C sequestration process in reducing GHG emissions has been emphasized in the literature (Holka and Bieńkowski 2020).

Among the analyzed technological operations in the grain maize production, the processes of mineral fertilization (from 36.7% in CT to 41.7% in RT), soil cultivation and sowing (from 31.2% in RT to 37.6% in CT) had the most significant share in shaping life-cycle costs. This resulted from the costs of mineral fertilizers, fuel, and machinery. Processes of harvesting and plant protection were of less importance (their shares were not higher than 17% in RT and 14% in CT) (Holka and Bieńkowski 2020).

The reduction of GHGs is a significant challenge for agriculture. There is a need to accurately identify the sources of emissions and to disseminate knowledge on agricultural practices that can contribute to reducing emissions in crop production (Holka and Bieńkowski 2020). The management of soil cultivation operations to increase the sequestration of organic carbon in the soil plays an important role in balancing GHG emission from agriculture (Holka and Bieńkowski 2020). The conducted research has shown that the use of no-tillage with a large amount of crop residues in the field significantly contributes to the reduction of GHG emissions in maize production. Regardless of the tillage system, the mineral fertilization process had the most potential impact on the GHG emission. Designing low-emission technologies requires considering the threats resulting particularly from the use of nitrogen fertilizers. To reduce emissions from fields and, at the same time, reduce the consumption of raw materials in fertilizer production, it is important to optimize fertilization (taking into account natural constraints, soil conditions) and the level of crop productivity (Holka and Bieńkowski 2020).

Farrell et al. (2006) compared six publications and found that the energy use and GHG emissions associated with corn (*Zea mays* L.) production ranged from 5728 to 12,066 MJ per ha per year and from 2441 to 4201 kg CO_2e per ha per year, respectively. One major factor causing variability in the results is the different energy and GHG emissions parameters used in the studies (Camargo et al. 2013).

Across all crops, the largest contributor to the total amount of GHG emissions was N_2O, which ranged from 157 kg CO_2e per ha per year (17% of the total) in soybeans to 1539 kg CO_2e per ha per year (45% of total) in corn silage. N production and on-farm fuel followed N_2O emissions, representing 16% and 14%, respectively, across all crops. When it was averaged across crops, N_2O emissions accounted for the greatest GHG contribution, with 44% of the total, followed by N production (16%), on-farm fuel (14%), lime (12%), K_2O (4%), P_2O_5 (3%), transportation of inputs (3%), seed (2%), herbicide (1%), drying (1%), and insecticide (0.6%) (Camargo et al. 2013).

4.3.7.1 Potential Effects on Greenhouse Gas Emissions, and Effects of Climate Change on Corn Cropping Systems

DP23211 corn, and the agricultural inputs and management practices that would be used in cultivation of this variety would contribute to GHG emissions, and the potential for carbon sequestration, as do other corn cropping systems. Any GHG emissions from cultivation of DP23211 corn would be the same as/similar to current crops emissions data (US-EPA 2020j). The agronomic practices and inputs are the same as for other corn varieties, and apart from the HR/IR traits, DP23211 corn is phenotypically the same as most other dent corn varieties—in growth and nutrient utilization requirements.

GHG emissions directly impact the environment in which farmers operate, and agriculture stands to be significantly influenced by the effects of climate change (USDA 2012). Extremes in precipitation; more severe storms; soil moisture; nighttime air temperature; heat waves; humidity; drought spells; crop-growing region migration; weed range and infestation intensity; migration and increased incidence in plant insect pests and pathogens; effects on insect generations per season; and effects on pollinators and pollinator management; are all factors that will be influenced by a changing, warming, climate, and in turn effect crop production (USDA 2012).

To help protect future crop production from, and adapt to, the effects of climate change the USDA contributes to climate assessments, provides analyses of adaptation and mitigation options, cost-benefit analyses, and tools to support agriculture, forests, grazing lands, and rural communities. The Climate Change Program Office (CCPO) operates within the Office of Energy and Environmental Policy (OEEP) to coordinate agricultural, rural, and forestry-related climate change program and policy issues across the USDA. The CCPO ensures that USDA is a source of objective, analytical assessments of the effects of climate change and proposed response strategies.

