Nuseed Americas Inc. Petition (17-236-01p) for Nonregulated Status for DHA Canola (*Brassica napus*)

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Final Environmental Assessment

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

2,4-D	2,4-Dichlrophenoxyacetic acid
ACCase	Acetyl CoA caboxylase
a.i.	Active ingredient
APHIS	Animal and Plant Health Inspection Service
Bar	Bialaphos resistance gene that occurs naturally in *Streptomyces hygroscopicus* and encodes for the phosphinothricin-N-acetyltransferase (PAT) enzyme
CAA	Clean Air Act
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations (U.S.)
CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
CWA	Clean Water Act
DHA	Docosahexaenoic acid
EA	Environmental Assessment
EFSA	European Food Safety Agency
EIS	Environmental Impact Statement
EO	Executive Order
EPA	Eicosapentaenoic acid
ESA	Endangered Species Act
EU	European Union
F1	First generation
F2	Second generation
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
FR	Federal Register
FSMA	Food Safety Modernization Act
GE	Genetically engineered
GHG	Greenhouse gas
GMO	Genetically modified organism
GRAS	Generally Recognized As Safe
Ha	Hectare
HR	Herbicide resistant
HEAR	High erucic acid rapeseed
IPM	Integrated pest management
IWM	Integrated weed management

ACRONYMS AND ABBREVIATIONS

K	Potassium
Kg	Kilogram
Lb	Pound (avoir.)
LC-PUFA	Long chain omega-3 polyunsaturated fatty acid
LEAR	Low erucic acid rapeseed
LLP	Low level presence
m^2	Square meters
MCPA	2-methyl-4-chlorophenoxyacetic acid
MOA	Mode of action
N	Nitrogen
N_2O	Nitrous oxide
NAAQS	National Ambient Air Quality Standards
NMFS	National Marine Fisheries Service
NASS	National Agricultural Statistics Service
NEPA	National Environmental Policy Act of 1969 and subsequent amendments
NHPA	National Historic Preservation Act
NNI	Neonicotinoid insecticide
NO_2	Nitrogen dioxide
NOx	Nitrogen oxides
NOP	National Organic Program
NPS	Non-point source (pollution)
ODA	Oregon Department of Agriculture
OECD	Organization for Economic Cooperation and Development
O_3	Ozone
P	Phosphorus
pat	gene that occurs naturally in Streptomyces hygroscopicus and encodes for the phosphinothricin-N-acetyltransferase (PAT) enzyme
PAT	phosphinothricin-N-acetyltransferase (PAT) enzyme
PIP	Plant incorporated protectant
OSHA	Occupational Safety and Health Administration
Pb	Lead
PDP	Pesticide Data Program
PM	Particulate matter
P_2O_5	Phosphorus pentoxide (fertilizer)
PPA	Plant Protection Act
PPRA	Plant pest risk assessment
PPA	Plant Protection Act
RI	Recommended daily dietary intake
RFS	Renewable fuel standard
RFS2	Second renewable fuel standard

ACRONYMS AND ABBREVIATIONS

S	Sulfur
SIP	State implementation plan
SO_2	Sulfur dioxide
SO_x	Sulfur oxides
t	Ton
T&E	Threatened & Endangered
TSCA	Toxic Substances Control Act
U.S.	United States and its territories and possessions
USDA	U.S. Department of Agriculture
USC	U.S. Code
USFWS	U.S. Fish & Wildlife Service
USGS	U.S. Geological Survey
U.S. EPA	U.S. Environmental Protection Agency
WHO	World Health Organization
WPS	Worker protection standards
WSSA	Weed Science Society of America
WTO	World Trade Organization

1 PURPOSE AND NEED

1.1 Background

In May 2017, Nuseed Americas Inc. (Nuseed) submitted a petition (17-236-01p) to the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), requesting that genetically engineered (GE) DHA canola, and any progeny derived from it, no longer be considered regulated articles under Title 7 of the Code of Federal Regulations part 340 (7 CFR part 340). DHA canola, which has been genetically engineered to produce omega-3 fatty acids, is currently regulated by APHIS because it was developed using the plant pest *Agrobacterium tumefaciens*; a regulated article under 7 CFR part 340.2.[1] As part of evaluation of Nuseed's petition for nonregulated status APHIS has developed this Environmental Assessment (EA) to inform APHIS' decision regarding the regulation of DHA canola.

1.2 Purpose of DHA Canola

Nuseed, in partnership with the Australian-based Commonwealth Scientific and Industrial Research Organis[z]ation (CSIRO), genetically engineered DHA canola to produce long chain omega-3 polyunsaturated fatty acids (LC-PUFAs) not otherwise present in canola seed, principally; docosahexaenoic acid (DHA) and eicosopentaenoic acid (EPA) (see Appendix A). DHA canola is intended to provide an additional source of these omega-3 fatty acids to help meet human and food animal (e.g., livestock, poultry, farmed fish) dietary needs.

Omega-3 fatty acids play important roles in the body as structural components of cell membranes. DHA, in particular, is particularly high in the retina and brain. In addition to their structural roles, omega-3 fatty acids (along with omega-6s) are used to form eicosanoids, signaling molecules that have wide-ranging functions in the body's cardiovascular, pulmonary, immune, and endocrine systems (NIH 2017).

Many studies have associated higher intakes of fish and other seafood, rich in EPA, DHA, and other omega-3 fatty acids, with improved health outcomes (NIH 2017). Finfish and mollusks are the primary dietary source of EPA and DHA for humans. Some foods, such as certain brands of eggs, yogurt, juices, milk, and soy beverages, are fortified with DHA and other omega-3s, and are also sources, as well as over the counter fish oil supplements. In general, current dietary sources of EPA and DHA are almost exclusively limited to finfish and mollusks, or fish oil supplements. There has been concern expressed by some scientists and health professionals that marine sources may not be sufficient to meet future human and food animal dietary needs for omega-3 LC-PUFAs (Adarme-Vega et al. 2012; Salem and Eggersdorfer 2015; Tocher 2015). For example, most EPA and DHA for human and food animal consumption is sourced from small fatty marine fish; as of the end of 2016, 38 fish stocks in U.S. coastal waters were listed as overfished (NOAA 2017). Due to limited/finite global fish stocks, recent research has been directed towards utilization and development of alternative sources of EPA and DHA. These include aquaculture with plant-based feeds, krill (small marine Crustaceans), farmed marine microalgae, yeasts, and GE plants (Adarme-Vega et al. 2012; Napier et al. 2015; Salem and Eggersdorfer 2015; Xie et al. 2015). DHA canola, genetically engineered to produce omega-3

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[1] Disarmed Agrobacterium is commonly used in the genetic modification of plants. Disarmed means the Agrobacterium is non-virulent.

LC-PUFAs, is intended to provide an additional source of EPA and DHA for use in food products, and livestock and aquaculture feed.

1.3 The Coordinated Framework for the Regulation of Biotechnology

Since 1986, the U.S. government has regulated GE organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (referred to as the Coordinated Framework). The Coordinated Framework, published by the Office of Science and Technology Policy, describes the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA-APHIS, the U.S. Environmental Protection Agency (U.S. EPA), and the U.S. Food and Drug Administration (FDA). The purpose of the Coordinated Framework is to ensure the safety of biotechnology products, and clarify how federal agencies use existing federal statutes to ensure public health and environmental safety, while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The Coordinated Framework is based on several important guiding principles: (1) agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities; (2) agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; (3) agencies are expected to exercise oversight of GE organisms only when there is evidence of "unreasonable" risk. A summary of the roles of each agency follows. A more detailed description can be found in the original 1986 policy statement (51 FR 23302) and in the 2017 Coordinated Framework update. [2]

1.3.1 USDA-APHIS

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to the Plant Protection Act (PPA), as amended (7 U.S. Code (U.S.C.) 7701–7772), govern the introduction (importation, interstate movement, and environmental release) of GE organisms that may pose a plant pest risk. A GE organism is considered a regulated article if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation (7 CFR § 340.2), such as *Agrobacterium tumefaciens*, which was used in development of DHA canola. A GE organism is also regulated under 7 CFR part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have sufficient information to determine if the GE organism is unlikely to pose a plant pest risk. A GE organism is no longer subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340 when APHIS determines that a GE organism is unlikely to pose a plant pest risk.

1.3.2 Environmental Protection Agency

The U.S. EPA is responsible for regulating the sale, distribution, and use of pesticides, including pesticides that are produced by GE organisms, termed plant incorporated protectants (PIP). The U.S. EPA regulates pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) and certain GE microorganisms (agricultural uses other than pesticides) under the Toxic Substances Control Act (TSCA) (15 U.S.C. 53 *et seq.*).

Before a pesticide may legally be used in the United States, the U.S. EPA must evaluate the pesticide to ensure that it will not result in an unreasonable risk to human health or the

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[2] See https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/update-coordinated-framework-regulation-biotechnology

environment. Pesticides that complete this evaluation are issued a "registration" that permits their sale and use according to requirements set by U.S. EPA. The U.S. EPA must approve the pesticide use label in accordance with 40 CFR part 158. It is a violation of federal law to use a pesticide in a manner inconsistent with its labeling. The courts consider a label to be a legal document. The purpose of the label is to provide clear directions for effective product use while minimizing risks to human health and the environment. The U.S. EPA reviews each registered pesticide at least every 15 years to determine whether it continues to meet the FIFRA standard for registration and safety (US-EPA 2015e).

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

1.3.3 Food and Drug Administration

The FDA regulates food and feed under the authority of the FFDCA and Food Safety Modernization Act. The FDA regulates food and feed from GE plants as they regulate all food. The existing FDA safety requirements impose a clear legal duty on everyone in the farm to table continuum to market safe foods to consumers, regardless of the process by which such foods are created. It is unlawful to produce, process, store, ship or sell to consumers unsafe foods.

The FDA created the Plant Biotechnology Consultation Program in the 1992 to cooperatively work with GE plant developers to help them ensure foods made from their new GE plant varieties are safe and lawful. [3] In this program, the FDA evaluates the safety of food from the new GE crop before it enters the market. Although the consultation program is voluntary, GE plant developers routinely participate in it before bringing a new GE plant to market. The FDA completed its first plant biotechnology consultation in 1994. Thus far, the FDA has evaluated more than 150 GE plant varieties through this program.

In June 2006, the FDA issued “*Guidance for Industry: Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use*” (US-FDA 2006), for the voluntary food safety evaluations for new non-pesticidal proteins produced by new plant varieties that are intended to be used as food or feed, including bioengineered plants. These early food safety evaluations help ensure the potential food safety issues related to a new protein in a new plant variety are addressed early in development. These evaluations are not intended as a replacement for a biotechnology consultation with the FDA, but the information may be used later in the biotechnology consultation.

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[3] FDA: Statement of Policy - Foods Derived from New Plant Varieties; http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096095.htm

As part of the FDA consultation process, Nuseed submitted a safety and nutritional assessment for food and feed derived from DHA canola to the FDA in March, 2017.

1.4 Purpose and Need for APHIS Action

As summarized above in 1.3.1, GE organisms that were developed using a plant pest, such as *Agrobacterium spp.*, are regulated articles under 7 CFR part 340. The regulations provide that any person may submit a petition to APHIS requesting that a GE organism should not be regulated, because it is unlikely to present a plant pest risk.[4] As required by 7 CFR § 340.6 APHIS must respond to petitioners with a regulatory status decision. A GE organism is no longer subject to the requirements of 7 CFR part 340 or the plant pest provisions of the PPA if APHIS determines through conduct of a Plant Pest Risk Assessment (PPRA) that it is unlikely to pose a plant pest risk.

As part of the evaluation of petitions for nonregulated status APHIS also conducts environmental analyses, such as this EA, pursuant the requirements of the National Environmental Policy Act (NEPA, 42 U.S.C. § 4321 et seq.); the Council of Environmental Quality's (CEQ) NEPA-implementing regulations (40 CFR parts 1500-1508); and USDA and APHIS NEPA-implementing regulations and procedures (7 CFR part 1b, and 7 CFR part 372). APHIS has prepared this EA to consider the potential impacts of a determination of nonregulated status for DHA canola on the human environment.[5]

1.5 Public Involvement

APHIS seeks public comment on EAs through notices published in the *Federal Register* and by other means. On March 6, 2012, APHIS announced in the *Federal Register* updated procedures for the way it solicits public comment when considering petitions for determinations of nonregulated status for GE organisms to allow for early public involvement in the process.[6] A summary of current practices follows.

1.5.1 First Opportunity for Public Involvement

Once APHIS deems a petition complete, the petition is made available for public comment for 60 days, providing the public an opportunity to provide input on the petition itself, and raise topics of concern that APHIS should consider in development of the draft EA and PPRA.

1.5.2 Second Opportunity for Public Involvement

Once the draft EA and PPRA are developed a notice of their availability for review and comment is published in a second *Federal Register* notice. This second notice follows one of two approaches for public involvement based on whether or not the petition for nonregulated status raises substantive new issues:

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[4] Petitioners are required (7 CFR § 340.6) to describe known and potential differences from the unmodified organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived.

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

[6] This notice can be accessed at: http://www.gpo.gov/fdsys/pkg/FR-2012-03-06/pdf/2012-5364.pdf

Approach 1: For GE organism that Raise No Substantive New Issues

This approach for public participation is followed when APHIS decides, based on review of the petition and evaluation of public comments received during the 60-day comment period, that the petition involves a GE organism that does not raise substantive new issues. This would include, for example, gene modifications that do not raise new biological, cultural, or ecological issues due to the nature of the modification or APHIS' familiarity with the recipient organism. Under this approach, APHIS will publish a notice in the *Federal Register* announcing its preliminary regulatory determination and the availability of the draft EA, draft PPRA and preliminary Finding of No Significant Impact (FONSI) for a 30-day public review period.

If no substantive information is received that would warrant substantial changes to APHIS' analysis or determination, APHIS' preliminary regulatory determination will become effective upon public notification through an announcement on its website. No further *Federal Register* notice will be published announcing the final regulatory determination.

Approach 2. For GE Organisms That Raise Substantive New Issues Not Previously Reviewed by APHIS

A second approach for public participation will be used when APHIS determines that the petition for a determination of nonregulated status raises substantive new issues, such as a GE organism that has not previously been determined by APHIS to have nonregulated status, or when genetic modifications raise substantive biological, cultural, or ecological issues not previously analyzed by APHIS. APHIS reviews the petition, and analyzes and evaluates public comments received during the 60-day comment period to determine if substantive issues have been identified.

APHIS will solicit comments on a draft EA and PPRA for 30 days through the publication of a *Federal Register* notice. Upon completion of the 30-day comment period, APHIS will review and evaluate all written comments received during the comment period and any other relevant information. After reviewing and evaluating the comments on the draft EA, draft PPRA, and other information, APHIS will revise the PPRA as necessary and prepare a final EA. Based on the final EA, APHIS will prepare a NEPA decision document – either a FONSI, or Notice of Intent (NOI) to prepare an Environmental Impact Statement (EIS). If a FONSI is reached, APHIS will provide a response to the petitioner, either approving or denying the petition. APHIS will publish a notice in the *Federal Register* announcing the regulatory status of the GE organism and the availability of APHIS' final EA, PPRA, FONSI, and regulatory determination.

1.5.3 Public Involvement for Petition 17-236-01p

Because the plant-trait combination for DHA canola is new and no prior GE canola varieties producing omega-3 fatty acids have been evaluated by APHIS, public involvement for petition 17-236-01p will follow the procedure described above for Approach 2. On December 11, 2017, APHIS announced in the *Federal Register* that it was making Nuseed's petition available for public review and comment to help identify potential environmental and interrelated economic impacts that APHIS should consider in evaluation of the petition.[7] APHIS accepted written comments on the petition for a period of 60 days, until midnight February 9, 2018. At the end of

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[7] Federal Register, Vol. 82, No. 236, Monday, December 11, 2017, p. 58167 - Nuseed Americas Inc., Availability of Petition for Determination of Nonregulated Status of Canola Genetically Engineered for Altered Oil Profile [Docket No. APHIS-2017-0096, www.regulations.gov].

the comment period APHIS had received a total of 4 comments – 2 were from individuals, and 2 were from the canola industry. APHIS evaluated the comments and integrated the concerns raised into this EA. All comments received on the petition are available for public review at www.regulations.gov, Docket ID: APHIS-2017-0096.

On June 26, 2018, APHIS announced in the *Federal Register* it was making available the draft EA and PPRA for a 30-day public review and comment period.[8] At the end of the comment period APHIS had received 2 public comments on the petition, 1 from the U.S. Canola Association and 1 from the Global Organization for EPA and DHA Omega-3s. Both were in support of Nuseed's petition for a determination of nonregulated status for DHA canola. No new information was presented to APHIS in the comments that contributed to or altered the analyses presented in the draft EA, thus, neither comment was deemed substantive in the sense that they warranted a formal response from APHIS. A full record of each comment received is available online at at www.regulations.gov, Docket ID: APHIS-2017-0096.[9]

1.5.4 Issues Considered in this EA

APHIS developed a list of topics for consideration in the EA based on issues identified in public comments on the petition, prior EAs for regulated canola varieties, public comments submitted for other EAs and EISs evaluating petitions for nonregulated status, the scientific literature on agricultural biotechnology, and issues identified by APHIS specific to wild and cultivated *Brassica* species. The following topics were identified as relevant to the scope of analysis (40 CFR § 1508.25):

Agricultural Production

- Acreage and Areas of Canola Production
- Agronomic Practices and Inputs

Physical Environment

- Soils
- Water Resources
- Air Quality

Biological Resources

- Soil Biota
- Animal and Plant Communities
- Gene Flow and Weediness
- Biodiversity

Human Health Considerations

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[8] Federal Register / Vol. 83, No. 123 / Tuesday, June 26, 2018 / Notices / Nuseed Americas Inc.; Availability of a Draft Plant Pest Risk Assessment and Draft Environmental Assessment for Canola Genetically Engineered for Altered Oil Profile, p. 29742

[9] https://www.regulations.gov/docket?D=APHIS-2017-0096

- Consumer Health and Worker Safety

Animal Health and Welfare

- Animal Feed and Livestock Health

Socioeconomic Considerations

- Domestic Economic Environment
- International Trade

In addition, potential cumulative impacts relative to these issues, and potential impacts on threatened and endangered (T&E) species are also considered, as wells as adherence of the regulatory status decision to Executive Orders, and environmental laws and regulations to which the action may be subject.

2 ALTERNATIVES

NEPA implementing regulations (40 C.F.R. § 1502.14) require agencies to evaluate all alternatives that appear reasonable and appropriate to the purpose and need for the Agency's action (in this case, a regulatory decision). Two alternatives are evaluated in this EA: (1) No Action, denial of the petition, which would result in the continued regulation of DHA canola, and (2) a determination of nonregulated status for DHA canola – approval of the petition, the Preferred Alternative.

2.1 No Action Alternative: Continuation as a Regulated Article

One of the alternatives that must be considered by APHIS is a "No Action Alternative," pursuant to CEQ regulations at 40 CFR part 1502.14. No Action in this instance means no change in regulatory status. Under the No Action Alternative APHIS would deny the petition request for nonregulated status and DHA canola and progeny derived from DHA canola would remain regulated articles under 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would be required for the introduction of DHA canola. Because APHIS concluded in its PPRA that DHA canola is unlikely to pose plant pest risk (USDA-APHIS 2018a), this is not APHIS' preferred alternative. Choosing this alternative would not be an appropriate response to the petition for nonregulated status, nor satisfactorily meet the purpose and need for making a science based regulatory status decision pursuant to the requirements of 7 CFR part 340.

2.2 Preferred Alternative: Determination of Nonregulated Status for DHA Canola

Under this alternative DHA canola and progeny derived from it would no longer be subject to APHIS regulation under 7 CFR part 340 because it was determined that, based on the scientific evidence before the Agency, DHA canola is unlikely to pose a plant pest risk (USDA-APHIS 2018a). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of DHA canola. This alternative best satisfies the purpose and need to respond appropriately to the petition for nonregulated status pursuant to the requirements of 7 CFR part 340.6 and the Agency's statutory authority under the PPA.

2.3 Alternatives Considered but Dismissed from Detailed Analysis

APHIS evaluated several other alternatives for consideration in the EA in light of the Agency's statutory authority under the PPA and APHIS implementing regulations at 7 CFR part 340, but dismissed these alternatives for analysis in the EA. The alternatives considered are summarized below along with the reasons for dismissal from detailed analysis.

2.3.1 Prohibit the Release of DHA Canola

APHIS could consider prohibiting the environmental release of DHA canola, including denying permits for field testing. However, this alternative would be inappropriate and legally challenging because APHIS determined that DHA canola is unlikely to pose a plant pest risk (USDA-APHIS 2018a). In enacting the PPA of 2000, Congress included findings that:

"decisions affecting imports, exports, and interstate movement of products regulated under [the PPA] shall be based on sound science;…" (7 U.S. C. §7701(4)) and that "The Secretary's determination on the petition shall be based on sound science" (§ 7711(3)(c)).

On March 11, 2011, in a Memorandum for the Heads of Executive Departments and Agencies, the White House Emerging Technologies Interagency Policy Coordination Committee developed broad principles, consistent with Executive Order 13563, to guide the development and implementation of policies for oversight of emerging technologies, such as genetic engineering, at the agency level. In accordance with this memorandum, agencies should adhere to Executive Order 13563 and, consistent with that Executive Order, the following principle, among others, to the extent permitted by law, when regulating emerging technologies:

"Decisions should be based on the best reasonably obtainable scientific, technical, economic, and other information, within the boundaries of the authorities and mandates of each agency"

Based on the PPRA for DHA canola (USDA-APHIS 2018a), experience with GE and non-GE canola varieties, and additional scientific information, APHIS concluded that DHA canola is unlikely to pose a plant pest risk. Accordingly, there is no scientific or legal basis for continuing the regulation nor prohibiting the release of DHA canola. Consequently, an alternative that would prohibit the environmental release of DHA canola was dismissed from analysis in the EA.

2.3.2 Approve the Request in Part

The regulations at 7 CFR § 340.6(d)(3)(i) provide that APHIS may "approve the petition in whole or in part." For example, a determination of nonregulated status in part may be appropriate if there is a plant pest risk associated with some, but not all lines described in a petition. APHIS has concluded that DHA canola is unlikely to pose a plant pest risk (USDA-APHIS 2018a). Because there must be a plant pest risk to deny the petition request, or approve the petition in part, it would be inconsistent with APHIS' statutory authority under the plant pest provisions of the PPA and regulations at 7 CFR part 340 to consider approval of the petition only in part. Consequently, this alternative was dismissed from detailed analysis.

2.3.3 Isolation of DHA Canola and Non-GE Canola Production Systems or Geographic Restriction

In response to public concerns regarding gene movement between GE and non-GE plants, APHIS could consider requiring isolation distances for separation of DHA canola from non-GE canola production systems. APHIS could also considered geographically restricting the production of DHA canola based on the location of production of non-GE canola, or organic production systems, or production systems for GE-sensitive markets. Because APHIS concluded that DHA canola is unlikely to pose a plant pest risk (USDA-APHIS 2018a), the Agency has no jurisdiction to continue regulating DHA canola. Consequently, prescribing isolation distances or geographic restrictions on production would be inconsistent with APHIS' statutory authority under the plant pest provisions of the PPA and regulations in 7 CFR part 340. Therefore, it would be unreasonable to evaluate an alternative to approval of the petition absent any jurisdiction to implement the alternative. For these reasons, this alternative was dismissed from detailed analysis.

While a determination that DHA canola is unlikely to present a plant pest risk means that APHIS has no further regulatory control over the planting, distribution, or other actions related to DHA canola, growers continue to be subject to any contract restrictions imposed by Nuseed, or the requirements of other federal or state agencies. Individual canola producers may also voluntarily choose to isolate or geographically restrict their GE and/or non-GE canola production systems, or use other management practices to minimize gene movement between canola fields.

2.3.4 Requirements to Test for DHA Canola

During comment periods for other petitions for nonregulated status, certain commenters requested that APHIS require and provide testing for the presence of GE material in non-GE production systems. Because there are no federal regulations describing testing criteria or quantitative thresholds for GE material in non-GE cropping systems or crop products, nationwide testing and monitoring would be extremely difficult to implement. Additionally, because DHA canola is unlikely to pose a plant pest risk (USDA-APHIS 2018a), the imposition of any type of testing requirements for DHA canola would be inconsistent with the plant pest provisions of the PPA, 7 CFR part 340, and federal regulatory policies embodied in the Coordinated Framework. Consequently, this alternative was dismissed from detailed analysis.

2.4 Comparison of the Alternatives Considered

Table 2-1 presents a summary of the environmental consequences associated with the No Action Alternative and Preferred Alternative that are evaluated in this EA. Detailed analysis of the affected environment and environmental consequences is discussed in Chapter 3 and Chapter 4, respectively.

Table 2-1. Summary of Potential Impacts for the Alternatives Considered

Analysis	No Action Alternative: Continue to Regulate DHA Canola as a Plant Pest	Preferred Alternative: Approve the Petition for Nonregulated Status for DHA Canola
Meets Purpose and Need	No	Yes
Unlikely to pose a plant pest risk	Addressed by the use of regulated field trials.	Determined by the plant pest risk assessment (USDA-APHIS 2018a).
Agricultural Production		
Acreage and Areas of Canola Production	Denial of the petition would have no effect on the areas or acreage utilized for canola production. There may be fluctuations in production areas and acreage relative to climate, pest and disease pressures, market demand for canola oil and meal, as well as availability of soybean oil and meal.	The potential impact of approval of the petition on the total number of U.S. acres planted to canola is difficult to determine with any degree of accuracy. Because DHA canola oil, enriched in EPA and DHA, would be a new commodity, it may entail use of additional cropland for production. Market forces, grower choices, consumer preference, and demand for vegetable and fish oils rich in EPA and DHA, across all markets (i.e., feed, food, and nutraceuticals), will, in combination, determine the market share and scale of adoption of DHA canola. Among these factors, consumer preference for a GE vegetable oil enriched in omega-3 fatty acids is

Table 2-1. Summary of Potential Impacts for the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate DHA Canola as a Plant Pest**	**Preferred Alternative: Approve the Petition for Nonregulated Status for DHA Canola**
		uncertain. Nuseed estimates that the market share of DHA canola oil in the fish oil food ingredient market is likely to be low initially, increasing over time and with market acceptance to as high as ~20 percent after 10 years (Nuseed 2017).
Agronomic Practices and Inputs	Agronomic practices and inputs used in canola crop production would remain unchanged.	Studies evaluating the phenotypic, phenologic, and agronomic properties of DHA canola indicate agronomic practices and inputs would be the same as for other varieties of canola (Nuseed 2017).
Physical Environment		
Soils	Agronomic practices, inputs, and other factors potentially impacting soils would be unaffected by denial of the petition.	The agronomic practices and inputs are the same for both DHA and existing canola varieties – potential direct and indirect impacts to soils would be unchanged.
Water Resources	Denial of the petition would have no effect on water resources in the United States.	Because DHA canola is agronomically similar to currently cultivated canola, and LC-PUFAs occur naturally in terrestrial and aquatic environments, approval of the petition and subsequent commercial production of DHA canola would present the same potential risks to water resources as currently cultivated canola varieties.
Air Quality	Emission sources, namely tillage and machinery combusting fossil fuels, and the level of emissions associated with canola production would be unaffected by denial of the petition.	Sources of potential impacts on air quality are the same as those under the No Action Alternative.
Biological Resources		
Soil Biota	Potential impacts on soil biota would be unaffected by denial of the petition.	Commercial production of DHA canola and DHA hybrid crops are not expected to present any risk to soil biota. Same or functionally similar elongase and desaturase enzymes, and the fatty acids they synthesize, are inherent to a variety of soil biota.
Animal Communities	Potential impacts on animal communities would be unaffected by denial of the petition. Canola fields can	Potential impacts on animal communities would be the same as that under the No Action Alternative. Fatty

Table 2-1. Summary of Potential Impacts for the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate DHA Canola as a Plant Pest**	**Preferred Alternative: Approve the Petition for Nonregulated Status for DHA Canola**
	contain several animal species. Some species (such as insect crop pests) may need to be controlled using a range of tools. These tools may be deployed within integrated pest management strategies. The U.S. EPA regulates pesticides and determines whether they pose an unacceptable risk to animal communities. It is violation of federal law to use a pesticide in a manner that is not in strict accordance with the instructions on its U.S. EPA-approved label.	acids are vital to the normal development and function of all organisms. The vast majority of fatty acids among eukaryotes and prokaryotes are common across taxa as are biosynthesis pathways. All wildlife consume or synthesize, and are comprised of, fatty acids found in DHA canola seed, to include the LC-PUFA EPA, and to some extent DHA. It is unlikely that DHA canola seed presents any risk to wildlife.
Plant Communities	Potential impacts on plant communities would be unaffected by denial of the petition. Plants (other than crop plants) in canola fields are considered weeds as they can impact crop yield and quality. Weeds are managed using a variety of methods, including tillage and herbicides. The U.S. EPA regulates and determines how pesticides can be used. U.S. EPA pesticide use requirements are intended to be protective of non-target plant communities and other plants, such as those in adjacent fields.	Potential impacts on plant communities would be the same as that for the No Action Alternative.
Gene Flow and Weediness	Pollen may flow from GE canola to sexually-compatible wild relatives i.e., *Brassica* spp. The progeny of this gene flow (hybrids) could spread to other areas and lead to the establishment of additional feral hybrid populations. Because of the general ecological requirements of *Brassica* spp., the establishment of feral hybrid populations is more likely in sites that are subject to frequent disturbances. Pollen dispersal is most likely to areas 300 feet or less from pollen sources. Rarely, outcrosses may occur at distances up to 2 miles away. APHIS	Based on the PPRA, APHIS concluded that it is unlikely that gene introgression from DHA canola to other organism with which it can interbreed will increase their weediness (USDA-APHIS 2018a). Consequently, the Preferred Alternative is not expected to substantially differ from the No Action Alternative in regard to the potential environmental impacts associated with gene flow and weediness.

Table 2-1. Summary of Potential Impacts for the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate DHA Canola as a Plant Pest**	**Preferred Alternative: Approve the Petition for Nonregulated Status for DHA Canola**
	recognizes interspecific and intraspecific hybridization will occur, although probably at a low frequencies. Gene flow is most likely to occur among *B. napus* crops grown in adjacent areas, and *B. napus* crops and wild relative *B. rapa* species.	
Biodiversity	Denial of the petition would have no effect on biodiversity in commercial canola cropping systems.	Because DHA canola is agronomically the same as currently cultivated canola varieties, potential impacts on biodiversity would be the same as under the No Action Alternative.
Human and Animal Health		
Human Health	Denial of the petition would no effect on human health.	As part of the FDA consultation process, Nuseed submitted a safety and nutritional assessment for food and feed derived from DHA canola to the FDA in March, 2017. If the petition is approved, DHA canola may become available to the commercial market. This would be considered a potential public health benefit, relative to potential uses of DHA canola oil by consumers and industry. A determination of nonregulated status for DHA canola would not be expected to have any effect on the U.S. EPA regulation of pesticides, or worker protection standards; potential risks and protections for workers would be no different than that of the No Action Alternative.
Animal Health and Welfare	Denial of the petition would have no effect on animal health and welfare. DHA canola will remain a regulated article, will not be available as an animal feed, and current canola based feed for livestock will remain unchanged.	DHA canola oil and whole seed would provide a supplemental source of omega-3 fatty acids in the production of animal feed, to include feeds for use in the aquaculture industry. Producers of livestock and farmed fish would be expected to utilize DHA canola oil and whole seed to the extent they determined it provided, as a dietary

Table 2-1. Summary of Potential Impacts for the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate DHA Canola as a Plant Pest**	**Preferred Alternative: Approve the Petition for Nonregulated Status for DHA Canola**
		component, optimal quality beef, swine, poultry, and farmed fish.
Socioeconomics		
Domestic Economic Environment	Denial of the petition would have no effect on the U.S. domestic canola oil, meal, or biodiesel markets.	A determination of nonregulated status for DHA canola is not expected to adversely impact domestic conventional, organic, or GE canola markets. DHA canola would be adopted to the extent DHA canola oil provided benefits in meeting market demand for food and feed with omega-3 fatty acids.
International Trade	There would be no impacts on trade under the No Action Alternative.	U.S. canola imports and exports would be unaffected by a determination of nonregulated status to DHA canola. Nuseed will seek international regulatory approvals in Australia, New Zealand, Canada, Mexico, Japan, South Korea, China, European Union, and other countries as required.
Cumulative Impacts		
Agriculture, Physical and Biological Resources, Public Health, Socioeconomic	No significant cumulative impacts on agronomic practices and inputs, the acreage and areas of canola production, the physical environment and biological resources, development of pest and weed resistance, gene flow and weediness, human and animal health, domestic markets, or international trade were identified.	DHA canola production would entail the use of pesticides and fertilizers, and to some extent tillage, which will contribute to potential cumulative impacts on water, soil, and air quality, as does current canola production. If total U.S. canola acreage increases due to DHA canola adoption in the market, there would be a commensurate increase in the contribution of agricultural inputs (pesticides and fertilizers) as well as NAAQS emissions, relative to the amount of increased acreage. If DHA canola is accepted by consumers, there may be a marginal increase in canola acreage, with commensurate cumulative effects on total agricultural inputs and NAAQS emissions, and the risk these may present to water and air quality, and soil resources.
Coordinated Framework		

Table 2-1. Summary of Potential Impacts for the Alternatives Considered		
Analysis	**No Action Alternative: Continue to Regulate DHA Canola as a Plant Pest**	**Preferred Alternative: Approve the Petition for Nonregulated Status for DHA Canola**
U.S. Regulatory Agencies	Denial of the petition would have no effect on the roles of the FDA and U.S. EPA in oversight of DHA canola.	Nuseed is consulting with the FDA on the food and feed safety of DHA canola. Changes to U.S. EPA registration of pesticides used on DHA canola would be unnecessary.
Regulatory and Policy Compliance		
ESA, CWA, CAA, SDWA, NHPA, EOs	Fully compliant	Fully compliant

3 AFFECTED ENVIRONMENT

This chapter provides an overview of those aspects of the human environment potentially affected by APHIS' decision to either approve or deny the petition. Broadly, those aspects considered are U.S. canola production, the physical environment, biological resources, public health, animal feed, and socioeconomics. Because the introduced genes are involved in the biosynthesis of omega-3 fatty acids, the primary focus of this EA is on: (1) potential impacts on human and animal (livestock/poultry) health, (2) effects on wildlife that may consume DHA canola or DHA canola hybrids (wild or commercial canola hybrids), and (3) gene flow and potential weediness.

3.1 Overview of Canola Production and Uses

3.1.1 Rapeseed and Canola Cultivars

The plant common names "rapeseed" and "canola" are often used interchangeably; however, canola and rapeseed are two different crop plants (the term "rape" derives from the Latin word for turnip, rapum). Both belong to the *Brassica* genus that is within the "mustard family" of plants (Al-Shehbaz 2001), which also comprises food crops such as kale, turnips, cabbage, and brussel sprouts, as well as plants considered to be weeds and/or wildflowers. While rapeseed and canola are of the same genus and species, *Brassica napus* L., they are distinct cultivars (subspecies), distinguished by the chemical composition of their seed oil (described in the following section).

Taxonomic experts offer a range of opinions, although the genus *Brassica* is typically divided into about 19 species (USDA-NRCS 2016; ITS 2017). The taxonomic relationship and common names used for these plants is summarized in Table 3-1. Rapeseed is the traditional name for *Brassica* oilseed crops, however, rapeseed may also be referred to as rape, oilseed rape, rapa, and rappi, as well as canola in some cases (USDA-NRCS 2016). The crop plant subject of petition 17-236-01p and this EA is *B. napus* L. subsp. *napus* (canola).

Table 3-1. Scientific and Common Names for Several Species in the Genus *Brassica*

Scientific Name	Common Name
Brassica napus L.	rapeseed, rape, rape, oilseed rape, rapa, rappi
Brassica napus L. subsp. *napus*	Argentine canola, canola, colza, oilseed rape, and rape
Brassica napus L. subsp. *napus* forma *napus*	Swede rape, winter rape
Brassica napus L. subsp. *napus* forma *annua*	annual rape, summer rape
Brassica napus L. subsp. *rapifera*	rutabaga, Swedish turnip

Source: (Wiersema and León 2013)

Various historic uses of rapeseed oil have been reported, such as for food, lamp oil, and as a steam engine lubricant due to its cold weather performance (Shahidi 1990). Rapeseed was cultivated in India well over 2000 years ago, spreading to China and Japan around 1000 years ago, with cultivation in Europe beginning around the 13th century (OECD 2012). In North America, cultivation of canola for vegetable oil and animal feed began in Canada in the 1950s, with economically significant U.S. production beginning the 1990s.

3.1.2 Canola

Canola is a particular variety of *Brassica* derived from the traditional (i.e., non-GE) breeding of *B. napus, B. rapa*, and *B. juncea.* This was done to reduce the levels of two types of nutritionally undesirable compounds that can occur in *Brassica* spp., erucic acid and glucosinolates. Erucic acid can represent around 20% to 50% of fatty acids found in rapeseed oil. Animal feeding studies examining the nutritional quality of rapeseed during the 1950s to the 1970s indicated that erucic acid was one of several fatty acids that are poorly metabolized by animals (Sauer and Kramer 1983), and if fed in large quantities was associated with heart disease (myocardial lipidosis) in animals (Sauer and Kramer 1983). In addition, most plants among the order Brassicales produce sulphur-containing compounds called glucosinolates, which are also undesirable in animal feeds.[10] While there are about 250 forms of glucosinolates that are produced by plants across 16 families of the order Brassicales, only about 20 glucosinolates are commonly found in *Brassica.* spp. (OECD 2012). A single *Brassica* species will often contain about four types of glucosinolates, although some may contain as many as 15 different glucosinolates. Glucosinolates occur in varying amounts in all tissues of the plant, and are one of the sources of the distinct flavor of many brassicaceous vegetables (e.g., cabbage, kale, collards) and the spicy/hot component of mustards (OECD 2012). High levels of glucosinolates can lower the value of rapeseed meal as animal feed, as many animals find the taste of glucosinolates unpleasant, leading to a reduced feed intake (Khajali and Slominski 2012). Very high levels of glucosinolates in animal feed have been associated with reduced growth rates in livestock and poultry (EFSA 2008; Khajali and Slominski 2012).

Historically, due to high levels of erucic acid and glucosinolates, rapeseed was not widely used as a food or animal feed crop in North America (Lin et al. 2013; CFIA 2016). During the 1960s, plant breeders, using traditional breeding, began developing low-erucic acid *B. napus*, *B. rapa*, and *B. juncea* varieties that also had low glucosinolate content (Lin et al. 2013; CFIA 2016). This was in response to research findings that indicated the nutritional value of rapeseed oil could be improved if the erucic acid and glucosinolate levels could be reduced (Sauer and Kramer 1983; Lin et al. 2013; CFIA 2016).

In 1978, the Western Canadian Oilseed Crushers Association registered low-erucic acid (LEAR) varieties with the name "canola," which is a blending of the words Canadian Oilseed. LEAR varieties of *B. napus*, *B. rapa*, and *B. juncea* are commonly referred to as “0-rapeseed,” or “low-rapeseed.” Those varieties that are both low in erucic acid and glucosinolates are called “00-rapeseed” or “double-low rapeseed.” The latter is the most common variety used today for the production of canola oil for human consumption, and canola meal for animal feed. The term “canola” is used to indicate a 00-rapeseed variety. The present definition of canola is an oil that must contain less than 2% erucic acid and the solid component of the seed must contain less than 30 micromoles per gram of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil free solid. This standard is approximately 18 micromoles per gram of seed on an air-dry basis (Button and Downey 2003).

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[10] It is not glucosinolates themselves that are directly undesirable in animal feeds. It is some of the breakdown products that are produced by the enzymatic action of a group of enzymes called β-thioglucosidases (myrosinases).

In North America, canola refers to edible canola grown for food oil and meal, whereas rapeseed refers to rapeseed oil used for industrial purposes. In other parts of the world, the term "rapeseed" may be used when referring to canola. In this EA, the terms rapeseed and canola will be used interchangeably when referencing global statistics and other information, consistent with the use of "rapeseed" outside of North America.

3.1.2.1 Canola Oil

Food use

Canola oil is the third largest source of vegetable oil in the world after soybean and palm oil (USDA-ERS 2016b), used for frying and as an ingredient in salad dressings, margarine, baked goods, and a variety of other food products. Canola oil appeals to certain consumers because it has no cholesterol, is low in saturated fat, and is a good source of monounsaturated fats.

Industrial Use

Generally, "industrial rapeseed" refers to high-erucic acid rapeseed (HEAR) oil, which has an erucic acid content of at least 45% in the seed oil. A small amount of HEAR oil is produced in the United States, used for a variety of purposes ranging from lubricants, hydraulic fluids, and penetrating oils to fuel, soap stock, and paints. HEAR oil is biodegradable and is used in applications requiring high heat stability where the risk of oil leaking into waterways or ground water is significant (USDA-ERS 2016c). In the United States, HEAR is grown under contract and is not introduced to the regular grain handling system (USDA-ERS 2016b).

Canola and rapeseed oil are also used for biodiesel production. In the United States, canola oil contribution to biodiesel production increased from 246 million pounds in 2011 to 745 million pounds in 2015, and 1.1 billion pounds in 2016. It has been third largest biodiesel source since 2014, and is expected to remain as such, behind soybean and corn oil (EIA 2017b). Biodiesel demand in the United States is driven primarily by the renewable fuel standards (Schwab et al. 2016), which were created by Congress in an effort to reduce greenhouse gas emissions, expand the United States renewable fuels resources, reduce reliance on imported oil, and reduce air pollution.

Unlike the United States, the majority of European cars and trucks run on diesel fuel, and in Europe, rapeseed is the most common plant stock for biodiesel production (Carré and Pouzet 2014). Currently, about 68% of European biodiesel production is derived from rapeseed, 15% from soybean and 6% from palm oil (EBIA 2017). With increasing biofuel mandates, industrial use of rapeseed oil has increased rapidly in the European Union (EU) from 4.2 million metric tons in 2000 to 10.2 million in 2015 (IndexMundi 2017).