In an effort to mitigate climate-related risks the USDA has established seven regional hubs for risk adaptation and mitigation to climate change (USDA 2020c). The USDA is taking steps to create modern solutions to the challenge of climate change. New uniform, science-based guidance on cover crop management helps producers prevent erosion, improve soil properties, supply nutrients to crops, suppress weeds, improve soil water content, and break pest cycles. The following are some of the USDA assessments that project climate impacts, adaptive strategies, and mitigation opportunities/strategies:

- Climate Change and Agriculture in the United States: Effects and Adaptation (USDA 2012)
- Climate Indicators for Agriculture (Walsh et al. 2020)
- Climate Change, Global Food Security, and the U.S. Food System (Brown et al. 2015)
- Impacts, Risks, and Adaptation in the United States: Fourth National Climate Assessment, Volume II (USGCRP 2018)

The USDA provides farmers guidance on tracking and mitigating GHG emissions. In 2014 the USDA released "Quantifying Greenhouse Gas Fluxes in Agriculture and Forestry: Methods for Entity-Scale Inventory" (Eve et al. 2014). Through the development of this guidance, the USDA prepared two primary products: 1. A comprehensive review of techniques currently in use for estimating GHG emissions and removals from agricultural and forestry activities; and 2. A technical report outlining the preferred science-based approach and specific methods for estimating GHG emissions at the farm or forest scale. The USDA also released a Carbon Management Evaluation Tool (COMET-FARM) to help producers calculate how much carbon their land's soil and vegetation can remove from the atmosphere (USDA 2020a).

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

4.3.8.1 Federal Laws and Regulations

The laws most relevant to APHIS determinations of regulatory status are the National Environmental Policy Act of 1969 (NEPA), the Clean Water Act of 1972 (CWA), the Safe Drinking Water Act of 1974 (SDWA), the Clean Air Act of 1970 (CAA), the Endangered Species Act of 1973 (ESA), and the National

Historic Preservation Act of 1966 (NHPA). Compliance with the requirements of the ESA has been addressed in Appendix 1. Compliance with the requirements of NEPA, CWA, SDWA, CAA, and NHPA, are specifically addressed in the following subsections.

4.3.8.1.1 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 Pioneer's petition, consistent with the requirements of NEPA and Council on Environmental Quality implementing regulations at 40 CFR parts 1500–1508.

4.3.8.1.2 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. As discussed in this EA, DP23211 corn production would entail the use of pesticides and fertilizers, and to some extent tillage. Because DP23211 corn is agronomically equivalent to currently utilized corn varieties, the potential impacts on water resources and air quality would be similar to that of other corn crops—both biotechnology-derived and conventionally bred corn cropping systems. APHIS assumes use of all pesticides on DP23211 corn will be compliant with EPA registration and label use requirements. There are several federal, state, and private sector collaborative initiatives to help farmers alleviate the impacts of crop production on water resources and air quality, for example, the EPA Mississippi River/Gulf of Mexico Hypoxia Task Force (US-EPA 2020a) and USDA National Water Quality Initiative (NWQI) (USDA-NRCS 2019c). The USDA and EPA provide guidance for farmers on how to best manage agricultural emissions sources (USDA-EPA 2012). Considering these factors, it is unlikely approval of the petition would lead to circumstances that resulted in non-compliance of DP23211 corn production with the requirements of the CAA, CWA, and SDWA.

4.3.8.1.3 National Historic Preservation Act

The NHPA 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. DP23211 corn would be cultivated on lands allocated or zoned for agricultural uses. As discussed in this EA, there are no weedy or invasive characteristics associated with DP23211 corn that could impact historic properties.

4.3.8.2 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 13985 – Executive Order on Advancing Racial Equity and Support for Underserved Communities Through the Federal Government**

  This EO requires federal agencies to advance equity for all, including people of color and others who have been historically underserved, marginalized, and adversely affected by persistent poverty and inequality. Because advancing equity requires a systematic approach to embedding fairness in decision-making processes, executive departments and agencies are required to recognize and work to redress inequities in their policies and programs that serve as barriers to equal opportunity. Consistent with these aims, each agency must assess whether, and to what extent, its programs and policies perpetuate systemic barriers to opportunities and benefits for people of color and other underserved groups. Such assessments will better equip agencies to develop policies and programs that deliver resources and benefits equitably to all.