3.1.2.2 Canola Meal

Canola meal is used extensively for animal feed and is second only to soybean as a source of protein meal. Because canola meal has a lower protein content than soybean meal (34-38% versus 44-49%), it is primarily used as feed for animals that do not have high energy or lysine (a type of essential amino acid) requirements, such as cattle, swine, and poultry (USDA-ERS 2016b).

3.2 Acreage and Areas of Canola Production

3.2.1 Global Production

Global canola/rapeseed [11] production grew rapidly over the past 40 years, rising from the sixth to the second largest oilseed crop in the world (Figure 3-1). During 2017, global rape/canola seed production was 73.1 million metric tons, following soybeans at 348.6 million metric tons (USDA-FAS 2017).

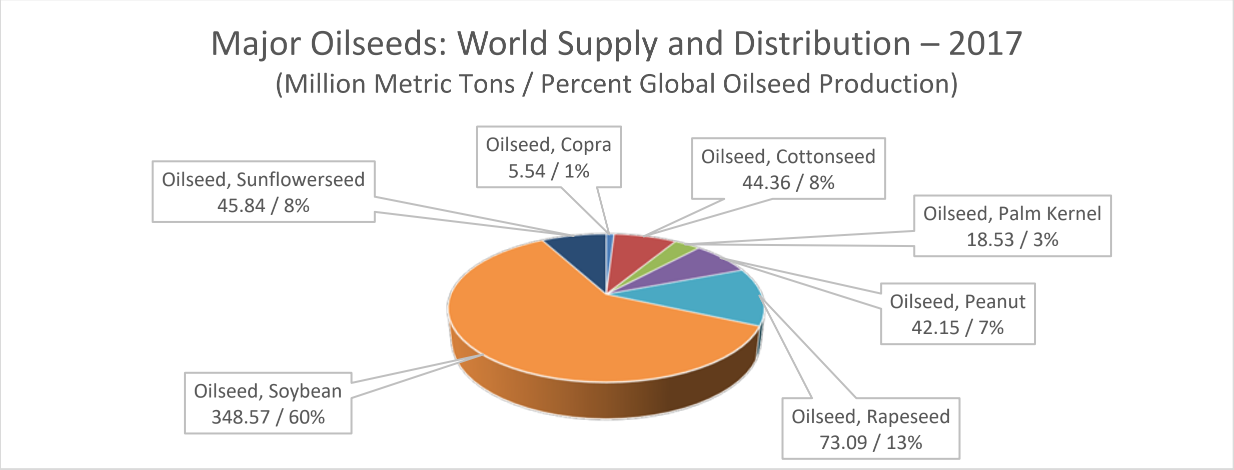

Figure 3-1. Global Oilseed Production by Source - 2017

Source: (USDA-FAS 2017)

Note: Rapeseed in reference to global production includes canola

Global canola oil production was 28.4 million metric tons, accounting for approximately 15% of global vegetable oil production, and global production of meal was 40.1 million metric tons, about 11% of all protein meals (Table 3-2). Canola meal is currently the second largest source of feed meal after soybean meal. The majority of production occurs in the EU, China, and Canada. U.S. canola production is relatively low compared with global output, with U.S. canola seed production at 1.4 million metric tons during 2017, comprising about 2% of the global supply.

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[11] Canola is called rapeseed in many countries, hence usage of the word rapeseed when referring to global markets. The term canola is used in the United States, Canada, and Australia.

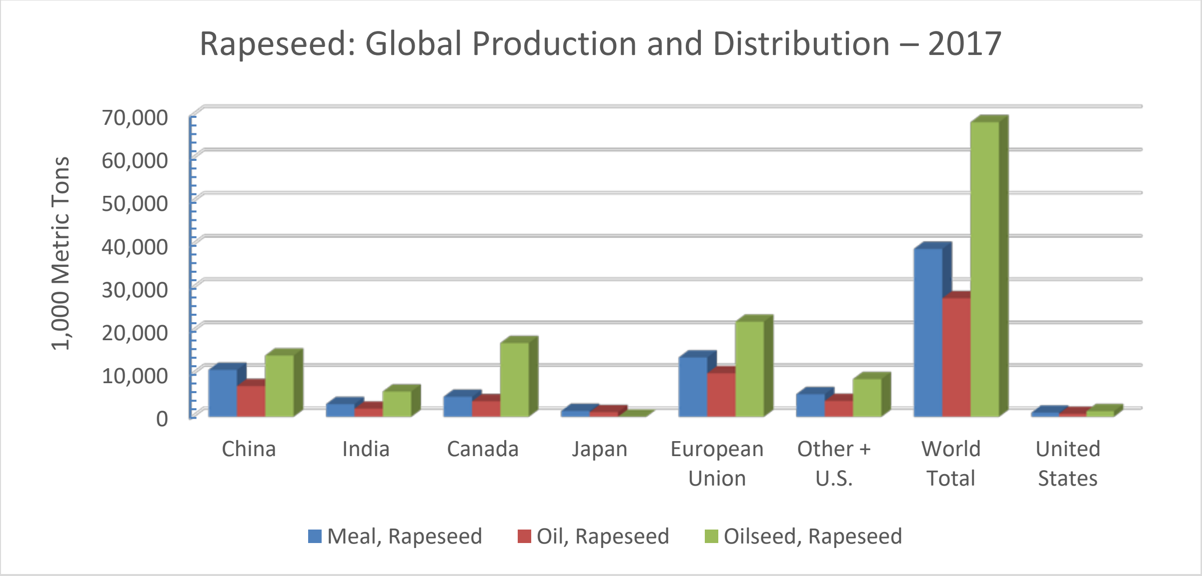

Figure 3-2. Global Production of Rapeseed – 2017
Source: (USDA-FAS 2017)
Note: Rapeseed in reference to global production includes canola

Table 3-2. Rapeseed and Products: World Supply and Distribution – 2017

	Meal	Oil	Oilseed
		Thousand Metric Tons	
China	10,236	6,745	13,100
India	3,350	2,100	6,500
Canada	5,200	4,020	21,500
Japan	1,360	1,075	4
European Union	14,250	10,450	22,100
Other + United States	5,655	3,964	9,887
World Total	40,051	28,354	73,091

Source: (USDA-FAS 2017)

3.2.2 U.S. Production: Conventional, GE, and Organic Canola

While the U.S. share of global canola production remains small, demand has increased. This is due in part to the fatty acid profile of canola oil – it contains no cholesterol, is low in saturated fats, and relatively high monounsaturated and polyunsaturated fats. The meal is also a good source of protein for animal feed. As of 2012 (latest census data) there were 3,995 canola farms across 34 states (USDA-NASS 2014b). In 2017, the area of canola acreage planted and harvested totaled about 2.0 million acres (USDA-NASS 2018a). While canola is produced in many states, approximately 90% of U.S. production occurs in North Dakota (Figure 3-3).

Canola is a cool season crop with both spring and winter varieties. The optimal temperature for canola growth and development is between 54° F and 86° F. In the United States, three different types of canola are grown:

- Spring canola

- Winter canola that requires vernalization (winter chilling to promote spring flowering)
- Winter canola that does not require vernalization

The vast majority of U.S. canola is spring canola, which is typically planted in March and harvested in September or October (NDSU 2011). Winter canola is planted in the fall, overwinters, and is harvested in summer (Brown et al. 2008). Winter cultivars requiring vernalization are generally produced in the Pacific Northwest, Great Plains, and Midwest regions of the United States. Winter cultivars that do not require vernalization are grown in the Southeast United States where they may be part of a double-crop production system and grown in the cooler portion of the year (Monsanto 2016). Primary canola producing states are listed in Table 3-3.

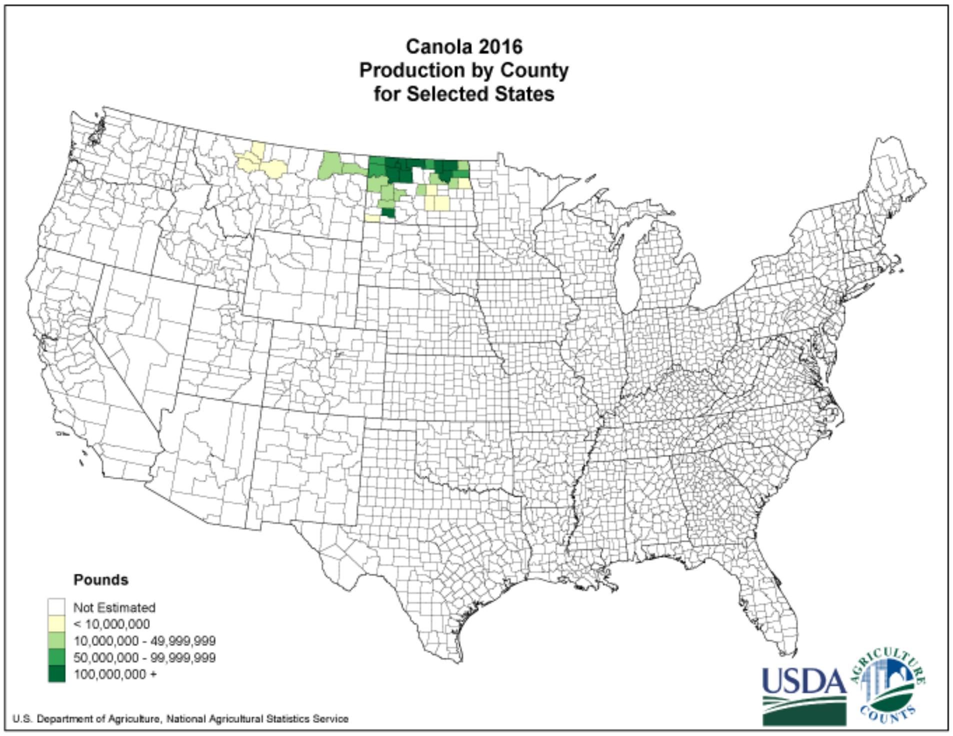

Figure 3-3. Primary Areas of Canola Production in the contiguous United States – 2016
Source: (USDA-NASS 2018b)

Table 3-3. U.S. Canola Area Planted, Harvested, and Production – 2017

State	Area planted	Area harvested	Production
	(1,000 acres)		(1,000 pounds)
North Dakota	1,590	1,560	2,542,800
Oklahoma	160	140	191,800
Montana	155	137	119,190
Washington	55	54	86,400
Idaho	23	22	34,565

Minnesota	36	35	70,725
Oregon	8	7	11,160
Other States	ND	ND	ND
U.S. Total	2,077	2,002	3,118,680

ND = No Data
Source: (USDA-NASS 2018a)

From 1991 to 2017, harvested canola acreage in the United States increased by about 1.8 million acres, (Figure 3-4). Yields have also steadily increased, from 1,300 lbs/acre in 1991 to 1,558 lbs/acre in 2017 (USDA-NASS 2016, 2018a).

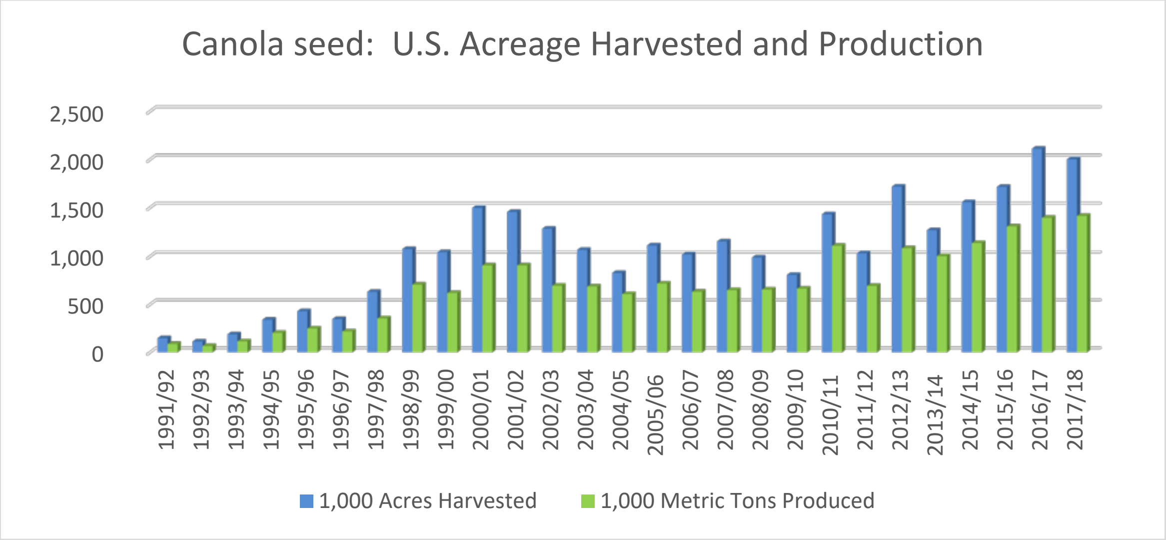

Figure 3-4. Harvested Canola Acreage and Production in the United States, 1991 – 2017
For oilseeds, the U.S. marketing year begins June 1 for canola (rapeseed), and September 1 for soybeans and sunflower seed. Dates given in dual year format (e.g., 1991/92) indicate the marketing year. Data for 2017/18 is estimated.
Source: (USDA-NASS 2016, 2018a)

3.2.2.1 Conventional Canola

Approximately 90% of the U.S. and Canadian canola crops are GE herbicide resistant (HR) varieties (Fernandez-Cornejo et al. 2016). However, there are several conventional cultivars produced in the United States, which have been bred for cultivation in specific areas such as the Pacific Northwest, Great Plains, and Southeast (see overview by (Brown et al. 2008)).

3.2.2.2 GE Canola in the United States

APHIS has issued determinations of nonregulated status for 10 varieties of GE canola (Table 3-4). Of these, all are either resistant to glyphosate or glufosinate, save for laurate canola (petition 94-090-01p).

Table 3-4. APHIS Determinations of Nonregulated Status for GE Canola

Petition	Petitioner	Petition Subject	GE Trait	Date of Determination

Table 3-4. APHIS Determinations of Nonregulated Status for GE Canola

94-090-01p	Calgene	pCGN3828-212/86-18 and pCGN3828-212/86-23	Laurate production	10/31/1994
97-205-01p	AgrEvo (Bayer CropScience)	T45	Phosphinothricin (glufosinate) tolerant *	1/29/1998
01-206-02p (Extension of 97-205-01p)	Aventis	Topas 19/2	Phosphinothricin (glufosinate) Tolerant & Pollination Control	1/23/2002
98-278-01p	AgrEvo (Bayer CropScience)	MS8 and RF3	Phosphinothricin (glufosinate) Tolerant and pollination control	1/22/1999
01-206-01p (Extension of 98-278-01p)	Aventis	MS1, RF1, RF2	Phosphinothricin (glufosinate) Tolerant	1/23/2002
98-216-01p	Monsanto	RT73	Glyphosate tolerant	1/27/1999
01-324-01p (Extension of 98-216-01p)	Monsanto	GT2000	Glyphosate tolerant	1/2/2003
11-188-01p	Monsanto	MON 88302	Glyphosate tolerant	9/25/2013
11-063-01p	Pioneer	73496	Glyphosate tolerant	7/18/2013
16-235-01p (Extension of 98-278-01p)	Bayer	MS11 Canola	Male Sterile, Glufosinate-Ammonium Resistant	7/26/2017

Source: (USDA-APHIS 2018b)
*Herbicides that contain glufosinate (also referred to as Phosphinothricin) or glyphosate may be sold under several product names.

3.2.2.3 Organic Canola Production

Currently, organic canola production in the United States is limited and comprises a small proportion of U.S. canola production. As of 2016 (latest organic census data) there were only 4 USDA certified organic canola farms in the United States; 2 in Pennsylvania, 1 in Indiana, and 1 in Iowa (USDA-NASS 2017d). Total production from these farms totaled 305,000 lbs of seed: This, compared to about 3.1 billion pounds of harvested conventional and GE canola (USDA-NASS 2018a). Organic sales data has been withheld over the last several years to avoid disclosing data for individual farms (USDA-NASS 2018a).

While organic canola production is limited, niche operations for provision of “non-GMO” verified canola oil and organic livestock feed are emerging (Vann 2017; Non-GMO-Project 2018). Organic and non-GMO canola production is further discussed in Section 3.8 – Socioeconomics.

3.3 Agronomic Practices and Inputs

Canola production involves the use of a range of agronomic practices and inputs, including crop rotation, crop monitoring, tillage, fertilizers, pesticides, seeding and harvesting, and in some cases, irrigation. The practices and inputs that are used by growers depends on several factors such as local conditions, soils, and the weeds and crops pests that may be present. Pesticide use and other practices are often necessary to protect crops from weeds and crop pests. However, certain agronomic inputs can potentially present environmental and human health risks when not properly used. It is unlawful to use a pesticide in a way that is not in strict accordance with its U.S. EPA approved label instructions. Those practices and inputs that can present environmental and human health risks are summarized below. This section considers the agronomic practices and inputs used in the production of canola.

3.3.1 Tillage

Tillage is primarily used to control weeds and soil-borne pests and disease, yet may also be used to dry and warm the soil prior to planting. Tillage types are commonly classified as conventional, reduced, and conservation tillage (to include no-till), which are characterized in part by the amount of plant residue 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 involves leaving at least 30% crop residue (USDA-ERS 2000).

Which tillage practices are used and to what extent can have substantial impacts on soil quality, erosion, and water and air quality. Tillage operations can also be costly and time-consuming to implement (Brown et al. 2008; Wallander 2015; Harper 2017). Over the long-term conventional tillage can lead to reduced soil quality, and result in soil erosion and run-off that can adversely affect surface waters (USDA-ERS 2000). Conservation tillage systems are the least intensive and, as the name implies, aim to improve or maintain soil quality and conserve topsoil (Roth 2015; Wallander 2015). Conservation tillage provides a variety of agronomic and economic benefits, such as reductions in fuel use and cultivation costs, preservation of soil organic matter and moisture, and reductions in soil erosion and water pollution (Fernandez-Cornejo et al. 2012; Roth 2015). No-till systems leave all crop residue on the field, unless those residues are removed for other reasons such as biomass production (USDA-ERS 2000).

Decisions concerning the amount, timing, and type of tillage to employ are some of the most important crop producers make. These decisions involve consideration of a wide range of interrelated factors such as the variety and extent of weeds and crop pests present, soil erosional capacity, fuel and other input costs, anticipated weather patterns, and potential air and water quality issues.

For U.S. canola production, conservation tillage and no-till are most commonly used (Gusta et al. 2011; Awada et al. 2014). Tillage practices in North Dakota are estimated to comprise around 75% no-till and 25% conventional till (S&T 2010). A survey of 571 GE canola farmers in Canada found that, likewise, many producers have moved to minimum or zero-tillage operations (Gusta et al. 2011), with more than half of those surveyed indicating they no longer use tillage in their cropping system.

3.3.2 Agronomic Inputs

3.3.2.1 Fertilizers

Fertilizers are necessary for good yield and product quality in some areas, but can present a problem when run-off carries these nutrients into surface waters such as rivers and lakes. Canola producers commonly apply nitrogen (N), phosphorus (P), sulfur (S), and potassium (K) before or at planting. Canola N, P, and K requirements are similar to those of small grains, although canola's S requirements are higher than most crops (Franzen and Lukach 2016). Fertilizer application rates vary depending on the region and inherent soil characteristics, and depend on the potential yield per acre of a given canola cultivar; the higher the yield potential, the greater the need for sufficient nutrients. For instance, the yield potential of winter canola is higher than spring canola, so soil fertility requirements are higher (KSU 2012).

3.3.2.2 Pesticides: Insecticides and Fungicides

Canola is subject to damage by a variety insects throughout its developmental stages with several species (i.e., beetles, moth larvae, midges) potentially feeding on the seeds, roots, stalk, leaf, or seed pod. In North America, the crucifer flea beetle (*Phyllotreta cruciferae*) and striped flea beetle (*Phyllotreta striolata*) are the most significant insect pests of canola (NDSU 2011; KSU 2012). Other insects that feed on canola include stinkbugs, cutworms, diamondback moths, root maggots, aphids, armyworms, and grasshoppers (Armstrong et al. 2012; Saroka et al. 2015; Alahakoon et al. 2016; Sekulic and Rempel 2016). Important canola diseases include the fungal diseases blackleg (*Leptosphaeria maculans*) and sclerotinia stem rot (*Schlerotina sclerotiorum*), and the protist disease clubfoot (*Plasmodiophora brassicae*) (NDSU 2011).

In many regions where canola is grown it is economically beneficial to use seeds treated with insecticides to prevent flea beetle damage, and fungicides to help control seed-borne blackleg disease, sclerotinia stem rot, and damping-off (wirestem) (Brown et al. 2008), and Strobilurin is used to control pathogenic fungi such as mildews, molds, and rusts (US-EPA 2016a). Triazole products such as ipconazole and metconazole are used as soil and foliar fungicides and fungicides containing cyazofamid are commonly used to control other plant disease in canola (US-EPA 2004, 2014, 2015b).