- **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. The EO emphasizes and pledges that federal agencies will communicate and collaborate with tribal officials when proposed federal actions have potential tribal implications.

Neither alternative evaluated in this EA is expected to have any disproportionate adverse impacts on minorities, low-income populations, or children, or adversely affect tribal entities. There are no challenges to establishing or maintaining equity for underserved communities identified with either alternative. As reviewed in this EA, there are no risks to human health, nor to food animal health and welfare, associated with the trait genes and gene products in DP23211 corn. DP23211 corn is compositionally and nutritionally comparable to other dent corn varieties. Tribal entities are recognized as independent governments and agricultural activities on tribal lands would only be conducted if approved by the tribe. Approval of the petition would have no effect on Indian tribal self-governance or sovereignty, tribal treaties, or other rights. APHIS conducted outreach to tribal nations informing tribes of the Pioneer petition for DP23211 corn. APHIS received one reply from the Ysleta del Sur Pueblo Tribe, stating that this project will not adversely affect traditional, religious, or culturally significant sites.

- **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. The 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 or spread into non-agricultural areas. Field corn (*Zea mays*) is a crop plant that was domesticated, bred for thousands of years, for large-scale food production. Domestication of *Zea mays* has rendered this cultivar less capable of survival in the wild, it is largely dependent on humans for persistence in the environment and not typically found outside areas of cultivation (OECD 2003).

APHIS evaluated the potential weediness and invasiveness of DP23211 corn and concluded that it is unlikely that DP23211 corn will become weedy or invasive in areas where it is grown (USDA-APHIS 2022b). As discussed in Section 4.3.3.4–Gene Flow and Weediness of Corn, the potential for a weedy or invasive species of corn to develop as a result of outcrossing of DP23211 corn with other sexually compatible species of corn, or wild *Tripsacum* species, is negligible. As APHIS concluded in its PPRA, the introduced trait genes in DP23211 corn are not expected to alter characteristics associated with reproductive biology—change the ability of the plant to interbreed with other plant species (USDA-APHIS 2022b).

- **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 cobs/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 highly unlikely the DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI proteins present any risks to the health of birds. Thus, it is unlikely that approval of the petition, and subsequent production of DP23211 corn, would present any hazard to migratory bird populations. Rather, DP23211 corn would likely provide a food source for some species of migratory birds.

4.3.8.3 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 crops 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 biotechnology-derived 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 crops within state boundaries.

Neither of the alternatives considered would affect APHIS partnerships with states in the oversight of biotechnology-derived crops, specifically in the regulation of environmental releases. 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.

4.3.9 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 nor denial of the petition.

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

Commercial crop production, whether a conventional, organic, or biotechnology-based cropping system will always has some degree of environmental impact (Robertson and Swinton 2005; NRC-IM 2015; Ritchie 2017). The potential introduction of pesticides and fertilizers to surface water or groundwater, soil erosion, fossil fuel use and emission of air pollutants, and effects on wildlife habitat and biodiversity are issues that all farmers, and the agricultural sector at large, work with in providing sufficient food, feed, fiber, fuel, 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 chemical/fertilizer inputs utilized; tillage practices; prevalence and diversity of insect pests and weeds; the efficacy of nutrient, insect pest, disease, and weed management programs; geography and proximity of surface waters and groundwater to crops; local biota; weather; and cover cropping and crop rotation practices. With around 360,000 corn farms utilizing around 90 million acres of the land in the United States (USDA-NASS 2019b), the scale of potential impacts, namely in an aggregate sense, necessitates integration of crop production with sustainability and conservation practices—for biotechnology-derived, conventionally bred, and organic crops alike. While implementing such practices can often result in significant mitigation of environmental impacts, not all impacts can be fully attenuated and accepting some degree of environmental impact/change in meeting the market demand for corn-based food, feed, fuel, and industrial commodities is inevitable (Robertson and Swinton 2005; NRC-IM 2015).

On approval of the petition, and subsequent grower adoption of DP23211 corn, the agronomic practices and inputs that would be used in the cultivation of DP23211 corn, and any contribution of these practices and inputs to impacts on soils, water quality, or air quality, as well as biological resources, would be similar to that of other corn crops currently cultivated. APHIS did not identify any significant changes to agronomic practices and inputs, nor in DP23211 corn physiology that would have effects on plant diseases and insect pests or their management. It is expected that DP23211 corn would be produced on lands already converted to cropland—replace other HR/IR corn crops currently cultivated. Hence, impacts on land use and wildlife habitats would be negligible.

There are various federal, state, and private sector collaborative initiatives that support sustainable agricultural practices to help alleviate the collective impacts of crop production on the physical environment, as well as biological resources—these are summarized below in Section 4.3.9.9.

4.3.9.2 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 utilization of natural resources over continued production cycles—namely topsoil, groundwater, beneficial insect populations such as plant pest predators and pollinators, the plants that support beneficial insects, and products such as pesticides and fertilizers that derive from natural resources. DP23211 corn is agronomically equivalent to other dent corn cultivars and would utilize the same types, and same/similar quantities of resources (e.g., groundwater, pesticides, fertilizers), as all other conventional and biotechnology-derived dent corn varieties. The annual production of DP23211 corn would face the same challenges in sustaining top-soils and soil quality, and air and water 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.

There would be, to some extent, reductions in insecticide and fossil fuel use with this corn variety—the utilization of the natural resources from which insecticides and fuels are derived. As a PIP based IR corn variety targeting *Diabrotica virgifera virgifera*, there would be a potential reduction in hazards to pollinators, and beneficial predator and parasitoid species (Bale et al. 2008; Barratt et al. 2018). This would be relative to how this IR corn variety facilitated reductions in, or limited use of, broad spectrum chemical insecticides, and the particular insecticides used (e.g., neonicotinoids, pyrethroids, or organophosphates). The novel RNAi (DvSSJ1 dsRNA) and IPD072Aa MOAs could facilitate management of development of insect resistance by broadening the variety of PIP MOAs in crops. These aspects of DP23211 corn would be expected to be of potential benefit to sustaining the long-term productivity of U.S. corn cropping systems, relative to the efficacy of implemented IWM and IPM programs.

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

An irreversible or irretrievable commitment of resources refers to impacts on or losses of resources that cannot be recovered or reversed. Irreversible commitments of resources involve those where the resources cannot be restored or returned to their original condition, such as the use of nonrenewable fossil fuel based products, and resources that are renewable only over long time spans. Irretrievable is a term that refers to those resources that, once used or utilized, would cause the resource to be unavailable for use by others or future generations (e.g., land use).

The production of corn and the food, feed, fuel, and industrial products derived from corn involves the irreversible and irretrievable utilization of resources. For example, 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 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 potentially contribute to wind and water erosion; cover cropping can help preserve and rebuild topsoil. Materials such as aluminum, steel, wood, and plastics would be utilized as part of the process of crop production. Most of these materials are non-renewable and would be irreversibly utilized if not recycled (plastics, metals). Crop production inherently entails the irretrievable removal of natural habitat and associated wildlife from the landscape; the relocation of associated wildlife to other landscapes.

DP23211 corn would be used to supply standard corn-based feed, fuel, food, and industrial commodities, and is physiologically and agronomically equivalent to other dent corn varieties (Pioneer 2020). Any irreversible or irretrievable commitments of resources in the production of DP23211 corn, and marketing of DP23211 corn commodities, would be similar to that of other dent corn cropping systems.

4.3.9.4 Whether the action would violate or conflict with a federal or state laws or local requirements governing protection of the environment and environmental justice.

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 DP23211 corn. Pioneer completed a food/feed safety consultation with the FDA in 2022; the FDA had no questions concerning human or animal food derived from DP23211 corn (US-FDA 2022).

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

There would be no conflicts with approval of the petition, and subsequent commercial production and marketing of DP23211 corn, with federal, state, tribal, or local land use plans or policies.

Federal Lands

There are four major federal land management agencies that administer around 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, nor federal oversight of these lands. Any cultivation of DP23211 corn on federal lands would require approval by a federal land management agency.

Tribal Nations, State, and Local Land Use Plans and Policies

As reviewed in Section 4.3.8–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 crops developed using genetic engineering, to include the production of DP23211 corn on state or county lands.

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

DP23211 corn is agronomically equivalent to other corn varieties—the energy requirements involved with the full life cycle of DP23211 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 2020c). Energy conservation estimation tools are also provided to help growers estimate costs and saving associated with irrigation, nitrogen use, and tillage.