Neonicotinoid insecticides (NNI) are nicotine-based compounds that are applied to seeds or soils which provide extended protection to crops during the early growth stages (Douglas and Tooker 2015; Sekulic and Rempel 2016). Due to their efficacy in the protection against major insect pests, such as flea beetles, NNI seed treatments are commonly used in canola production in Western Canada (Sekulic and Rempel 2016) and there is an increased use in the United States since 2003 (Douglas and Tooker 2015). The use of NNI can reduce the number of foliar insecticide applications required each season. In general, foliar applications are at much greater per-acre application rates and may pose more hazards to non-target organisms than soil or seed treatments (Sekulic and Rempel 2016).

There is, however, some evidence that NNIs can adversely affect certain sensitive bird species (Gibbons et al. 2015), particularly smaller species such as house sparrows (*Passer domesticus*) and canaries (*Serinus canaria*). The U.S. EPA reported that the NNI clothianidin, when used to treat canola, could reduce the survival of certain small birds (DeCant and Barrett 2010; Gibbons et al. 2015). A review by Mineau and Palmer suggests that the risks of acute intoxication with imidacloprid (also a NNI) applied on oilseeds or cereals are such that birds need only ingest a small amount of treated seeds (Mineau and Palmer 2013). Incidents of bird poisoning by imidacloprid-treated seed have been documented, suggesting that NNI treated seeds can present risks to certain avian species (Gibbons et al. 2015).

NNIs are also potentially harmful to pollinator species such as honey bees (Douglas and Tooker 2015; Sekulic and Rempel 2016). In June 2014, the White House issued a memorandum establishing a Pollinator Health Task Force, co-chaired by the USDA and the U.S. EPA, to create a National Pollinator Health Strategy that promotes the health of honey bees and other pollinators, such as birds, bats, butterflies, and insects. In January 2017, the U.S. EPA issued preliminary pollinator-only risk assessments for the neonicotinoid insecticides clothianidin, thiamethoxam, and dinotefuran and an update to its preliminary risk assessment for imidacloprid (US-EPA 2017a). The U.S. EPA also issued final policy that describes methods for addressing acute risks to bees from pesticides (US-EPA 2017j). Applications of acutely toxic pesticides would be prohibited under certain conditions when bees are most likely to be present. While the restrictions focus on managed bees, the U.S. EPA believes that these measures will also protect native bees and other pollinators that are in and around treatment areas. New label language is expected to protect managed bees under contract to provide crop pollination services.

3.3.2.3 Pesticides: Herbicides

Weed management is essential for obtaining optimal yield and net returns in canola production (CCoC 2016b). Weeds are highly competitive and use up resources — soil moisture, nutrients, access to sunlight — that would otherwise be available to the crop plant. Yield loss from weed competition can be significant. Weeds can also:

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

The most common weeds in the major canola production areas of North Dakota include those listed in Table 3-5.

Table 3-5. Agricultural Weeds in North Dakota

barnyardgrass	mallow, common	quackgrass
buckwheat, wild	marshelder	ragweed, common
cocklebur, common	mustard, wild	smartweed, annual
foxtail, green	mustard, annual	sunflower
foxtail, yellow	nightshade, black	thistle, Canada
horseweed (marestail)	nightshade, hairy	thistle, Russian
kochia	pigweed, redroot	volunteer cereals (wheat, barley)
lambsquarters	pigweed/waterhemp	wild oat
lanceleaf sage	prickly lettuce	wormwood, biennial

Source: (NDSU 2011)

A combination of preventive, cultural, mechanical, biological, and chemical methods are recommended for effective weed management. The coordinated use of these methods is termed integrated weed management (IWM). Because herbicides are effective, and because weeds can cause severe crop losses, they remain the most commonly used among management tools. In the United States, herbicides accounted for 57% of all pesticide uses, and 58% of pesticide expenditures, costing farmers roughly $5.1 billion in 2012 (Atwood and Paisley-Jones 2017). One of the advantages of herbicides is that they allow crops to be planted with less tillage (Lingenfelter 2018). Due to reduced tillage, soil erosion is reduced, limiting soil and agricultural run-off from entering waterways and decreasing the quality of the nation's surface waters (see 3.4.2 – Water Resources). Without herbicide use, no-till agriculture is not possible. Herbicides registered by the U.S. EPA for use on canola and their mode of action (MOA) are summarized in Table 3-6.

Table 3-6. U.S. EPA Registered Herbicides for Use on Canola

Herbicide (Trade Name)	Application	Weeds	Mode of Action
Ethalfluralin (Sonalan®)	Preplant incorporated	Annual broadleaf, foxtail, barnyard grass	Microtubule inhibitor
Trifluralin (Treflan™)	Preplant incorporated	Annual broadleaf, foxtail, barnyard grass	Microtubule inhibitor
Clopyralid (Stinger®)	Foliar spray at 2 to 6 leaf stage	Annual and perennial broadleaf	Synthetic auxins (plant growth hormone)
Quizalofop (**Assure® II**)	Foliar spray	Annual grasses	Inhibition of acetyl CoA carboxylase (ACCase)
Sethoxydim (Poast®)	Foliar spray	Annual grasses	Inhibition of acetyl CoA carboxylase (ACCase)
Clethodim (Select®)	Foliar prior to bolting	Annual grasses	Inhibition of acetyl CoA carboxylase (ACCase)
Glufosinate - for LibertyLink® Cultivars	Foliar until early plant bolting	Annual broadleaf and grasses	Glutamine synthase inhibitor
Glyphopsate - for RoundupReady® cultivars	Foliar from seed emergence to plant bolting	Annual broadleaf and grasses	EPSP synthase inhibitor
Imazamox (Beyond®) - for imazamox tolerant cultivars	Apply foliar from seedling emergence to full bloom	Many annual broadleafs and grasses	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)

Source: (Brown et al. 2008)

3.3.2.3.1 Herbicide Resistant Weed Development and Management

While the importance of herbicides in commercial crop production is well recognized, herbicide use can also presents some risks that include potential environmental, ecological, and human health effects. In terms of ecological effects, various weeds can be inherently (naturally) resistant to an herbicide active ingredient (a.i.), or rather, its MOA (Owen 2011; Owen 2012; Vencill et al. 2012). Herbicides impart selection pressures on these types of plant populations resulting in survival of those plants resistant to one or more active ingredients in an herbicide. Weed populations can also develop “evolved” resistance to an herbicide a.i., adaptation of the weed to an external chemical stressor. Over-reliance on herbicides for weed control and problems with development of herbicide resistant weeds has sparked debate on how to best incorporate herbicides into sustainable cropping systems (Mortensen et al. 2012; Vencill et al. 2012; Duke 2015).

Herbicide resistant weed populations naturally emerge in fields when the resistant individuals survive and reproduce after repeated exposure to an herbicide, passing the inherent (non-GE) herbicide resistant trait on to their progeny. Over time this lack of “herbicide diversity” can result in the development of herbicide resistant weed populations that are resistance to one, two, or more herbicide active MOAs (Kniss 2017). When HR weeds are present, weed control practices that once worked can begin to fail.

In terms of evolved resistance, an herbicide a.i. can promote the evolution of resistance mechanisms that are either involved in the herbicide MOA (target site resistance), or not (non-target site resistance) (Rey-Caballero et al. 2017). These types of evolved resistance emerge from mutations in genes/proteins involved the herbicide MOA – target site resistance (e.g, (Yang et al. 2016)) or mutations that confer resistance to the herbicide a.i. (e.g., (Yang et al. 2016; Rey-Caballero et al. 2017)). For example, certain non-target based resistance is the result of the weeds ability to rapidly metabolize the herbicide a.i. (Christopher et al. 1991). It should be noted that these are adaptive responses, mutations in the weed genome, these are not the result of gene flow from GE crop plants to weeds. APHIS is not aware of any GE HR trait being passed from a GE crop plant to a non-crop plant, resulting in the establishment of an HR weed population.

The development of herbicide resistance weeds is not a recent phenomenon nor is it unique to GE crops. Herbicide resistant weed populations have been evolving (selected for) since the advent and widespread use of chemical herbicides in the 1950s. Overreliance and repeated use of an herbicide with a single herbicide MOA can place significant selection pressure on weed populations. When only one herbicide MOA is consistently used year after year as the primary means of weed control, the weed population selected will be for those inherently resistant to the herbicide MOA, or those that have evolved resistance. This type of selection pressure has resulted in the emergence of numerous HR weed populations in the United States, and worldwide (Wilson et al. 2009; Shaw et al. 2011; Vencill et al. 2012). HR weeds are becoming increasingly common in the United States and a primary concern for crop producers.

In theory, control methods that result in total weed population mortality do not exert a selection pressure because there are no weeds that survive and reproduce. However, 100% control is rarely achieved in the field. Herbicide resistant weed populations can become ever more prevalent year-to-year as HR weeds differentially survive and reproduce and non-resistant weed populations are suppressed. It should be noted that herbicide resistant weeds may also be transported and spread among fields, for example, as seeds hitchhiking on farm equipment.

Table 3-7 summarizes resistant weeds in the primary canola production area of North Dakota. As evident, numerous weed populations are resistant to ALS inhibitors and EPSPS inhibitors, with significantly fewer resistant to mitotic inhibitors, growth regulators, and photosystem II inhibitors. There are 34 other states with commercial canola farms (USDA-NASS 2014b). Each of these other states will likewise have populations of HR weeds unique to the area, and particular type of crop production system.

While development and management of HR weeds populations are a concern for many crop producers, to date, HR weeds in canola have not emerged as a significant problem in the United States. No HR weeds have been reported for canola crops in North Dakota. During the last 15 years, the only reports for U.S. canola crops have been HR Italian ryegrass in Idaho and HR green foxtail in Montana – both of these reported in 2005 (Heap, 2017). However, herbicide resistant biotypes of green foxtail, wild oat, wild mustard, false cleavers, field pennycress, and kochia have been reported in neighboring canola croplands in Canada (Heap, 2017).

Table 3-7. Herbicide Resistant Weeds in North Dakota

	ACCase inhibitors	ALS inhibitors	Mitotic inhibitors	Growth regulators	Photosystem II inhibitors	EPSPS inhibitor (glyphosate)
HR Weed	**Mode of Action**					
Wild Oat *	X	X				
Green Foxtail	X		X			
Kochia *		X		X	X	X
Waterhemp *		X				X
Common ragweed *		X				X
Marshelder		X				
Wild mustard		X				
Black nightshade		X				
Redroot pigweed						
Horeseweed						X
Lambsquarters						X

Source: (NDSU 2016)

* Denotes multiple resistance – weeds resistant to two or more herbicide MOAs.

Strategies for managing and avoiding the development of HR weeds populations in U.S. agriculture are continually being refined. The majority of crop producers using herbicides, including canola producers, employ IWM strategies to address HR weed management concerns, practices recommended by the crop protection and seed industries, the USDA, university extension services, the U.S. EPA, state departments of agriculture, the Weed Science Society of America (WSSA), and others (WSSA 2016b).

In 2017, the U.S. EPA issued PR Notice 2017-2, *Guidance for Herbicide-Resistance Management, Labeling, Education, Training and Stewardship* (US-EPA 2017c), which provides registrants and growers detailed information on slowing the development and spread of herbicide resistant weeds. The U.S. EPA guidance is part of a more holistic, proactive approach involving crop consultants, agricultural commodity organizations, professional /scientific societies, researchers, and the pesticide registrants themselves.

3.3.3 Pest and Pathogen Resistance Management

As with most all crops, a key strategy for controlling pests and disease is the use of canola cultivars with resistance to the pest or pathogen, as resistant cultivars are the most effective means for reducing crop losses. Various canola cultivars have been developed through traditional breeding that are resistant to common pathogens such as sclerotinia and clubroot disease (BrettYoung 2017). DHA canola was derived from the parent line AV Jade canola, which was bred for resistance to blackleg disease.

In addition to the use of disease resistant canola cultivars, as discussed above, canola producers use insecticides and fungicides to control pests and disease (NDSU 2011; KSU 2012). Concomitant with the increased acreage of canola over the last 20 years, and often shorter crop rotations, pesticide resistant strains of *Leptosphaeria maculans* (blackleg disease) have evolved. Because resistance among *Schlerotina sclerotiorum* and *Plasmodiophora* (clubfoot) may likewise evolve, plant breeders continually strive to develop new canola varieties resistant to potential pathogen variants (Minogue 2016).

In 2017, the U.S. EPA issued PRN 2017-1, *Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling* (US-EPA 2017e). PRN 2017-1 revises and updates PRN 2001-5, and applies to all conventional pesticides (i.e., fungicides, bactericides, insecticides, and acaricides). The guidance is intended to provide:

- additional guidance for resistance management on pesticide labels;
- references to external technical resources for guidance on resistance management; and
- updated instructions on how to submit changes to existing labels in order to enhance resistance-management language.

In the U.S. EPA's *Guidance on FIFRA Section 6(a)(2) Regulations for Pesticide Product Registrants*, any substantiated incidents of pest resistance for any regulated pesticide product must be reported to the U.S. EPA.[12] This reporting requirement is in accordance with FIFRA Adverse Effects Reporting Section 6(a)(2), which requires pesticide product registrants to submit adverse-effects information about their products to the U.S. EPA.

3.3.4 Volunteer Management

Seeds from previous crops that have been left on the soil may emerge voluntarily in subsequent crops. Volunteer crop plants can reduce crop yield, act as hosts for plant pests and pathogens, and generally are considered weeds (DuPont-Pioneer 2015; CCoC 2016b). Volunteers are common among many cropping systems (e.g., corn, wheat, soybean, canola) and are controlled primarily with herbicides, when they occur. For canola, due to seed number and size (1 to 2.5 mm in diameter, 3 to 6 grams per 1,000 seeds) seed loss at harvest can be 10% or more of the seeds produced (Gulden 2007; Fleury 2015). With the expansion of canola production and acreage in the United States and Canada, particularly herbicide resistant varieties that comprise around 90% or more of the canola acreage in both countries, and the fact that canola requires rotation with other crops for successful production, volunteer management has emerged as a frequently required component of canola production (e.g., see (Gulden et al. 2003; DuPont-Pioneer 2015; CCoC 2016b)). Because there can be multiple flushes of volunteers during the growing season, volunteer canola control may require the use of herbicides with residual activity, and/or multiple applications of an herbicide or herbicides without residual activity to provide season-long control (Gulden 2007; Fleury 2015). The importance of volunteer management in canola is further discussed in Section 3.5.3 – Gene Flow and Weediness of Canola.

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[12] See https://www.epa.gov/pesticide-registration/prn-98-3-guidance-final-fifra-6a2-regulations-pesticide-product-registrants

3.3.5 Pesticide Toxicity

While many of the more problematic pesticides have been removed from the commercial market the potential unintended risks pesticides can present to human health and wildlife remain a concern for many citizens, as well as federal and state regulatory agencies. The U.S. EPA evaluates the potential acute and chronic toxicity of a pesticide active ingredient via human health and environmental risks assessments. These assessment are conducted prior to a pesticide registration and inform the U.S. EPA label use requirements (US-EPA 2018a). Adherence to the U.S. EPA pesticide label instruction is not only a legal requirement, it is fundamental to the safe use of the product. Pesticides can be hazardous if the recommended safe dose, duration, and frequency of exposure is exceeded. Information on the potential acute and chronic toxicity of each pesticide on the market is publicly available from various online resources, such as: (1) the U.S. EPA's ecotoxicology knowledgebase (ECOTOX), which provides single chemical environmental toxicity data on aquatic life, terrestrial plants, and wildlife (US-EPA 2016b); (2) the Pesticide Action Network (PAN 2018); (3) the Integrated Risk Information System (US-EPA 2018b); (4) Toxicology Data Network (US-NIH 2018);and, (5) Agency for Toxic Substances and Disease Registry (ATSDR 2018).

When used according to U.S. EPA label requirements, as required by law, pesticides are not considered a significant risk to the environment or human health. However, depending on the active ingredient and its toxicity, pesticides can present risks to pesticide applicators. Use of pesticides on canola crops is regulated by the U.S. EPA, as further described in subsection 3.6 – Human Health.

3.4 Physical Environment

3.4.1 Soils

In an agricultural setting, concerns regarding soils are the potential for agronomic practices and inputs to affect soil fertility; erosional capacity; off-site transport of sediments, pesticides, and fertilizers; and disturbance of soil biodiversity. Tillage, cover crops, crop rotation, and pesticide and fertilizer inputs can influence the biological, physical, and chemical properties of soil, which in turn can affect fertility, crop yield potential, and soil erosional capacity (Baumhardt et al. 2015). While soil erosion occurs through natural processes, the rates of which are determined by soil type, local ecology, and weather, tillage is the primary practice that can facilitate topsoil loss via wind and water erosion; a process that can take centuries to reverse. 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, Southern High Plains of Texas, and Northern Plain States, to include certain areas of North Dakota where canola production is concentrated (Figure 3-5).

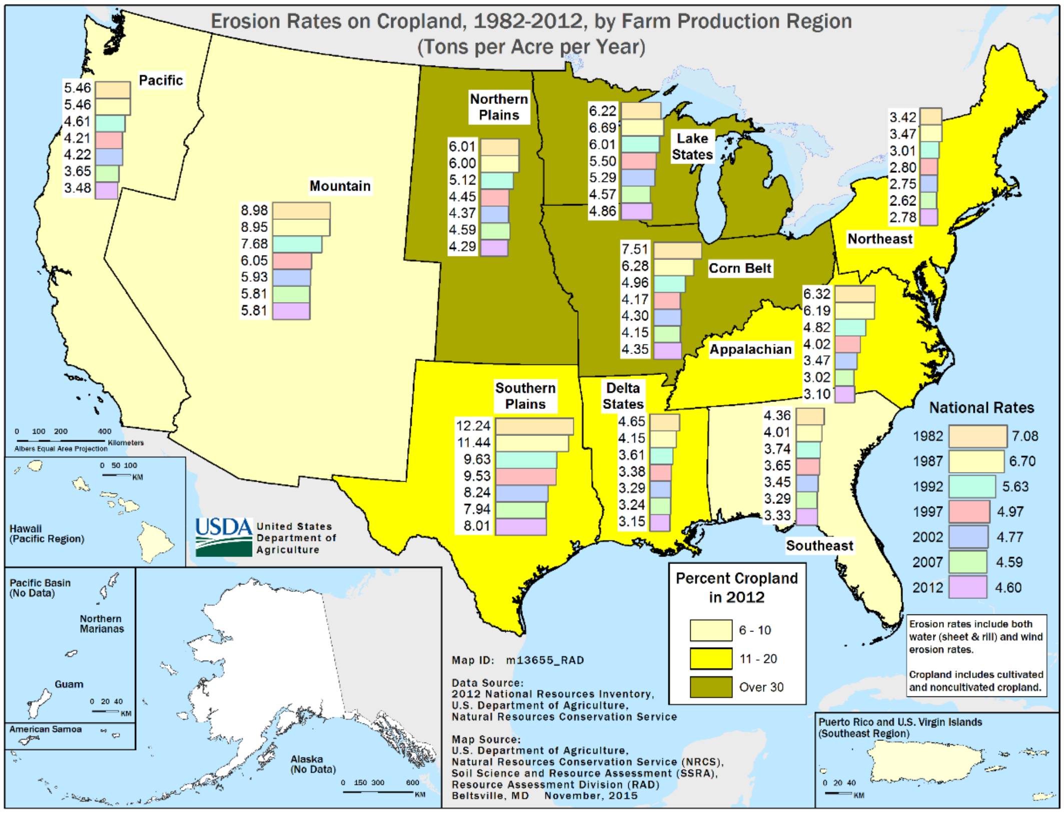

Figure 3-5. Locations and Status of U.S. Croplands Subject to Erosion
Source: (USDA-NRCS 2018)

Since 1985, conservation programs have specifically targeted highly erodible lands in the United States. As part of these efforts use of conservation tillage on U.S. cropland increased from around 16% in 1979 to about 36% in 1996. As of 2011 (latest data), around 40% of cropland, on average, was under conservation tillage (USDA-NASS 2014a; Wade et al. 2015). No-till/strip-till was used on 39% of total acreage in major crops, including 31% of corn, 46% of soybeans, 33% of cotton, and 43% of wheat (Wade et al. 2015). Roughly 23% of land in corn, soybeans, wheat, and cotton was on a farm where no-till or strip-till was used on every acre (full adopters) (Wade et al. 2015). About 56% of cropland used for corn, soybeans, wheat, and cotton was under no-till/strip-till on at least part of the cropland in 2011, 23% of cropland was on farms that used no-till/strip-till on all cropland, and 33% was on farms that used a mix of no-till, strip-till, and other tillage practices (Wade et al. 2015).

As conservation tillage and no-till practices increased, total soil loss on erodible croplands in the United States decreased. Soil erosion on cropland decreased 44% between 1982 and 2012. Water (sheet and rill) erosion declined from 1.59 billion tons per year to 0.96 billion tons per year, and erosion due to wind decreased from 1.38 billion tons per year to 0.71 billion tons per year, over the same time period (USDA-NRCS 2015).

As discussed in Section 3.3 – Agronomic Practices, canola farmers largely use minimum or no-till systems, which provide sufficient levels of weed control in canola crops (Beckie H.J et al.

2011; Gusta et al. 2011). In general, as of 2012, oilseed and grain farming accounted for 84% of the no-till acres and 81% of conservation tillage acres (USDA-NASS 2014a). In North Dakota, tillage practices used in canola production are estimated to be around 75% no-till and 25% conventional tillage (S&T 2010). In Canada, over 50% of farmers use no-till practices in GE HR canola production. The current reduced and no-till practices that are predominant in U.S. and Canadian canola production are considered beneficial to cropland soils where canola is cultivated, limiting the impacts of canola production on soil erosion and soil quality in these areas. Beneficial in this context meaning relative to conventional tillage.

3.4.2 Water Resources

Crop Irrigation

Few canola farms employ irrigation. In 2012, 133 out of 3,995 canola producers (0.03%) irrigated their crops, which comprised a total of 26,894 out of 1.7 million planted acres (USDA-NASS 2014b). Canola needs about 18 to 22 inches of water through its growing season to produce good yields, with water use varying from a low of 0.1 inch per day at the rosette stage to a peak of 0.3 inch per day during flowering (Herbek et al. 1992). This puts canola water use in the range of most grain crops. The Northern Plain states of North Dakota and Montana, where most canola is grown, generally average around 10-24 inches of annual precipitation. Canola is not considered a reliable crop with less than 8 inches (250 mm) of plant available water (George et al. 2015). To achieve high yields, some supplemental irrigation may be required during prolonged dry spells or in areas that receive limited annual precipitation. Canola is however sensitive to waterlogging and excess soil moisture can cause crop lodging (bending over of the stems near the ground level in grain crops), which makes them difficult to harvest and can reduce yield. The timing and amount of irrigation therefore needs to be well managed to avoid this problem (George et al. 2015).

Water Quality

Tillage and agronomic inputs can potentially lead to the impairment of surface waters through soil erosion and run-off, and impairment of groundwater through the leaching of pesticides and fertilizers. Agricultural run-off, which can be a problem for all crops, is a primary source of non-point source (NPS) contaminants that can impact surface waters such as rivers and lakes, and is the third most noted cause of impairment to estuaries (US-EPA 2015d, 2017h). The most common NPS contaminants in agricultural run-off are sediment, nutrients such as nitrogen and phosphorus, and pesticides; all of which can adversely affect aquatic ecosystems. For rivers and streams, the U.S. EPA lists sediments as the second most frequent cause of impairment of streams and rivers, nutrients third, and pesticides sixteenth (US-EPA 2017g). For lakes, reservoirs, and ponds, nutrients are second, sediments twelfth, and pesticides thirteenth (US-EPA 2017g). In North Dakota specifically, where the majority of canola production occurs, for rivers and streams, sedimentation is listed as fourth and nutrients as the fifteenth leading cause of water quality impairment; pesticides are not listed (US-EPA 2017f). For lakes, reservoirs, and ponds, nutrients are second, and sediments the fourth leading cause of impairment (US-EPA 2017f). In general, sediment and nutrient loading are the principal NPS concerns in crop production, to include canola, although pesticides will always remain a monitored agronomic input due to their potential to adversely affect both aquatic and terrestrial biota.

Due to the potential impacts of agriculture on water resources, various national and regional efforts are underway to reduce NPS contaminants in agricultural run-off, and run-off itself, such as the U.S. EPA's Mississippi River/Gulf of Mexico Hypoxia Task Force (US-EPA 2017h) and USDA-NRCS National Water Quality Initiative (NWQI) (USDA-NRCS 2017). For example, through the NWQI, the NRCS and partners (e.g., local and state agencies, nongovernmental organizations) work with producers and landowners to implement voluntary conservation practices that improve water quality in high-priority watersheds, while maintaining agricultural productivity.

3.4.3 Air Quality

The U.S. EPA establishes National Ambient Air Quality Standards (NAAQSs) pursuant to the Clean Air Act (CAA) that are intended to protect public health and the environment. 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). States enforce the NAAQS through creation of state implementation plans (SIPs), which are designed to achieve NAAQS.

Some canola crop production practices can generate NAAQS pollutants and may contribute to challenges in maintaining regional NAAQS. Agricultural emission sources associated with canola production include fossil fuels used with farm equipment (e.g., pesticide application, harvest, tillage); soil particulates from tillage (PM); and pesticide volatilization or drift (Aneja et al. 2009; US-EPA 2013).

Spray drift, and volatilization of pesticides from soil and plant surfaces, can result in the introduction of constituent chemicals into the air. Volatilization is dependent on pesticide chemistry, soil wetness, and temperature. Drift is dependent on wind conditions and applicator practices, to include application equipment features such as nozzle size. 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.

While the U.S. EPA establishes NAAQS, the standards do not set emission control requirements for any particular industry, including agriculture. The USDA and the U.S. 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 U.S. EPA and USDA provide guidance to the agriculture sector for limiting NAAQS emissions. The USDA Environmental Quality Incentives Program Air Quality Initiative provides financial and technical assistance to help farmers and ranchers limit air pollution. The U.S. EPA has developed USDA-approved measures to help manage air emissions from cropping systems to help satisfy SIP requirements. The U.S. 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 may be implemented to limit emissions.

The U.S. EPA's Office of Pesticide Programs, which regulates the use of pesticides, introduced initiatives to help pesticide applicators minimize off-target pesticide drift. The U.S. EPA's voluntary Drift Reduction Technology Program was developed to encourage the manufacture, marketing, and use of spray technologies that reduce pesticide drift. The U.S. EPA is also working with pesticide manufacturers through the registration and registration review programs on improvements to pesticide label instructions to reduce drift and volatilization (e.g., see (US-EPA 2015c).

3.5 Biological Resources

3.5.1 Soil Biota

Soil biota (i.e., earthworms, nematodes, fungi, bacteria) play key roles in soil formation, structure, organic matter content, biodegradation of pesticides, nutrient cycling, suppression of plant pathogens, promotion of plant growth, and a wide range of biochemical soil processes (Parikh and James 2012). Some soil based microorganisms are plant pathogens and can cause plant diseases that can result in substantial yield and economic losses. For canola, these include various fungal, bacterial, and viral plant pathogens (CCoC 2016a).

The main factors affecting soil biota populations and diversity are soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), plant type (that provides specific carbon and energy sources into the soil), and agricultural management practices, such as crop rotation, tillage, pesticide and fertilizer application, and irrigation (Garbeva et al. 2004; Gupta et al. 2007). Climate, particularly the water and heat content of soil, is a principal determinant of soil biological activity. Pesticides used on canola crops can, relative to the dose, duration, and frequency of exposure, potentially impact soil communities, and are required by law to be used according U.S. EPA label requirements.

Certain crop and soil management practices, such conservation tillage, cover cropping, and crop rotation increase soil organic matter and plant residues, and impart attributes to soil that can enhance pesticide degradation, hinder pesticide movement, and facilitate the natural cycles of soil nutrients.

3.5.2 Animal and Plant Communities

3.5.2.1 Animals

While the species of animals found in and around commercial crop fields are typically less diverse as compared to non-cropland areas, canola fields do provide food and habitat for some species of wildlife, primarily birds and large and small mammals. Geese and blackbirds, for example, feed on canola seeds (Boyles et al. 2012; Schillinger and Werner 2016). Horned larks feed on the cotyledons of emerging canola, but they typically do not eat the stem or seed (Schillinger and Werner 2016). Most animals that use canola fields are ground-foraging omnivores that feed on the remaining plant matter following harvest. Small mammals of the Great Plains that may be associated with canola fields include sagebrush voles (*Lemmiscus curtatus*), meadow voles (*Microtus pennsylvanicus*), shrews (*Blarina brevicauda*), and deer mice (*Peromyscus maniculatus*) (Heisler et al. 2013; Heisler et al. 2014). It is likely that predators (e.g., raptors, reptiles) of small mammals utilize canola fields and surrounding areas as hunting grounds. Large mammals such as white-tailed deer, mule deer, moose, and elk and smaller mammals like coyotes, foxes, rabbits, and prairie dogs are common in North Dakota and may transit canola fields (NDGFD 2017).

Although many arthropods are considered pests in crop fields, there are beneficial arthropods which are natural enemies of both weeds and insect 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 the predatory mite (*Phytoseiulus persimilis*) (Landis et al. 2005; Shelton 2011). Many insect and related arthropod species perform valuable functions; they pollinate plants, contribute to the decay and processing of organic matter, reduce weed seed populations through predation, cycle soil nutrients, and attack other insects and mites that are considered plant pests. Common pollinators attracted to canola include honey, bumble, and leafcutter bees (Nichols and Altieri 2012; Kamel et al. 2015; Sekulic and Rempel 2016). The yellow coloration, and visible nectar of canola flowers also attracts pollinators such as butterflies (Kamel et al. 2015; Sekulic and Rempel 2016).

3.5.2.2 Vegetation Associated with Canola Fields

Canola crops are generally bordered by other canola or other crops, woodlands, rangelands, pasture, or grassland areas. Plant communities among these varied habitats may be impacted by agricultural operations, both beneficially and adversely. Fertilizers and water may run-off into adjacent areas, serving as nutrients for flora outside the field margins. Herbicides can potentially drift if sprayed and damage flora in the vicinity of the crop.

Plant diversity is an important component of a sustainable agricultural system (Scherr and McNeely 2008; CBD 2015b), and hedgerows, woodlands, fields, and other surrounding habitat serve as important reservoirs for beneficial insects and other animals. By providing habitats, pollen and nectar resources, and serving as hosts, plants adjacent to canola fields help support a suite of beneficial arthropod species, including pollinators and biological control agents that prey on agricultural plant pests (Scherr and McNeely 2008; Nichols and Altieri 2012). Surrounding plant communities can also help regulate runoff, reduce soil erosion, and improve water quality (Egan et al. 2014a). In general, surrounding habitat and plant communities provide invaluable ecosystem services such as pollination, pest control, and control of run-off.

Declining plant diversity in agroecosystems has often been attributed to use of herbicides; they are among those most often implicated in drift complaints – situations where herbicides float off-site and cause unintended harm to sensitive plant species in areas adjacent to crops. All herbicides have some degree of environmental mobility, and vegetation outside of the treated crop can be exposed through a variety of mechanisms, including spray drift, volatilization, surface and subsurface water flow, and deposition in rainfall (Egan et al. 2014a). Given these diverse routes of exposure, it is likely that plants growing in habitats adjacent to crops routinely experience contact with a variety of herbicides at a range of phytotoxically active doses (Egan et al. 2014a). The structure and function of plant and associated arthropod communities are nuanced and will depend on species composition, successional patterns, and to some degree the timing of herbicide exposure.

These factors considered, recent studies have found that herbicides alone are not the causative factor in shaping plant communities proximate to crops. Rather, for the purposes of conserving plant species diversity in agricultural landscape, other strategies like preserving habitats such as woodlots, pastures, and riparian buffers may be more effective than reducing herbicide use (Egan et al. 2014a). While herbicides will continue to play a fundamental role in weed management programs and can affect surrounding vegetation, how surrounding habitats are managed (Egan et al. 2014b) likewise determines the diversity of plants, pollinators, and natural predators of plant pests (Nichols and Altieri 2012; Egan et al. 2014b).

3.5.3 Gene Flow and Weediness of Canola

3.5.3.1 GE Canola as a Weed & Volunteer Canola

Potential concerns regarding GE canola include naturalization of the plant, and the transfer of trait genes to sexually compatible relatives through hybridization and introgression (Schafer et al. 2011). Incorporation of DHA canola trait genes into populations of sexually compatible wild relative species via hybridization, and particularly introgression into the genome of wild populations, could present an ecological concern, as well as an economic concern to producers of canola crops (Knispel and McLachlan 2010). GE canola can present as a volunteer in subsequent crops, form feral populations, and hybridize with wild relative species, either through seed dispersal during harvest or transport, or pollen flow from GE canola fields.

While GE canola can present as a volunteer and hybridize with wild relative species, *Brassica napus* L., is not a federally or state listed weed in the United States (USDA-NRCS 2016). *Brassica* spp. is listed on the Michigan weed list, but not specifically cultivated canola (*B. napus*). It should be noted that currently, in the United States, *B. napus*, *B. rapa*, *B. nigr*a, *B. juncea*, *B. adpressa* and *R. raphanistrum* are listed as weeds by the Weed Science Society of America (WSSA 2016a).

Volunteer canola (Section 3.3.4) can serve as a source of gene flow in subsequent canola crops (Gulden 2007; Fleury 2015), and potentially to sexually compatible wild relative species. The primary factors contributing to volunteer canola are seed loss at harvest and insufficient intervals between crop rotations. Relative to other crops, canola seeds are small, some 2 millimeters (0.08 inches) in diameter, and in conjunction with high seed losses at harvest (3% to 10%) can result in large seedbank inputs, around 3,000 seeds/m^2; many times the normal seeding rate for canola (Gulden 2007). In general, around 40% – 45% of canola seed in the soil seedbank will persist for one winter, 1.4 % for two winters, and less than 0.5% for three winters (Gulden et al. 2003; Gulden 2007). While the percentage of seed persistence declines rapidly over time, due to the sheer numbers of seeds deposited (3,000/m^2), even low soil seedbank persistence can potentially lead to a high number of volunteers in subsequent years (Gulden 2007; Bailleul et al. 2016). Canola seeds can also develop secondary dormancy under sub-optimal germination conditions (i.e., water stress, heat, hypoxia), which can lead to persistence in the soil seedbank for several years (Gulden 2007). Dormancy is removed by a complex of environmental conditions including short exposure to cool temperatures (35-39° F)(Gulden 2007). These factors, collectively, can contribute to high levels of soil seedbank persistence, and the presence of volunteer canola populations for several years after the last canola crop was grown (Gulden 2007).

Crop rotations on a 4 to 5 year cycle are now widely recommended for management of volunteer canola, as volunteer canola is more often a problem in tight canola rotations, which can exacerbate seedbank replenishment (Gulden 2007; Fleury 2015; CCoC 2016b). The tighter the canola rotation, the more difficult eradication or minimizing seedbank replenishment will be. Crop rotations on a 4 to 5 year cycle can reduce the incidence and prevalence of volunteers (Gulden 2007; Fleury 2015). Avoiding tillage is also recommended. Tillage can bury seed, which can facilitate seedbank persistence for several years (Gulden 2007). Cultural practices such as reducing seed loss at harvest, sufficient intervals in crop rotations, and tillage, are critical to limiting seedbank inputs and minimizing volunteer plants.

Various herbicide regimes are used to manage volunteer canola (Gulden 2007; CCoC 2016b). Volunteer canola control can require herbicides with residual activity, or multiple applications of herbicide without residual activity, as there can often be multiple flushes of volunteers during the growing season (Gulden 2007; Fleury 2015). For control of volunteer canola, both a pre-seed and in-crop treatment can be required. Scouting and early identification of volunteers is critical as herbicides are most effective at the early stages of growth, generally at the 3 leaf stage or less (Gulden 2007).

3.5.3.2 Hybridization and Introgression among Brassica and Related Species

Hybridization can occur between two subspecies (intraspecific), two different species (interspecific), and two different genera (intergeneric). While rare, interfamilial hybrids have also been known to occur. For a trait to become incorporated into a species genome (introgression), survival and recurrent backcrossing of hybrids with parental species is necessary (OECD 2006). In the absence of introgression, hybrids may persist for many generations, contributing to gene flow among populations of sexually compatible plants.

Gene flow among GE canola populations and plants among *Brassica* and other genera has been fairly well studied. However, as noted by many investigators, hybridization and introgression among *Brassica* and related genera can be somewhat complex due to the various species and subspecies involved, and environmental factors governing hybridization (e.g., see (FitzJohn et al. 2007; Ellstrand et al. 2013; Harrison and Larson 2014)). Provided here is a synopsis of the sexual compatibility of canola (*B. napus*) and related species, and the propensity for hybridization of *B. napus* with other species. A more thorough discussion on this topic can be found in the literature cited in this section. *Brassica napus* plants readily outcross with plants of the same species, and potentially with the related species listed in Table 3-8.

Table 3-8. Outcrossing Potential of *B. napus* with Related Species in the United States

Genus/Species [a]	**Crop**	**Weed** [b]	**Hand Pollination (successes:failures)** [c]		**Spontaneous and Natural Hybridization** [c]		**Presence in Winter Canola Growing Areas** [d]	**Presence in Spring Canola Growing Areas** [d]
			B.napus **as Male**	***B.napus*** **as Female**	***B.napus*** **as Male**	***B.napus*** **as Female**		
Brassica carinata	Y	X	4:1	7:0				
Brassica elongata		Y					Y	Y
Brassica fruticulosa		Y	0:1	1:1			Y	X
Brassica juncea	Y	Y	25:1	13:4	Y	Y	Y	Y
Brassica nigra	Y	Y	2:2	4:2	X	X	Y	Y
Brassica oleracea	Y	X	3:11	9:17	Y	X	Y	Y
Brassica rapa	Y	Y	55:8	84:0	Y	Y	Y	Y
Brassica tournefortii		Y	0:1	1:1			Y	X
Camelina sativa	Y	X	0:1	0:1			Y	Y
Capsella bursa- pastoris		Y	0:1	0:1			Y	Y
Coincya monensis		Y					Y	Y
Conringia orientalis		Y	0:1	0:1			Y	Y
Diplotaxis erucoides		X	1:1				Y	Y

Table 3-8. Outcrossing Potential of *B. napus* with Related Species in the United States

Diplotaxis muralis		Y	3:0	1:1			Y	Y
Diplotaxis siifolia		X	0:3	0:1			X	X
Diplotaxis tenuifolia		Y	0:3	1:1			Y	Y
Eruca vesicaria (E.sativa)		Y	2:0				Y	Y
Erucastrum gallicum		Y	0:1	1:0	X		Y	Y
Hirschfeldia incana		Y	1:2	1:2	Y	Y	Y	X
Moricandia arvensis		X	0:2	0:2			X	X
Myagrum perfoliatum		X	0:1	0:1			Y	X
Raphanus raphanistrum		Y	0:4	3:2	Y	Y	Y	Y
Raphanus sativus	Y	Y	1:5	1:2			Y	Y
Rapistrum rugosum		Y		1:0			Y	Y
Rorippa islandica		Y		1:0			X	X
Sinapis alba	Y	Y	0:6	1:2	X		Y	Y
Sinapis arvensis		Y	1:10	5:8	X	Y	Y	Y
Sisymbrium irio		Y	0:1	0:1			Y	X
Sisymbrium orientale		Y					Y	Y

X = No
Y = Yes, to some degree

a. Species highlighted in shaded cells have at least one report of successful hybridization with *B. napus*
b. Weed Science Society of America or USDA NRCS list of noxious weeds
c. (Andersson and de Vicente 2010), (USDA-NRCS 2016)
d. USDA PLANTS Database
Blank cells = no data available

Intraspecific crosses among *B. napus*, and interspecific crosses among *B. napus* and *B. rapa* (field mustard) occur readily (Table 3-9). To a lesser extent, interspecific crosses between *B. napus* and *B. rapa*, and *B. napus* and *B. oleracea* (cabbages), *B. juncea* (brown mustard), *B. carinita* (Ethiopian mustard), and *B. nigra* (black mustard), are possible. Intergeneric crosses between *B. napus and Diplotaxis muralis* (annual wallrocket), *Raphanus raphanistrum* (wild radish), *Sinapis arvensis* (charlock mustard) and *Erucastrum gallicum* (common dogmustard) may occur, but far less frequently.

Gene flow is most likely to occur among *B. napus* and *B. rapa* canola crops, and *B. napus* and *B. rapa* crops with weeds of the *Brassica* genus occurring in or around crop fields (Beckie et al. 2003; Legere 2005; CFIA 2011, 2016). For example, gene flow from GE glyphosate resistant canola (*B. napus*) to wild populations of bird's rape (*B. rapa*) in eastern Canada has been documented (Beckie et al. 2006). Introgression between *B.napus* and *B.rapa* populations under natural conditions has also been observed (Hansen et al. 2001; Legere 2005; Myers 2006).

Table 3-9. Summary: Outcrossing of Brassica and Related Species in the United States

Intraspecific crosses readily occur among the following

B. napus	rapeseed, rape, canola (*Brassica napus*)
B. rapa	field mustard (*Brassica rapa*)

Table 3-9. Summary: Outcrossing of Brassica and Related Species in the United States

Interspecific crosses can occur among the following	
Occur readily	
B. napus	field mustard (*Brassica rapa*)
Occur more rarely	
B. napus or *B. rapa*	field crops of *B. oleracea* (cabbage, kohlrabi, Brussels sprouts, broccoli, cauliflower, collards, and kale) brown mustard (*Brassica juncea*) black mustard (*Brassica nigra*) Ethiopian mustard (*Brassica carinita*)
Intergeneric crosses are possible with varying degrees of probability	
B. napus or *B. rapa*	wild and cultivated radish (*Raphanus raphanistrum* and *R. sativus*) wild/charlock mustard (*Sinapis arvensis* L) common dog mustard (*Erucastrum gallicum*) annual wallrocket *(Diplotaxis muralis)*

Source: (CFIA 2011; Myers 2015; CFIA 2016; USDA-NRCS 2016)

The frequency of hybridization among *B. napus* and relative species has been assessed in greenhouse and field experiments, and under commercial cropping conditions. Interspecific hybridization between *B. napus* and *B. rapa* has been observed to average 7% (range: 0–36%) in field experiments to 13.6% in commercial fields (Warwick et al. 2003). Jorgensen and Andersen reported higher frequencies of *B. napus* x *B. rapa* hybridization, up to 60%, when flowering of *B. rapa* and *B. napus* was synchronized, while the frequency decreased to 13% to 22% when *B. rapa* flowered 1 week earlier than *B. napus* (Jorgensen and Andersen 1994). Katsuta et al. (2015) reported that, when cultivated together, the frequency of crossing between GE HR *B. napus* and *B. rapa* was around 0.4% to 17.5%.