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

There are no depletable resource requirements unique to the production and marketing of DP23211 corn. Use of natural resources (e.g., irrigation water, soils, production or herbicides and 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 resource to help mitigate potential environmental impacts, such as those summarized below in 4.3.9.9, would likewise not differ.

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

As reviewed in 4.3.3.4– Gene Flow and Potential Weediness of DP23211 Corn, there are no weedy or invasive characteristics associated with DP23211 corn that could impact historic properties—result in alteration of the character or use of historic properties protected under the NHPA. The design of the built environment in relation to crop production activities would be resolved at the state and local levels of governance (e.g., city, county, and/or state authorities governing land use).

4.3.9.9 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 water quality protection, conservation of cropland biodiversity, and nutrient management. Each contribute in some way to environmental stewardship and long-term farm sustainability. A more detailed description of USDA sustainability and conservation initiatives are provided in the references below.

The EPA Mississippi River/Gulf of Mexico Hypoxia Task Force (US-EPA 2020a) and USDA Natural Resources Conservation Service (NRCS) National Water Quality Initiative (NWQI) (USDA-NRCS 2019c) 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 Climate Change Program Office (CCPO) operates within the Office of Energy and Environmental Policy (OEEP) to coordinate agricultural, rural, and forestry-related climate change program and policy issues across USDA. OEEP's CCPO works across USDA to help ensure that the effects of climate change on working lands and rural communities are understood across the Department and that adaptation is integrated into USDA programs, policies and operations based on the most up-to-date science. OCE also provides data, tools and information to assist land managers, stakeholders and USDA agencies and mission areas with adaptation assessments, planning and implementation (USDA 2020d).

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: It 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).

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

Economic considerations have been evaluated in Section 4.3.6–Socioeconomics. The economic impacts associated with the utilization of DP23211 corn for production of food, feed, fuel, and industrial commodities would be potentially beneficial, to both farmers and corn commodities markets.

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

Based on APHIS' evaluation provided in Appendix 1 of this EA, a determination of nonregulated status of DP23211 corn, and subsequent commercial production of this corn variety, would have no effect on listed species or species proposed for listing, nor would it affect designated habitat or habitat proposed for designation.

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

As reviewed in Section 4.3.4–Human Health, approval of the petition and subsequent availability of DP23211 to commercial markets would not present any risks to public health or worker safety.

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

Approval of the petition would provide for the commercial production of DP23211 corn, subject to any FDA, EPA, state, or tribal requirements. APHIS maintains a publicly available list of petitions and determinations of nonregulated status on its website (USDA-APHIS 2022a). Insect and herbicide resistant varieties of corn were first deregulated in 1995, with adoption rates increasing rapidly in the years that followed. As of November 2022, APHIS has issued determinations of nonregulated status in response to 41 petitions for biotechnology-derived corn varieties. Currently, over 90% of U.S. corn acreage is comprised of transgenic varieties (USDA-ERS 2022a).

Farmers generally adopt a biotechnology-derived crop based on the benefits they can derive from it, such as effective insect pest or/and weed control, maximal crop yields per acre, increased farm net returns, and time savings (Fernandez-Cornejo et al. 2014b; Brookes and Barfoot 2018b). Potential net benefits are a function of the particular crop farmed and geographic location; pest and weed pressures; agronomic input and market commodity prices; and efficacy of on-farm crop production practices (e.g., IWM, IRM).

Advances in agricultural biotechnology are expected to continue, and refine the precision with which crop varieties will be developed, leading 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 years, traits likely to be introduced 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 any adverse environmental impacts that are unique, or differ from conventional crops and cropping systems (e.g., (Sanvido et al. 2007; Klümper and Qaim 2014; Brookes and Barfoot 2018b; Brookes and Barfoot 2020), and others). Generally, to date, biotechnology-derived crops, which undergo evaluation by USDA, the EPA, and FDA under the Coordinated Framework (ETIPCC 2017; USDA-APHIS 2020b), have been found to have no more or fewer adverse effects on the environment than conventionally bred crops (NRC 2010; NAS 2016; Brookes and Barfoot 2020).