Field experiments examining spontaneous hybridization between *B. juncea* x *B. napus* observed the frequency of hybrids to range from 0.14% to 5.91%. *B. juncea* x *B. napus* interspecific crosses produced on average 2.1% hybrids, and the *B. napus* x *B. juncea* cross produced 0.2% hybrids (Heenan et al. 2007). Male fecundity for the *B. juncea* x *B. napus* F1 hybrids from both hand pollination and spontaneous field pollination studies was observed to range from 20.3% and 27.9% viability. Interspecific crossing between GE HR *B. napus* and *B. juncea* has been reported to occur at frequencies of around 0.1–3.3%, when cultivated together (Katsuta et al. 2015). Other studies have reported similar hybridization frequencies (Tsuda et al. 2014).

The probability of gene flow from *B. napus* canola to the wild relatives of *R. raphanistrum*, *S. arvensis*, or *E. gallicum* is considered to be low (Warwick et al. 2003). Hybrids between *B. napus* and *Raphanus raphanistrum* have been observed in the field, although rarely. For example, in field studies conducted during 2000, a single *R. raphanistrum* x *B. napus* first generation (F1) hybrid was detected out of 32,821 seedlings examined (Warwick et al. 2003). Similar hybridization rates between *R. raphanistrum* and *B. napus* have been reported from Australian ($<4 \times 10^{-8}$), French (10^{-7} to 10^{-5}), and Canadian studies (3×10^{-5}) (see review by (Legere 2005)). The hybridization frequency between *B. napus* and *S. arvensis* has been observed to be less than 2×10^{-5}, and that of *B. napus* by *E. gallicum* is less than 5×10^{-5} (Warwick et al. 2003).

Interspecific hand crosses between *B. napus* and *B. nigra* have been difficult to obtain, and in controlled crosses hybridization levels have been observed to be extremely low (hybridization rate of 0 – 0.09). No hybrids have been found in natural crosses when *B. nigra* was the female (OECD 2012). *B. napus* × *D. muralis* hybridization occurs, albeit rarely, with the likelihood of introgression considered very low (OECD 2012).

B. carinata can hybridized under controlled and field conditions with *B. napus*, either with *B. napus* as the male or female parent (Warwick et al. 2009b; Séguin-Swartz et al. 2013). A field study with *B. carinata* and glyphosate-resistant *B. napus* demonstrated these species can cross with each other under field conditions, albeit at a low rate. Overall, field hybridization levels detected with glyphosate resistance *B. napus* and *B. carinata* were 0.005% in an adjacent field (up to 150 m), and 0.002% in a separated field (up to 65 m). Pollen viability of hybrid plants was 14% and 8% for the two sites, and average seed set was 1.5 and 3.8 seeds per plant, respectively (Séguin-Swartz et al., 2013). Other studies have reported an average of 4.1% hybridization in crosses of *B. napus* and *B. carinata* (Roy 1980), and a hybridization frequency of 0.08 seeds per pollination (Getinet et al. 1997). In the latter study, Getinet et al. (1997) reported F1 interspecific hybrids to be highly sterile. Based on fitness information under controlled and field conditions, the fertility of hybrid plants is expected to be low (Séguin-Swartz et al. 2013).

Hybrids commonly exhibit inferior fitness relative to parental lines, with the fitness of F1 *B. rapa* x *B. napus* hybrids intermediate to the parent plants (Hauser et al. 1998; Legere 2005; Warwick et al. 2008). *B. rapa* x *B. napus* F1 hybrids are generally observed to have reduced pollen viability, around 55% (Warwick et al. 2003; Legere 2005). While hybrids exhibit less fitness, the lower fitness of second generation (F2) and backcross offspring may deter, but would not necessarily prevent introgression of genes/transgenes from *B. napus* into wild *B. rapa* populations (Hauser et al. 1998; Legere 2005). This assumption is supported by studies conducted by Warwick et al. (2008), who reported putative introgression of a glyphosate resistance transgene from *B. napus* into the gene pool of *B. rapa* under commercial cropping conditions. In this study, populations of GE HR *B. napus* x *B. rapa* hybrids were observed to significantly decline over a 3-year period (2002-2005), from 85 to 5 plants, out of a total of 200. Most hybrids, in both F1 and backcross generations, had reduced male fertility, and intermediate genome structure (Warwick et al. 2008). Although hybrid numbers rapidly declined from 2002 to 2005, the HR transgene persisted in one of the two *B. rapa* populations studied. Persistence of the HR trait occurred over a 6-year period, in the absence of herbicide selection pressure (with the possible exception of exposure to glyphosate in 2002), and in spite of the fitness cost associated with hybridization (Warwick et al. 2008). Similarly, yet under controlled experimental conditions, the glufosinate resistance trait gene was shown to be stably incorporated from *B. napus* into the *B. rapa* genome, and survival and seed production per plant were noted to be similar for GE HR and non-GE HR plants (Snow et al. 1999). Hence, where *B. napus* x *B. rapa* hybrids may have reduced fitness, which may deter introgression of transgenes into wild populations, such reduced fitness would likely preclude transgene introgression where conditions were favorable for sustaining hybrid populations over the long-term (Legere 2005).

3.5.3.3 Feral GE Canola Populations

Feral populations of GE HR canola have developed along transport routes as a result of seed spill, and occur in areas adjacent to crops as a result of pollen dispersal via wind and insects. Feral populations of GE HR canola exist in the United States (Katsuta et al. 2015) and Canada

(Knispel and McLachlan 2010), Japan (Katsuta et al. 2015), and have been reported in Switzerland (Schulze et al. 2014) and Australia (Busi and Powles 2016). Feral populations of GE HR canola are extensive in North Dakota, statewide. Schafer et al. (2011) conducted roadside surveys to quantify the presence and abundance of feral GE and non-GE canola populations in North Dakota during June and July of 2010. *Brassica napus* was present at 45% (n=634) of the surveyed sites (3,479 total miles, 39 miles of sampling sites), of which 80% expressed at least one transgene; 41% positive for only glyphosate resistance (CP4 EPSPS); 39% for only glufosinate resistance (PAT); and 0.7% comprised of both transgenes – a hybrid phenotype not produced by seed companies at that time (Schafer et al. 2011). Densities of *B. napus* plants at collection sites ranged from 0 to 30 plants/m^2 and averaged around 0.3 plants/m^2. Populations of feral GE canola were denser along major transportation routes, at construction sites, and near areas of canola cultivation (Schafer et al. 2011). Seed spill during transport is the presumed mechanism of dispersal along transportation routes, and wind and insect dispersal for those areas in proximity to commercial canola fields.

In western Canada, feral GE HR canola is found along roadsides and field edge habitats, with a large proportion of the plants glyphosate and glufosinate resistant (93% - 100%) (Knispel and McLachlan 2010). Average GE HR canola counts within 110 m^2 sampling areas ranged from 0.7 to 60.6 plants in roadside habitats to 1.0 to 49.5 plants in field edge habitats (Knispel and McLachlan 2010). As a result of the scale of cultivation, and seed and pollen dispersal, escaped GE HR canola plants have become a permanent feature of agricultural landscapes in western Canada (Knispel and McLachlan 2010). While small local populations may be prone to extinction, consistent dispersal of seed during transport, and pollen dispersal via insects and wind, enables the ongoing establishment of new populations (Knispel and McLachlan 2010).

As a consequence of ongoing cultivation and transport of GE HR canola seed, feral populations of GE canola are persistent along roadways, in the areas of ports, and other transportation routes, as well as in areas proximate to GE canola crop fields, wherever GE canola is cultivated. Roadside populations of GE canola in the United States and Canada, as well as feral populations in semi-natural and natural habitats proximate to crop fields are persistent from year to year, and contribute to the spread of transgenes outside areas of cultivation (Schafer et al. 2011).

While feral populations of GE canola have been well documented outside area of cultivation, worldwide (e.g., see (Warwick et al. 2009a; Devos et al. 2012; Luijten et al. 2015)), clear evidence of adverse environmental impacts has not, to date, been reported in the scientific literature. This may be in part due to the fact that *B. napus* is most commonly associated with managed or disturbed environments (CFIA 1994). Unless habitats are disturbed on a regular basis, populations of *B. napus* can become displaced by plants that form more stable climax communities, such as perennial grasses, tree species, and perennial shrubs (CFIA 2016). The current literature also indicates that without constant replenishment of the soil seedbank, which is supported by pollen flow and/or seed dispersal, feral GE HR canola populations are unlikely to successfully establish and persist outside of crop fields (e.g., see (Knispel et al. 2008; Warwick et al. 2008; Beckie and Warwick 2010; Devos et al. 2012; Bailleul et al. 2016)).

Considering the information reviewed above: As a result of commercial production, feral populations of GE HR canola are expected to persist in areas outside of cultivation, although largely limited to areas in close proximity to GE HR canola fields (i.e., within the range of successful pollen flow), and areas that border canola seed transportation routes; namely roads and railway lines. It is expected that outcrossing to sexually compatible wild plants on the order of 1% to 10% will occur within about a 30ft range (10 meters), and from 0.1% to 0.01% to plants within around 300ft (100 meters) (Myers 2006; EFSA 2013). This considered, pollen flow via wind has been reported at distances of up to 3 km (1.9 miles) (Warwick et al. 2008), albeit rarely.

Introgression of transgenes from GE HR canola into wild populations appears to be limited, with few instances of introgression being documented to date (Warwick et al. 2008; Luijten et al. 2015; Bailleul et al. 2016; Belter 2016; Busi and Powles 2016). Whether this is due to lack of detection, occurrence, or combination of both, has not been well elucidated.

3.5.3.4 Trait-Stacking in Feral GE Canola

Gene flow from cultivated GE HR canola to wild relative species has resulted in trait stacking in feral canola plants in North Dakota. This includes feral canola comprised of glyphosate and glufosinate resistant traits (Schafer et al. 2011). The diversity of feral populations, which emerges from hybridization among feral GE HR canola plants, and feral GE HR canola and wild plants, can increase over time as a result of continued loss of seed during transport, dispersal of pollen by insects and wind, and survival of seed in the soil seed bank (Legere 2005; Allainguillaume et al. 2006; Warwick et al. 2008; Knispel and McLachlan 2010; Schafer et al. 2011; Bailleul et al. 2016).

3.5.3.5 Trait-Stacking in Volunteer Canola

As with trait-stacking in feral GE canola, stacking in canola volunteers in western Canada is common, derived from pollen flow among varieties of GE HR and non-GE HR canola in commercial production, and an increasing management problem in cultivated fields in Canada (Beckie et al. 2003; Beckie and Warwick 2010; Knispel and McLachlan 2010). For example, hybrid canola plants with dual-HR traits (glyphosate–glufosinate, glufosinate–imazethapyr) and triple-HR traits (glyphosate–imazethapyr–glufosinate) have been identified in Canada since the early 2000s (Simard et al. 2005). The persistence of dual-HR and triple-HR hybrid canola volunteers renders volunteer canola a weed problem for canola producers in certain areas of Canada.

Persistence of GE HR canola volunteers have been observed for up to 7 years in Canada (Beckie and Warwick 2010), and 15 years in Germany. A recent study by Belter (2016) found that at two former field trial sites in Germany, in-field GE HR oilseed rape volunteers were observed up to fifteen years after harvest. While volunteer plants were persistent, observations over the entire monitoring period of 15 years showed that, based on the former field trial sites and cropland, there was no dispersal of GE canola to surrounding areas (Belter 2016).

In the absence of introduction via seed dispersal by animals and humans, persistence of volunteer populations largely depends upon volunteers completing their life cycle, returning viable seed to the soil seed bank, and seed dormancy. In general, the persistence of volunteers is characterized by exponential decline, but with a relatively long "tail;" the length of that tail largely dependent on seed bank replenishment, which, in turn, is influenced by landscape, environmental conditions (e.g., wind, precipitation), management practices, and potential for canola seed to develop secondary dormancy (Beckie and Warwick 2010; Haile and Shirtliffe 2014). The potential for secondary dormancy, an important factor, is controlled by canola genetics, environmental conditions, seed size, and harvest practices (Haile and Shirtliffe 2014).

While stacked-trait volunteer canola can be controlled with existing herbicides, their persistence will influence the agronomic practices employed in subsequent crops, such as the choice of herbicides or mechanical means used for volunteer control, and may impose restrictions on the choice of crops used in canola rotations (Legere 2005). Alternative herbicides, those other than glyphosate and glufosinate, such as such as metribuzin, 2,4-D, or MCPA, are required to control current varieties of volunteer GE HR canola (Beckie and Warwick 2010; Knispel and McLachlan 2010). Ultimately, contamination of a canola crop with volunteers comprised of non-crop traits can compromise the marketability of harvested seed. With crops of the *Brassica* family, because of the small seed size and large number of seeds produced by the crop, poor management practices can result in severe volunteer problems in succeeding crops.

3.5.3.6 Summary

Transportation of canola seed and canola cultivation, in tandem with seed and pollen dispersal processes contribute to the spread and persistence of feral and volunteer plants (Knispel and McLachlan 2010). Seed losses at canola harvest can potentially contribute round 3,000 seeds m^2 to the soil seed bank (Gulden et al. 2003), and viable seeds can persist in the soil for several years, although the majority of seeds germinate in the first year after harvest.

Canola seed yields range from around 1,200 to 3,000 per plant. Seeds are spherical and about 1 – 2 millimeter in diameter. Even with best management practices employed, seed size and plant fecundity will inevitably lead to seed loss during harvest. Likewise, due to size, seed loss during transport is probable. Herbicide tolerant canola, both GE and non-GE, establishes outside of agricultural environments via pollen flow and seed spillage. Feral GE HR canola is reported from most areas where the crop is grown or seed transported, including Canada, the United States, Europe, Australia, and Japan. It is highly likely that if GE canola varieties are grown feral populations will establish, via pollen flow, in areas proximate to the crop. Likewise, establishment of feral populations along seed transport routes appears difficult to prevent. Feral canola will hybridize with wild relative species and while hybridization rates are low, it is probable that GE traits will be transferred to other *Brassica* species (Warwick et al. 2008; Knispel and McLachlan 2010; Smyth et al. 2011; Bailleul et al. 2016). Outcrossing among feral canola populations has led to the stacking of HR traits in wild *Brassica* populations (Warwick et al. 2008; Beckie and Warwick 2010; Knispel and McLachlan 2010).

Based on hybridization frequencies and fitness data summarized above, where feral GE canola persist in a given habitat on an annual basis, and the following species are present; the potential for hybridization with wild *B. rapa* and *B. juncea* is high (OECD 2012). Hybridization with *B. oleracea*, *B. nigra*, and *B. carinata* is possible, although current literature suggests the potential for successful crosses is low. Intergeneric crosses of *B. napus* with *R. raphanistrum*, *S. arvensis*, *E. gallicum*, and *D. muralis* would occur very rarely (OECD 2012). Introgression of transgenes from GE HR canola into wild populations appears to be limited, with few instances of introgression being documented to date (Warwick et al. 2008; Luijten et al. 2015; Bailleul et al. 2016; Belter 2016; Busi and Powles 2016). Whether this is due to lack of detection, occurrence, or combination of both, has not been well elucidated.

In areas of Canada where GE HR canola has been grown for over 20 years, 2007 surveys found the majority of growers do not consider volunteer canola a particular nuisance (Smyth et al. 2010; Gusta et al. 2011). Around 74% of those surveyed reported they were able to control volunteer canola more easily or about the same as compared to 10 years prior, with 26% reporting volunteer canola control was more difficult. Nine percent of producers reported loss in yields due to volunteer canola (Smyth et al. 2010). Crop rotations of 4 years or more, scouting and early detection, and appropriate herbicide regimes are required to manage GE HR volunteer populations in most crops. However, controlling volunteer populations in some rotational crops may be more challenging due to crop injury from residual herbicides, or where the GE HR crop has the same HR mode of action as the volunteer canola population (Gulden 2007).

While canola hybridizes with wild relative species, *B. napus* does not have invasive or weedy characteristics, and is not a federally or state listed weed in the United States.[13] *Brassica* spp. is listed on the Michigan weed list, but not specifically cultivated canola, *B. napus*.

3.5.4 Biodiversity

As a highly managed landscape biodiversity in and around large-scale cropping systems is limited. The homogeneity of the plants in a crop (monoculture), and frequent disturbance of land through planting, harvesting, cover cropping, tillage, pesticide application, scouting, and related production activities limit the diversity of plants and animals in and around crop fields (Altieri 1999; Landis et al. 2005; Sharpe 2010; Towery and Werblow 2010). While biodiversity will be inherently limited, growers, as well as federal and state agencies/programs, well recognize the need for environmental stewardship and maintenance of some degree of cropland biodiversity, which is essential to sustainable farming (SARE 2012). Pollinators (e.g., bees, butterflies), those species that beneficially or adversely affect pollinators, and species that control plant pests and diseases are vital components of crop production. For instance, bees can have a positive impact on canola production. Pollinators can not only enhance the yield of canola crops, they promote more uniform flowering and earlier pod setting, and can increase the number of pods per plant and seeds per pod, as well as the seed weight (Gavloski). Canola in particular is considered a good food source for honey bees (CCoC 2017):

- Canola flowers produce high amounts of nectar that has a good sugar profile for honey production.
- The large amounts of pollen offer a good nutritional balance of amino acids, protein, and fats.
- Plentiful canola blooms allow bees to feed efficiently, without covering large distances.
- Canola fields bloom for relatively long periods, so one field can provide bees with a good source of nectar for up to a month.

A variety of federally supported programs, such as the USDA funded Sustainable Agriculture Research and Education Program, and partnership programs among the U.S. EPA and the agricultural community, support sustainable agricultural practices that are intended to protect the

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[13] USDA Plants Database: http://plants.usda.gov/core/profile?symbol=BRRA

environment, conserve natural resources, and promote cropland biodiversity (i.e.,(US-EPA 2017b; USDA-NIFA 2017)).

3.6 Human Health

Human health considerations associated with GE crops are those related to (1) the safety and nutritional value of GE crops and their products to consumers, and (2) the potential health effects of pesticides that may be used in association with GE crops. As for food safety, consumer health concerns are in regard to the potential toxicity or allergenicity of the introduced genes/proteins, the potential for altered levels of existing allergens in plants, or the expression of new antigenic proteins. Consumers may also be concerned about the potential consumption of pesticides on/in foods derived from GE crops. Occupational exposure to pesticides is also a concern.

The safety assessment of GE crop plants, summarized following, 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 potential health effects of food derived from the GE crop plant.

3.6.1 Consumer Health

3.6.1.1 Food Safety

The FDA regulates human and animal food from GE plants like they regulate all food. The existing FDA safety requirements impose a clear legal duty on everyone in the farm to table continuum to market safe foods to consumers, regardless of the process by which such foods are created. It is unlawful to produce, process, store, ship or sell to consumers unsafe foods.

As discussed in Section 1.3.3, the FDA created the voluntary plant biotechnology consultation process in the 1990’s, which crop developers can use to ensure the safety of food derived from new GE crops before they enter the market.[14] Under this policy, the FDA implements a voluntary consultation process to ensure that human and animal food safety issues or other regulatory issues are resolved before commercial distribution of food derived from GE plants. In such a consultation, a developer who intends to commercialize food or feed derived from a GE plant meets with the FDA to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the food/feed product(s). Although the consultation program is voluntary, GE plant developers routinely participate in it before bringing a new GE plant to market. The FDA completed its first plant biotechnology consultation in 1994. Thus far, the FDA has evaluated more than 150 GE plant varieties through this program.

In addition to the FDA consultation, foods derived from GE plants undergo a safety evaluation among international agencies before entering foreign markets, such as reviews by the European Food Safety Agency and the Australia and New Zealand Food Standards Agency. The Codex Alimentarius, established by the World Health Organization and Food and Agriculture Organization of the United Nations, is a set of international standards, principles, and guidelines for the safety assessment of foods derived from modern biotechnology. These standards help countries coordinate and harmonize review and regulation of foods derived from GE plants to

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[14] FDA: Statement of Policy - Foods Derived from New Plant Varieties; http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Biotechnology/ucm096095.htm

ensure public safety and facilitate international trade (WHO-FAO 2009). Currently, the Codex Alimentarius Commission is comprised of 187 member countries, to include the United States.15 Most governments incorporate Codex principles and guidelines in their review of foods derived from GE crop plants.