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APPENDIX 1: THREATENED AND ENDANGERED SPECIES

The Endangered Species Act (ESA) of 1973, as amended, is one of the most far-reaching wildlife conservation laws ever enacted by any nation. Congress passed the ESA to prevent extinctions facing many species of fish, wildlife, and plants. The purpose of the ESA is to conserve threatened and endangered and species and the ecosystems on which they depend as key components of America's heritage. To implement the ESA, the U.S. Fish & Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS), other federal, state, and local agencies, tribes, non-governmental organizations, and private citizens. Before a plant or animal species can receive the protection provided by the ESA, it must first be added to the federal list of threatened and endangered wildlife and plants.

A species is added to the list when it is determined by the USFWS/NMFS to be threatened or endangered 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 an animal or plant is added to the list, in accordance with the ESA, protective measures apply to the species and its habitat. These measures include protection from adverse effects of federal activities.

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 of 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 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, Pioneer has requested that the USDA-APHIS consider that DP23211 corn is not a plant pest as defined by the PPA. After completing a PPRA, if APHIS determines that DP23211 corn seeds, plants, or parts thereof do not pose a plant pest risk, then this DP23211 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 DP23211 corn is not subject regulation. As part of this EA, APHIS analyzed the potential effects of DP23211 corn on TES and critical habitat. APHIS thoroughly reviewed data related to DP23211 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 the PPA and APHIS' 7 CFR part 340 regulations, APHIS only has the authority to regulate DP23211 corn and other organism developed using genetic engineering as long as APHIS believes they may pose a plant pest risk. APHIS has no regulatory jurisdiction over any other risks associated with organisms developed using genetic engineering, including risks resulting from the use of pesticides on those organisms.

Relative to herbicide use with DP23211 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 2021).

2 Potential Effects of DP23211 Corn on TES

Based on the information submitted by Pioneer and reviewed by APHIS, DP23211 corn, with the exception of insect resistance and resistance to glufosinate-ammonium, is agronomically and compositionally comparable to conventional corn (Pioneer 2020). Pioneer has presented results of agronomic field trials for DP23211 corn. The results of these field trials demonstrate that there are no differences in agronomic practices between DP23211 corn and conventional corn, apart from the specific use of use of glufosinate-ammonium, and potential reduction in chemical insecticide use. The common agricultural practices that would be carried out in the cultivation of DP23211 corn are not expected to deviate from current practices. DP23211 corn is not expected to directly cause a change in U.S. corn acreage or the areas devoted to corn production (see Subsection 4.3.1—U.S. Corn Production). It is expected that DP23211 corn will replace other varieties of HR/IR corn without expanding the acreage or area of corn production.

Corn can be grown in all 50 states and U.S. territories. The issues discussed herein focus on the potential environmental consequences of approving the request for nonregulated status of DP23211 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 2021).

For its analysis on TES plants and critical habitat, APHIS focused on the agronomic differences between DP23211 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 effects on TES animals, APHIS focused on the implications of exposure to the DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI proteins expressed in DP23211 corn, and the ability of the plants to serve as a host for a TES.

2.1 Threatened and Endangered Plant Species and Critical Habitat

The agronomic data provided by Pioneer were used in the APHIS analysis of the weediness potential for DP23211 corn and evaluated for the potential to impact TES and critical habitat. Agronomic studies conducted by Pioneer evaluated the weediness and invasiveness potential of DP23211 corn with respect to conventional corn (Pioneer 2020). The results obtained from these studies demonstrated that the agronomic characteristics of DP23211 corn were comparable to those of conventional corn. Statistically significant differences were identified in days to flowering and final population, with 39 of 47 observations for days to flowering and 46 of 47 observations for final population within the reference range. These differences are not anticipated to be biologically meaningful as they are within the normal ranges of variation in conventionally bred corn varieties. The differences observed for days to flowering and final stand count are unlikely to result in DP23211 corn with increased weediness potential or survivability, compared to conventional corn, which is not considered a weedy or invasive plant. As discussed in this EA and APHIS' PPRA (USDA-APHIS 2022b), there are no weed risks associated with DP23211 corn. Volunteer corn plants can be easily controlled with herbicides or mechanical means if needed.

APHIS evaluated the potential of DP23211 corn to cross with a listed species. There are no federally listed *Zea* or *Tripsacum* species in the United States (USFWS 2021), genera with which corn (*Zea mays*) may interbreed. As discussed in Gene Flow and Weediness (Section 4.3.3.4), the potential for gene movement between DP23211 corn and related teosinte (*Zea*) species is limited. Teosinte do not appear to be present in the United States other than in botanical gardens or at research stations. Three species of *Tripsacum* have been identified in the United States: Eastern gamagrass, Mexican gamagrass, and Florida gamagrass. Eastern gamagrass is the only Tripsacum species of widespread occurrence (USDA-NRCS 2002). The potential for hybridization and successful introgression of *Z. mays* genes into *Tripsacum* populations is, however, 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. Based on these factors, APHIS determined that DP23211 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 DP23211 corn would be those TES that inhabit corn fields and feed on DP23211 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, although 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, cover, or roosting. 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.

Of the 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, is known to forge in, and roost near, agricultural fields during migration (CWS-USFWS 2007). 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 2021). Listed populations of Columbian white-tailed deer (*Odocoileus virginianus leucurus*) are found in certain areas associated with the Columbia River in Washington State (USFWS 2021). These locations are well south and west, respectively, of the regions where corn crops are typically planted (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 2021); the Florida salt marsh vole (*Microtus pennsylvanicus dukecampbelli*), which occurs in salt marsh habitat on the Gulf Coast of Florida (USFWS 2021); the endangered Key Largo woodrat (*Neotoma floridana smalli*) of Florida Key's climax hardwood hammocks (USFWS 2021); and the northern and southern subspecies of the endangered, tidal marsh dwelling, salt marsh harvest mouse (*Reithrodontomys raviventris*) (USFWS 2013, 2021).

APHIS considered the risks to threatened and endangered animals from consuming DP23211 corn. Pioneer presented information on the food and feed safety of DP23211 corn, comparing the DP23211 corn variety with conventional varieties currently grown. There are no toxins or allergens associated with this plant (Pioneer 2020). The forage assessment included proximate, fiber, and mineral analytes. The grain assessment included proximate, fiber, fatty acid, amino acid, mineral, vitamin, secondary metabolite, and anti-nutrient analytes. The analytes included for the compositional assessment were based on the OECD consensus document on compositional considerations for new varieties of corn (OECD

2003). Results presented by Pioneer show that the introduced genetic material in DP23211 corn does not result in any significant compositional differences between DP23211 corn and non-transgenic corn.

As discussed in those sections addressing human health, animal health and welfare, and wildlife; there are no health hazards associated with the DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI proteins. Therefore, there is no expectation that exposure to the DvSSJ1 dsRNA, and IPD072Aa, PAT, and PMI proteins will have any effect on TES that may consume DP23211 corn. As part of the FDA voluntary biotechnology consultation program, Pioneer completed a food/feed safety consultation with the FDA in 2022; the FDA had no questions concerning human or animal food derived from DP23211 corn (US-FDA 2022).

APHIS considered the possibility that DP23211 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). APHIS is not aware of any TES for which corn serves as a host plant (USFWS 2021).

Considering the compositional similarity between DP23211 corn and other corn varieties currently grown, and the lack of any hazard presented by the introduced IR and HR trait genes and gene products, apart from targeted lethality for the plant pest *Diabrotica virgifera virgifera*, APHIS has concluded that exposure to and consumption of DP23211 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 DP23211 corn, APHIS has not identified any stressor that could affect listed TES or species proposed for listing. APHIS also considered the potential effect of DP23211 corn on designated critical habitat and habitat proposed for designation, and could identify no differences from effects that would occur from the production of other corn varieties. Corn is not sexually compatible with, nor serves as a host species for, any listed species or species proposed for listing. Consumption of DP23211 corn by any listed species or species proposed for listing would pose no health risks. DP23211 corn material, including pollen, are practically non-toxic (aside to the target pest; *Diabrotica*) to lepidopteran, coleoptera, and other invertebrates.

Based on these factors, APHIS has concluded that a determination of nonregulated status of DP23211 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.

APPENIDX 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 ▪ 22 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, Integrated Toxicology and Environmental Health, Duke University ▪ M.S., Marine Science/Oceans and Human Health, University of North Carolina at Wilmington ▪ B.S., Biology, Adelphi University ▪ 22 years of experience in environmental/human health risk assessment and 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

APPENDIX 3: REFERENCES

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