3.6.1.2 Safety of Canola Oil

Some consumers have expressed concern, largely on blogs and in non-peer reviewed literature, that canola oil may not be safe for human consumption. However, other peer-reviewed sources indicate that canola oil is one of the healthier food oils; being low in cholesterol and containing the lowest amount of saturated fat of all vegetable oils. A literature review of 270 research articles examining the effects of canola oil (00-rapeseed oil) consumption on coronary heart disease, insulin sensitivity, lipid peroxidation, inflammation, energy metabolism, and cancer cell growth concluded that available evidence shows a number of potential health benefits may derive from canola oil consumption (Lin et al. 2013). Benefits include substantial reductions in total cholesterol and low-density lipoprotein (LDL) cholesterol, as well as beneficial tocopherol levels, as compared with consumption of other dietary fat sources (Lin et al. 2013). The American Heart Association recommends use of cooking oils lowest in saturated fats, trans-fats, and cholesterol – such as canola oil, corn oil, and olive oil.[16]

The FDA classified canola oil produced from LEAR varieties as Generally Recognized as Safe on January 1, 1985 (US-FDA 1985). In 2006, the FDA authorized a qualified health claim for canola characterizing the relationship between the consumption of unsaturated fatty acids in canola oil and a reduction in risk of coronary heart disease. The FDA concluded, on review of scientific peer reviewed literature, that "limited and not conclusive scientific evidence suggests that eating about 1 ½ tablespoons (19 grams) of canola oil daily may reduce the risk of coronary heart disease due to the unsaturated fat content in canola oil."[17] The FDA provision for this health claim applies to canola-oil and canola oil containing foods.

3.6.1.3 Pesticides Used on Food and Feed Crops

As discussed in Section 1.3, the U.S. EPA establishes maximum allowable pesticide residue limits, more commonly referred to as tolerances, for residues in or on food for human consumption, or establishes an exemption for a tolerance (21 U.S. Code § 346a). The Federal government will seize and remove any crops or products if pesticide residues are found above the tolerance limits.

The USDA and FDA enforce tolerances to ensure the safety of the nation's food supply. The USDA enforces tolerances established for meat, poultry, and some egg products, and the FDA enforces tolerances established for other foods (USDA-FSIS 2018). The USDA's Pesticide Data Program (PDP) is a national pesticide residue monitoring program and produces the most comprehensive pesticide residue database in the United States (USDA-AMS 2015). The

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[15] Codex Members and Observers: http://www.fao.org/fao-who-codexalimentarius/members-observers/en/

[16] AHA - Healthy Cooking Oils: http://www.heart.org/HEARTORG/HealthyLiving/HealthyEating/SimpleCookingwithHeart/Healthy-Cooking-Oils_UCM_445179_Article.jsp#.V_Jpc3Lr2Uk

[17] FDA - Qualified Health Claims: Letter of Enforcement Discretion - Unsaturated Fatty Acids from Canola Oil and Reduced Risk of Coronary Heart Disease (Docket No. 2006Q-0091): http://www.fda.gov/Food/IngredientsPackagingLabeling/LabelingNutrition/ucm072958.htm

Monitoring Programs Division administers PDP activities, including the sampling, testing, and reporting of pesticide residues on agricultural commodities in the U.S. food supply, with an emphasis on those commodities highly consumed by infants and children. The NRP also collects and uses national data on chemical residues to support risk assessment, enforcement, and educational activities. The program is implemented through cooperation with State agriculture departments and other Federal agencies. PDP data:

- Enable the U.S. EPA to assess dietary exposure.
- Facilitate the global marketing of U.S. agricultural products.
- Provide guidance for the FDA and other governmental agencies to make informed decisions.

The U.S. EPA conducts periodic pesticide reregistration reviews for each pesticide every 15 years, as required by FIFRA to ensure that each continues to meet the statutory standard of no unreasonable adverse effects.

3.6.2 Worker Safety

Agriculture is considered one of the most hazardous industries in the United States. Worker hazards include those associated with the operation of farm machinery, vehicles, and pesticide application. Agricultural operations are covered by several Occupational Safety and Health Administration (OSHA) standards including Agriculture (29 CFR part 1928), General Industry (29 CFR part 1910), and the General Duty Clause. Further protections are provided through the National Institute of Occupational Safety and Health, which in 1990 began development of an extensive agricultural safety and health program to address the high risks of injuries and illnesses experienced by workers and families in agriculture.

In consideration of the risk of pesticide exposure to field workers, the U.S. EPA's Worker Protection Standard (WPS) (40 CFR part 170) was issued in 1992 to require actions to reduce the risk of pesticide poisonings and injuries among agricultural workers and pesticide handlers. The WPS contains requirements for pesticide safety training, notification of pesticide applications, use of personal protective equipment, restricted entry intervals following pesticide application, decontamination supplies, and emergency medical assistance. The OSHA also requires employers to protect their employees from hazards associated with pesticides and herbicides.

In November 2015, the U.S. EPA issued revisions to the WPS regulations intended to enhance the protections provided to agricultural workers, pesticide handlers, and other persons by strengthening elements of the existing WPS such as training, pesticide safety and hazard communication information, use of personal protective equipment, and the providing of supplies for routine washing and emergency decontamination (80 FR 211, November 2, 2015, p. 67496). Most of the revised WPS requirements became effective during 2017 and early 2018. By the end of FY 2018, the EPA expects to publish a Notice of Proposed Rulemaking to solicit public input on proposed revisions to the WPS requirements for minimum ages, designated representatives, and application exclusion zones (82 FR 60576, December 21, 2017, p. 60576).

In September, 2016 the U.S. EPA in conjunction with the Pesticide Educational Resources Collaborative made available a guide to help users of agricultural pesticides comply with the requirements of the 2015 revised federal Worker Protection Standard. Agricultural workers and handlers, owners/managers of agricultural establishments, commercial (for-hire) pesticide handling establishments, and crop production consultants are advised to employ this guidance. The updated 2016 WPS How to Comply Manual supersedes the 2005 version.[18]

3.7 Animal Feed

Animal feed derived from canola is in the form of canola meal, which is an oilseed meal similar to linseed, soybean, and other oilseed meals. Canola seeds are first crushed to remove the oil, yielding a cake as the by-product. The cake is further processed for use in animal feeds. Most of the canola meal in the United States is fed to cattle and pigs as part of a feed rotation. It can also be used as feed for poultry, aquaculture, and specialty animals (Jacob 2013; CCC 2015; USDA-ERS 2016a). As with human foods, it is the responsibility of feed manufacturers to ensure that the products they introduce into commerce are safe for animal consumption. Feed derived from GE canola must comply with all applicable legal and regulatory requirements, and, as described for human health considerations, may undergo a voluntary consultation process with the FDA before being released to the market. The U.S. EPA establishes tolerance limits for feed under Section 408 of the FFDCA and Section 405 of FQPA, which is the maximum amount of pesticide residue that can remain on or in animal feed (US-EPA 2015a).

3.8 Socioeconomics

3.8.1 Domestic Economic Environment

Canola oil, meal, and biodiesel are the primary commodities derived from canola. Meal is also used, to a small extent, as a fertilizer and for weed control. After crushing, canola seeds yield about 40% oil and 60% meal.[19] The meal that remains after oil extraction is utilized by the livestock industry as feed.

Canola Oil

Canola oil is used as cooking/salad oil, as a food and cosmetics ingredient, and for conversion into biodiesel. Consumer preference for healthy cooking oils and foods low in saturated fat and cholesterol has made canola oil a popular commodity in the United States and abroad, a trend that is expected to continue. Canola oil is the third most consumed vegetable oil in the world after soybean and palm oil, and number two by volume in the United States (USDA-ERS 2016b).

Canola Meal

Canola meal is one of the most widely used protein sources for livestock, poultry, and fish; it is the second-most widely traded protein ingredient after soybean meal (CCC 2015). Canola meal is primarily fed to cattle and pigs as part of a feed ration. The majority of canola meal in the

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[18] https://www.epa.gov/pesticide-worker-safety/pesticide-worker-protection-standard-how-comply-manual

[19] Crushing is an industrial process that segregates oilseed into crude oil and meal, both of which are further refined for food, feed, or biofuel use purposes.

United States is fed to dairy cows because the high fat content of the meal enhances milk production.

3.8.1.1 U.S. Canola Production

Currently, around 90% of U.S. canola acreage is comprised of GE HR varieties (Figure 3-6). There is no production of GE canola with a modified nutritional profile, anything comparable to DHA canola. The choice to cultivate GE HR canola is largely attributed to the net benefits that be derived from these varieties, which can include reductions in the cost of production through reduced expenditure on herbicides, modest increases in yields/acre, and some savings in fuel and labor costs associated with herbicide application and tillage (Beckie H.J et al. 2011; Gusta et al. 2011; Brookes and Barfoot 2015a).

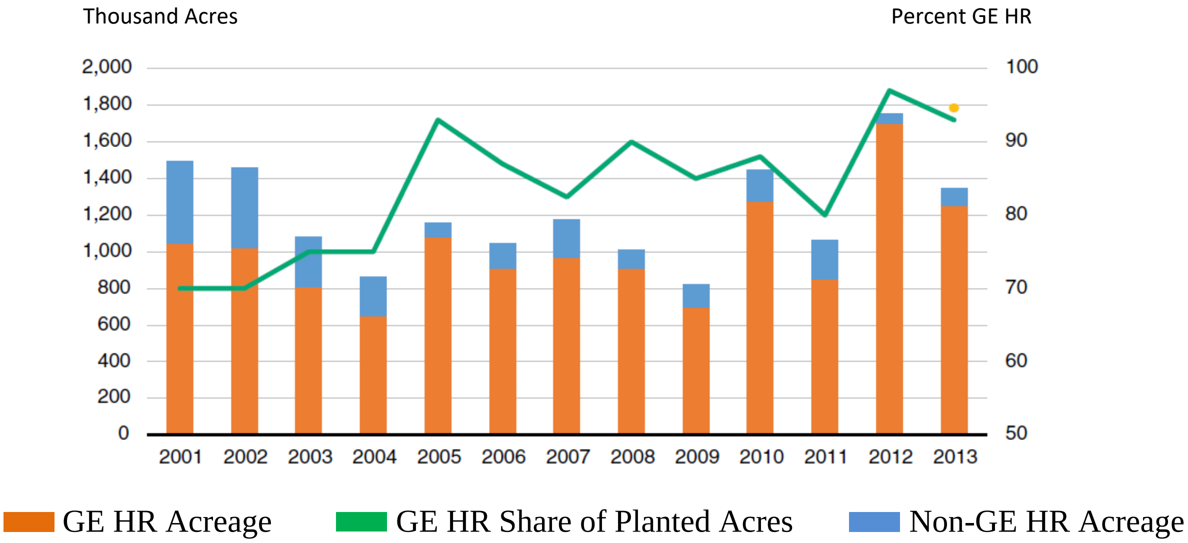

Figure 3-6. Adoption of GE HR Canola in the United States: 2001 – 2013
Source: (Fernandez-Cornejo et al. 2016)

Overall, U.S. canola production and market value has increased substantially since the early 1990s responding to domestic and international demand for canola oil, meal, and biodiesel. From 1991 to 2016, total U.S. acres of canola seed harvested increased from around 147,000 to 1.7 million acres (USDA-NASS 2017a), and market value of annual harvest increased from $18.6 to $494 million (USDA-NASS 2017b). Over this time frame, annual canola oil production increased 43 fold, from around 36 million to 1.6 billion pounds, and price per pound from 24 to 35 cents per pound, from a peak of 66 cents per pound during 2007/08. Annual canola meal production increased from around 29,000 tons to about 1.2 million tons (~ 2.4 billion pounds), and price from $130 to $300 per ton (USDA-ERS 2017). Due to demand for vegetable oil, biodiesel, and feedstock, demand for canola is expected to remain high or increase.

As of 2012 (latest census data), there were a total of 34 states with commercial canola production (USDA-NASS 2014b). Most U.S. canola production is located in the northern tier states contiguous with Canada, primarily North Dakota (USDA-NASS 2014b). Of the 1.7 million acres harvested during the 2016 growing season, 1.4 million acres were in North Dakota (USDA-

NASS 2017a). The 2016 market value of the North Dakota canola harvest was around $436 million (USDA-NASS 2017c).

3.8.1.2 Organic Canola Markets

As of 2016, there were only 4 USDA certified organic canola farms in the United States; 2 in Pennsylvania, 1 in Indiana, and 1 in Iowa (USDA-NASS 2017d). There is little reported data for the four certified organic canola farms. In 2016, total production from these farms totaled 305,000 lbs of seed: This, compared to about 3.1 billion pounds of harvested conventional and GE canola (USDA-NASS 2018a). Organic sales data has been withheld over the last several years to avoid disclosing data for individual farms (USDA-NASS 2018a).

A significant proportion of canola use is in the non-food sector (biofuels, industrial lubricants, animal feed) where there is a limited market for organic canola oil. Further, the large variety of vegetable oils available to consumers (e.g., canola, safflower, soybean, corn, flax, olive) means that the lowest cost oils will dominate market use, which contributes to limiting the price premium obtainable for organic canola oil (Brookes and Barfoot 2004). In brief, currently, organic canola production in the United States is limited, reflective of market demand.

Similar to canola commodities that may be organically produced, there is a market for “non-GMO” commodities. For example, canola oil derived from conventionally bred crop plants, or even if it is produced organically, can be marketed as “non-GMO” through verification programs. For companies that want to have their commodity verified as being free of material derived from GE crop plants, there is a Non-GMO Project Verified seal administered by the Non-GMO Project. [20]

3.8.1.3 Biodiesel

Currently, biomass accounts for about half of all renewable energy consumed and 5% of total U.S. energy consumed (Figure 3-7). Among biomass energy commodities, the market for biodiesel[21] is relatively small, but has been growing over the past five years, and currently accounts for approximately 2% of the 50 billion gallon annual diesel market (Schwab et al. 2016).

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[20] The Non-GMO Project: http://www.nongmoproject.org/

[21] Biodiesel is blended with petroleum based diesel up to 5% or 20% by volume (referred to as B5 and B20, respectively). Biodiesel diesel meets specifications for use in existing infrastructure and diesel engines, so it is not subject to any blending limitations.

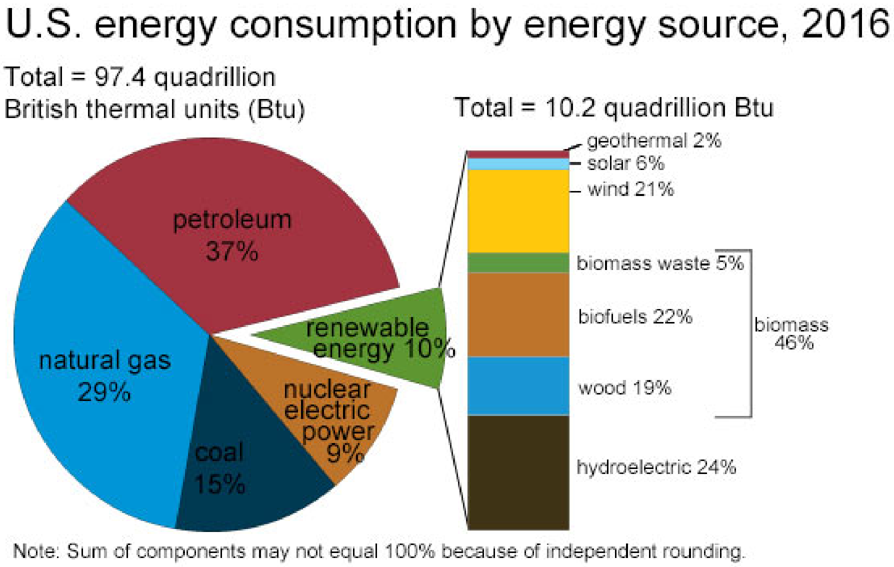

Figure 3-7. Biomass Energy Consumption in the United States, 2016
Source: (EIA 2017a)

Over 80% of biodiesel is made from vegetable oil with the primary source oils being soybean, corn, and canola, and to a lesser extent of sunflower seed, cottonseed, and camelina. Canola oil inputs to biodiesel production markedly increased from 246 million pounds in 2011 to 1.13 billion pounds in 2016 (Table 3-10), comprising the third largest biodiesel source in the United States (EIA 2017b).

Table 3-10. U.S. Inputs to Biodiesel Production

Feedstock inputs (million lbs)

	Vegetable oils						Animal fats				Recycled feeds				Other Inputs	
Year	Canola	Corn	Cotton	Palm	Soybean	Other	Poultry	Tallow	White grease	Other	Yellow grease	Other	Algae	Other	Alcohol	Catalysts
2015	745	1,057	W	W	4,908	W	197	429	589	56	1,254	122	0	122	982	170
2016	1,130	1,306	S	S	6,096	W	220	332	578	W	1,389	W	S	W	1,279	213

Source: (EIA 2017b)
W – Withheld to avoid disclosure of individual company data
S – Value is less than 0.5 of the table metric, but value is included in any associated total.

Biodiesel demand in the United States is driven primarily by the renewable fuel standards (RFS) under two subcategories; biomass-based diesel and other advanced biofuels (Schwab et al. 2016). Congress created the RFS program in an effort to reduce greenhouse gas emissions, expand the U.S. renewable fuels resources, reduce reliance on imported oil, and reduce air pollution. The RFS program was created under the Energy Policy Act of 2005, which amended the CAA. The Energy Independence and Security Act of 2007 further amended the CAA by expanding the RFS program to increase biofuel production to 36 billion gallons by 2022. Of the latter goal, 21 billion gallons must come from cellulosic biofuel or advanced biofuels derived from feedstocks other than cornstarch.

The U.S. EPA implements the RFS program in consultation with the USDA and U.S. Department of Energy. The RFS program requires a certain volume of renewable fuel to replace or reduce petroleum-based fuel, heating oil, or jet fuel. As part of this requirement the RFS requires petroleum refiners and importers to blend a certain percentage of biofuels into their fuels.[22] The four renewable fuel categories under the RFS are:

- Biomass-based diesel (canola)
- Cellulosic biofuel
- Advanced biofuel (canola)
- Total renewable fuel

The first RFS issued in 2007 applied mainly to gasoline and ethanol, and the second RFS (RFS2) took effect for biodiesel in July of 2010. U.S. EPA analyses determined that canola oil biodiesel meets the lifecycle greenhouse gas emission reduction threshold of 50% required by the Energy Independence and Security Act of 2007. Canola oil biodiesel also qualifies as both an advanced biofuel and as biomass based diesel.

According to U.S. Energy Information Administration data 4,906 million pounds of soy bean oil and 1,044 million pounds of corn oil were used to produce biodiesel in the United States in 2015. Other significant sources of feedstock were yellow grease (1,232 million pounds), canola oil (745 million pounds), white grease (588 million pounds), tallow (429 million pounds), and poultry fat (190 million pounds).

3.8.1.4 Costs of Volunteer and Feral Canola

Volunteer canola plants can introduce unwanted traits into a seed crop through cross pollination and physical seed contamination, both of which can result in rejection of the seed crop and economic loss to the grower and the seed company.

Costs may also be incurred in controlling volunteer and feral canola (Beckie and Warwick 2010; Schafer et al. 2011; Munier et al. 2012). U.S. data for the costs of volunteer control is lacking– most reports derive from Canada. Potential external costs associated with control of feral canola has not been systematically studied and no data are available.

In a 2007 survey of 571 Canadian GE canola producers, Gusta et al. (2011) found that more than 94% of respondents reported that weed control was the same or had improved, with 62% reporting no difference in practices required for controlling volunteer GE canola, although 8% indicated that they viewed volunteer GE canola to be one of the top five weeds in need of control. Based on 2007 data, the estimated cost of controlling volunteer canola in Canada was determined to be from $2.00/acre (Gusta et al. 2011) to $4.23/acre (Smyth et al. 2010).

While volunteer GE HR canola can present challenges (Schafer et al. 2011; Munier et al. 2012), overall, most studies have found the benefits of growing GE HR canola varieties to be greater

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[22] Biodiesel is most often blended with petroleum diesel in ratios of 2% (B2), 5% (B5), or 20% (B20). Biodiesel can also be used as pure biodiesel (B100).

than that of conventional varieties, outweighing the control costs for volunteers (Smyth et al. 2010; Gusta et al. 2011; Brookes and Barfoot 2015b). This would be consistent with the empirical observation that canola producers in the United States and Canada continue to produce GE HR canola, in lieu of a substantial number of options among conventionally bred cultivars. Management of volunteer GE HR canola is now standard practice for most canola producers (Gulden 2007; Smyth et al. 2010; Fleury 2015; CCoC 2016b), and substantive guidance for implementation of volunteer management programs exist, for example, from the Canola Council of Canada[23], DuPont Pioneer Agronomy Sciences[24], and Extension Services.[25]

3.8.1.5 Costs to Non-GE Producers

Contamination of non-GE canola crops, to include certified seed crops, via a GE canola crop or volunteer plants from the GE crop can compromise the marketability of the non-GE crop product (Knispel and McLachlan 2010). The two primary sources of contamination are through pollen and seed. Pollen from GE plants may fertilize a non-GE crop plant. Pollen flow and contamination is primarily a concern with cross-pollinated crops such as corn and canola. While there is a risk for potential contamination of non-GE crops, there are no reports of substantive losses associated with the unintended presence of GE HR canola in non-GE canola crops or crop commodities in the United States. Contamination of non-GE certified canola seed by GE HR canola was reported in Canada in 2002, although no monetary value of potential losses due to contamination was assessed (Friesen L.F. et al. 2003).

In general, contamination of non-GE canola with GE canola has not presented as a significant problem and the risk of contamination appears to be isolated to specific instances. This is likely due in part to the fact that around 90% of cultivated canola is GE, and cultivation of non-GE canola is limited. Nevertheless, given the large proportion of GE HR canola varieties in cultivation, the risk of contamination of non-GE canola crops and seed lot contamination exists. Such concerns can be seen in Oregon legislation. In 2013, Oregon signed into law a bill banning the commercial production of canola (rapeseed) until 2019 inside a two million acre Willamette Valley Protected District, one of the world's largest vegetable seed producing regions. Producers desiring to grow canola are required to apply for a contract with the Oregon Department of Agriculture (ODA) that contains requirements for managing the canola crop. In general, ODA's rule limits how much canola can be grown in the Willamette Valley, where it can be grown, and requires management practices for production by controlling inadvertent spread of canola seed (Oregon Revised Statute 570.405).

3.8.2 International Trade

The EU, China, Canada, and India are the largest producers of canola. Canada accounts for more than half of world trade in canola seed, meal, and oil. The EU does not produce GE canola, but it does import GE canola. In 2016, the EU represented approximately 33% of global production, followed by Canada (22%), China (20%), India (10%), Australia (5%), Ukraine (3%), and the United States and Russia at 2% each (USDA-FAS 2017).

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[23] http://cdnseed.org/archive/pdfs/Fact%20Sheets/VolunteerCanolaFactSheet.pdf

[24] https://ca.pioneer.com/west/media/1882/management-of-volunteer-canola.pdf

[25] https://www.ag.ndsu.edu/extensionentomology/recent-publications-main/publications/A-1280-canola-production-field-guide

Canada is by far the largest exporter of canola seed at around 65% of the global share over the last several years, with Australia at 17%, Ukraine at 14%, the EU at 4%, and the United States and Russia at around 1% each (Jervais 2015). Exports of canola oil follow a similar trend, with Canada, the EU, Russia, the UAE, the United States, Australia, and Belarus, being the largest exporters (USDA-FAS 2017).

Identity protection is important in international trade. The low level presence (LLP) and adventitious presence (AP) of GE trait material in internationally traded conventional or organic commodities are important considerations in the trade of canola. Asynchronous Approvals and zero tolerance policies can result in the diversion of trade by some exporters, and rejection or market withdrawals by importers of canola. 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 2014a).

The challenges associated with maintaining variety identity in international trade can increase costs, as well as the premiums paid, for some GE crops. GE canola is excluded by some countries sensitive to the importation of food or feed derived from GE plants, and other countries may lag approval of new GE canola varieties. In general, LLP or compromise of canola commodity identity can cause disruptions in international trade when GE trait material is inadvertently incorporated into food or feed shipments. As such, GE crop producing countries are required to take those measures necessary in the production, harvesting, transportation, storage, and post-harvest processing of GE crops to avoid the potential for LLP in conventional or organic commodities.

3.8.2.1 Canola Oil and Meal

Demand for canola oil is expected to remain strong because of growing use of vegetable oils in China and India, and canola oil based biodiesel use in the EU, United States, and Canada. The United States is the primary importer of Canadian canola oil and meal due its proximity and the ease of cross-border trade (USDA-ERS 2016c). As Canada's nearest neighbor and fellow North American Free Trade Agreement member, the United States will likely continue to purchase the majority of Canadian exports of canola oil and meal (USDA-ERS 2016c). U.S. imports of canola oil from Canada are projected to grow strongly through 2024, augmenting the U.S. edible oil supplies for domestic consumption (USDA-OCE 2015).

As the global demand for meat increases, so does the demand for animal feed. Protein meal consumption is expected to continue to grow at 1.6% per year through 2024, the majority of this is anticipated to be soybean based (USDA-OCE 2015). Projected increases in meat production, and slowing production of canola meal for feed are expected to lead to projected gains in domestic demand for soybean meal in the coming decade (USDA-OCE 2015). In general, trade in canola meal is limited due to the abundance of higher quality soybean meal and the high cost of transportation relative to the value of canola meal (USDA-ERS 2016c).

3.8.2.2 Biodiesel

In the past few years, fuel standards policy and mandates in the EU and United States have increased demand for canola oil as a source of biodiesel, and seed crushing capacity has expanded considerably. Globally, the EU is the largest producer and consumer of biodiesel, with mandated targets of a minimum of 10% in all member states by 2020 (Lonza et al. 2011). It is projected that the use of vegetable oil as feedstock for biodiesel, globally, will increase by 2.1% per year over the next ten years, with the share of vegetable oil used to produce biodiesel expected to be around 13% of world vegetable oil demand in 2024 (OECD/FAO 2015). In the United States, EU, Argentina, and Brazil, soybean oil is the most dominant biodiesel feedstock. As a result of increasing and inelastic canola oil demand for food use, it is unlikely that significant amounts of canola/rapeseed oil will be traded for biodiesel purposes. Instead, soybean oil and tallow will be preferred for their lower prices and wider availability (USDA-ERS 2016b).

4 ENVIRONMENTAL CONSEQUENCES

This chapter provides an evaluation of the potential environmental consequences that could derive from the alternatives considered in this EA; denying the petition, or issuing a determination of nonregulated status for DHA canola. In evaluating potential impacts, APHIS considers the likelihood that an impact will occur, and the potential to cause a significant impact if it does occur. Pursuant to CEQ regulations APHIS considers the direct, indirect, and cumulative impacts of both alternatives. Potential direct and indirect impacts are discussed in this chapter, and potential cumulative impacts in Chapter 5.

4.1 Scope of Analysis

An impact would be any change, beneficial or adverse, from existing (baseline) conditions described for the affected environment in Chapter 3. A direct impact derives from an Agency action without intermediate steps or processes. A direct impact would be, on approval of the petition, the availability of DHA canola to commercial markets, its use subject to any U.S. EPA requirements, FDA consultation, and/or state requirements. Indirect impacts are those related to, but removed from the Agency's decision in space and time. Examples would include emissions of air pollutants from farm equipment used in DHA canola production, and potential impacts on water quality resulting from agricultural run-off comprised of soil sediment, pesticides, and/or fertilizers.

4.2 Acreage and Areas of Canola Production

No Action Alternative: Acreage and Area of Canola Production

Denial of the petition would have no impact on the acreage required or areas utilized for U.S. canola production. Acreage is determined by domestic and international demand for oilseed products, independent of APHIS' regulatory status decision for DHA canola. In general, demand for canola products is expected to increase through 2024 commensurate with an increasing U.S. and global population (Westcott and Hansen 2015). There may be increased canola production, to some extent, in areas outside of North Dakota, as there are regional reports of farmers planting winter canola on land that would otherwise be left fallow in the Southeastern United States (Cebert and Ward 2014).

Preferred Alternative: Acreage and Area of Canola Production

The potential impact of approval of the petition on the total number of U.S. acres planted to canola is difficult to determine with any degree of accuracy. Because DHA canola oil would be a new commodity, marketed as a specialty canola oil containing EPA and DHA, production may entail use of additional cropland. Market forces, consumer preference, grower choices, and demand for vegetable and fish oils rich in EPA and DHA, across all markets (i.e., feed, food, and nutraceuticals), will determine the market share and scale of adoption of DHA canola. Because there is uncertainty among these factors the future scale of production cannot be precisely estimated. Principally, consumer preference for a GE vegetable oil enriched in omega-3 fatty acids is uncertain. Nuseed estimates that the market share of DHA canola oil in the fish oil food ingredient market is likely to be low initially, increasing over time and with market acceptance to as high as ~20% after 10 years (Nuseed 2017). It is anticipated that initial use may be limited to the livestock and aquaculture feed industries. In general, it is foreseeable and possible that, if

DHA canola eventually becomes a preferred source of food oil and meal, as well as a source for production of EPA/DHA supplements, an increase in canola acreage could follow.

Although the extent to which DHA canola will be adopted in the market, and acreage used for production cannot be foreseen with accuracy, APHIS provides an upward bounded estimate of acreage based on assumptions of low yields and high rates of product utilization. DHA canola could yield around 0.75 to 1.0 metric tons of oil/hectare (750 to 1,000 kg/ha).[26] EPA/DHA content in the oil fraction ranges from about 5% – 10% (Napier et al. 2015; Nuseed 2017), this equates to provision of around 35 to 75 kg EPA/DHA per hectare (assuming lower yield of 0.75 metric tons/ha). This estimate does not account for losses during extraction, separation, processing, packaging, and shipping. Many health authorities worldwide recommend a daily intake (RI) of > 500 mg/day EPA/DHA. The National Academy of Medicine in the United States recommends a daily intake (RI) of > 1 g/day of EPA/DHA (NIH 2017). If DHA canola were exclusively used to fulfill 20% of EPA/DHA supply for the entire U.S. population, at a RI of 500 mg/day this could entail use of around 160,000 hectares, or 400,000 acres (Table 4-1). It should be noted that this estimate does not include use by the animal feed/aquaculture industry and other uses. These uses could not be estimated due to lack of quantitative data on the amount of canola oil and whole seed that is or would be used for livestock and aquaculture feeds. However, these additional uses and any losses of product would increase the land area needed to meet this hypothetical RI demand.

Table 4-1. DHA Canola Acreage – Upward Bounded Estimates

US Population	EPA/DHA RI - Kg	EPA/DHA Required per Day - Kg	Day/Yr	Total EPA/DHA Required per Year - Kg	EPA/DHA per Year at 20% - Kg	EPA/DHA Yield per Hectare - Kg	Total Hectares	Total Acres
325,000,000	0.0005	162,500.0	365	59,312,500.0	11,862,500.0	75	158,166.7	390,671.7

For perspective in scale, in 2017, both corn and soybean crops were planted to around 90 million acres (36.4 million ha) each. All U.S. oilseed crops (including soybeans), collectively, comprised a total of 93.4 million acres in 2017; hence, 400,000 acres of DHA canola would represent around 0.4% of total oilseed crops, and 19% of U.S. canola cropland (2.16 million acres).

Generally, any increased acreage allotted to DHA canola production is expected to be limited, as (1) surging demand for fish products as an EPA/DHA source is anticipated to be met by growth in supply from aquaculture production, which is expected to reach 102 million tons by 2025, 39 percent higher than the 2013-2015 period level (FAO 2016) and (2) other emerging sources of EPA/DHA such as GE camelina (Tejera et al. 2016), GE EPA/DHA producing canola (Walsh et al. 2016), EPA/DHA producing yeasts (Xie et al. 2015), and farmed marine algae (Salem and Eggersdorfer 2015), may also become available, subject to any required regulatory evaluations and approvals. These factors, conjoined with what is anticipated to be limited consumer

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[26] Average canola yield in North Dakota is around 0.82 metric tons. Note that the meal, which is about 55% - 60% of the seed after crushing, is not anticipated to provide a substantial source of EPA/DHA, and not considered in the estimated acreage.

preference for GE canola oil enriched in omega-3 fatty acids, at least initially, will likely serve to limit the acreage utilized for DHA canola crop production over the next decade.

In terms of potential areas of production: Soybeans produce their best yields in hot, wet, and humid climates, while canola, safflower, sunflower, and flax tend to be planted in more arid regions. Consequently, canola does not typically compete for acreage with soybeans because they require different climates (USSEC 2011). DHA canola, if adopted, would more likely be planted in areas currently utilized for canola production (North Dakota), safflower (Montana, South Dakota), sunflower (North Dakota, South Dakota), and flaxseed (North Dakota).

4.3 Agronomic Practices and Inputs

No Acton Alternative: Agronomic Practices and Inputs

The potential environmental impacts associated with the agronomic practices and inputs used for the production of GE and non-GE canola varieties such as tillage, volunteer management, pest and weed management, and pest and weed resistance management would be unaffected by continued regulation of DHA canola.

Preferred Alternative: Agronomic Practices and Inputs

The agronomic practices and inputs used for production of DHA canola would be the same as those currently used for other canola varieties. Data presented by Nuseed demonstrate that, except for the fatty acid profile, DHA canola is agronomically and phenologically [27] equivalent to the non-GE parental line (AV Jade) and other conventional canola varieties (Nuseed 2017). Agronomic performance assessments were conducted to evaluate characteristics such as emergence, seedling vigor, plant height, lodging, and yield. Under the parameters evaluated, DHA canola generally showed similar germination, vigor, and yield compared to its parental canola line, AV Jade (Nuseed 2017).

Field trials also examined disease and insect stressors. Data from field trials demonstrate that pathogen susceptibility and disease resistance characteristics of DHA canola were unchanged compared to those of the non-GE varieties (Nuseed 2017). There were no differences in DHA canola compared to the non-transformed parent line (AV Jade) with respect to interaction with various diseases and insect pests (Nuseed 2017). Consequently, changes in pesticide use are unlikely. DHA canola is inherently resistant or tolerant to blackleg (*Leptosphaeria maculans* + L. *biglobosa*), or rather, the parent line, AV Jade, was bred for resistance. This could potentially help curtail the use of certain fungicides in cultivation of DHA canola, albeit not preclude fungicide use for treatment of other pathogens.

4.4 Physical Environment

4.4.1 Soils

No Action Alternative: Soil Quality

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[27] Phenology refers to the timing of biological events, such as flowering and seed set, in relation to environmental conditions, such as day length and temperature.

As a regulated article DHA canola may be field tested under APHIS authority. Potential impacts of DHA canola cultivation on soils (i.e., tillage, pesticide use) would be short-term and minor, as discussed in Section 3.4.1 – Soils.

Preferred Alternative: Soil Quality

The agronomic practices and inputs used for DHA canola production that can impact soil quality would be no different from those currently used for production of existing canola cultivars. Consequently, potential impacts on soil quality would be the same or similar for both the Preferred Alternative and No Action Alternative.

4.4.2 Water Resources

No Action Alternative: Water Resources

The potential impacts of canola crop production on water resources in the United States would be unaffected by denial of the petition. Growers would continue to cultivate GE HR and non-GE canola varieties currently available, employing the agronomic practices and inputs associated with these varieties. These include the more commonly used herbicides glyphosate, glufosinate, and imidazolinones, fertilizers (e.g., N, P, K, S), as well as insecticides and fungicides. All of these inputs can potentially impair surface and groundwater quality. The conservation tillage and no-till practices commonly used in canola production help to reduce agricultural runoff, and are largely considered favorable to water resources, relative to cropping systems using conventional tillage. For example, tillage practices in North Dakota for canola production are estimated to be around 75% no-till and 25% conventional till (S&T 2010).

Preferred Alternative: Water Resources

Crop Irrigation

Few canola farms currently employ irrigation due to the climate of the northern tier states where most canola is grown. In 2012 (latest data), 133 out of 3,995 canola producers (0.03%) irrigated their crops, which comprised a total of 26,894 out of 1.74 million planted acres (USDA-NASS 2014b). Water use requirements for DHA canola are no different than other canola varieties. Consequently, considering average irrigation practices for canola, potential impacts to groundwater and surface water supplies are unlikely.

Water Quality

Because the agronomic practices and inputs utilized for DHA canola production would not change, sources of potential impacts on water resources, namely NPS pollutants in agricultural run-off, would not be expected to substantially differ. There are no novel impacts to water resources identified with cultivation of DHA canola.

The U.S. EPA determines use requirements for pesticides that are intended to be protective of water quality.[28,29] The U.S. EPA provides label use restrictions and guidance for product handling intended to prevent impacts to surface and groundwater. Similarly, national and local programs to reduce NPS contaminants in agricultural run-off, and run-off itself, would continue,

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[28] For example, EPA - Aquatic Life Benchmarks for Pesticide Registration: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-pesticide-registration

[29] For example, EPA - Drinking Water and Pesticides: https://www.epa.gov/safepestcontrol/drinking-water-and-pesticides

such as the USDA-NRCS National Water Quality Initiative (NWQI) (USDA-NRCS 2017) and the North Dakota Department of Agriculture Pesticide Water Quality Program (NDDA 2017).

4.4.3 Air Quality

No Action: Air Quality

The emission sources associated with canola cultivation would be unaffected by a decision to deny the petition. Air quality would continue to be affected along current trends by emission sources such as tillage (PM), pesticide application (aerosols, spray drift), and use of farm equipment that combusts fossil fuels (NAAQS pollutants – O_3, NO_2, CO, SO_2, Pb, PM). The U.S. EPA and USDA efforts to reduce emissions, along with state and local efforts, would likewise continue (US-EPA 2017d). Conservation and no-till practices commonly used in canola production (Smyth et al. 2011) limit soil and fuel based emissions – relative to conventional tillage, and are expected to continue as currently practiced.

Preferred Alternative: Air Quality

Because agronomic practices and inputs would remain unchanged, no changes to emission sources (i.e., tillage, fossil fuel burning equipment, the application of fertilizers and pesticides) are expected. The U.S. EPA provides label use restrictions and guidance intended to minimize spray drift and aerosolization. An increase in acreage used for DHA canola production would result in a commensurate increase in NAAQS emissions, however, the probability and extent of increased acreage is highly uncertain (see 4.2 – Acreage and Areas of Canola Production). If there is increased acreage utilized for DHA canola production, it is expected to develop slowly over the next decade or longer – immediate increases in canola acreage are unlikely (see cumulative impacts discussion for acreage and air quality). In general, approval of the petition is unlikely to result in any significant increase in production area and associated emissions of NAAQS pollutants.

4.5 Biological Resources

Potential impacts to biological resources considered in this EA are the effects of the GE trait genes and their gene products through gene flow to wild relative species, and consumption of DHA canola by wildlife. The potential for GE canola to act as a weedy or invasive species are also considered.

4.5.1 Soil Biota

No Action Alternative: Soil Biota

Denial of the petition would have no effect on the relationship between canola production and soil biota. Potential impacts on soil biota in canola croplands, beneficial and adverse, would continue along current trends. Limited cultivation of DHA canola in field trials as a regulated article may have short-term minor impacts on soil communities at field sites where pesticides are used, although long-term impacts are unlikely.

Preferred Alternative: Soil Biota

Because DHA canola is agronomically equivalent to the parental line (AV Jade) and other canola varieties (Nuseed 2017), the potential effects of agronomic practices and inputs used for DHA canola production on soil biota would be the same as that for the No Action Alternative. Use of pesticides on DHA canola and any hybrid progeny is not expected to be any different than that currently used in canola production. All pesticide use on DHA canola would be subject to U.S. EPA requirements.

While DHA canola differs from other canola varieties in the fatty acid profile of the seed, this difference is not expected to have any effect on soil biota or community structures. A diverse array of fatty acids are common across terrestrial animals, plants, and microorganisms, as are the mechanisms of fatty acid biosynthesis (Řezanka and Sigler 2009; Ruess and Chamberlain 2010). Functionally similar elongase and desaturase enzymes and fatty acid biosynthesis pathways (Appendix A) in DHA canola seed are extant in many soil dwelling organisms (Stanley-Samuelson et al. 1988; Řezanka and Sigler 2009; Ruess and Chamberlain 2010). Fatty acid profiles have been characterized in a variety of species of soil bacteria, yeasts, fungi, and soil dwelling insects (Řezanka and Sigler 2009; Ruess and Chamberlain 2010). Numerous straight-chain saturated, straight-chain monounsaturated, straight-chain polyunsaturated, and branched-chain fatty acids have been isolated from soils (Sampedro et al. 2006) – among these fatty acids are those produced by DHA canola (Ruess and Chamberlain 2010). Due to the ubiquity and diversity of fatty acids in soil, fatty acids are in fact used as biomarkers to study soil food webs. For example, fatty acid profiles indicating specific dietary components (i.e., soil bacteria, fungi, plant litter) of the soil dwelling arthropod Collembola have been used to elucidate feeding strategies (Ruess and Chamberlain 2010).

Considering that soil biota are already exposed to the fatty acids in DHA canola via the biosynthesis and trophic transfer of fatty acids among soil bacteria, fungi, yeasts, earthworms, soil dwelling insects, and those derived from decayed plants and animals (Sampedro et al. 2006; Řezanka and Sigler 2009; Ruess and Chamberlain 2010), disturbance of the soil ecosystem as a result of DHA canola cultivation is highly unlikely.

4.5.2 Wildlife Communities

No Action Alternative: Wildlife Communities

Under the No Action Alternative, conventional and GE canola production will continue as currently practiced while DHA canola remains a regulated article. Cultivation of other GE and non-GE canola varieties will continue following the trends summarized in Chapter 3. Potential impacts of GE and non-GE canola production on wildlife communities would be unchanged.

Preferred Alternative: Wildlife Communities

Approval of the petition, and subsequent commercial production of DHA canola, would not be expected to affect animal communities adjacent to or within DHA canola cropping systems any differently from that of current canola cropping systems. DHA canola is agronomically and phenotypically the same as other canola varieties, and the seed has a similar fatty acid profile to other canola apart from the longer chain omega-3 fatty acid profile (Table 4-2). Thus, conceptually, the only potential risk to wildlife, as a matter of hazard assessment, would be from exposure to the desaturase and elongase enzymes and fatty acids they synthesize via consumption of the seed, this type of feeding is largely limited to birds, granivorous insects, and foraging rodents. Caterpillars of certain species of Lepidoptera, such as armyworms, seedpod weevils, grasshoppers, and Lygus bugs feed on canola seed pods. Voles, shrews, mice and other rodents may likewise consume DHA canola seed. Consumption of EPA and DHA via DHA canola seed has the potential, conceptually, to impact primary consumers (e.g., granivorous insects, rodents, birds), as well as secondary (e.g., insectivorous birds, bats, and insects etc.) and tertiary consumers (e.g., foxes, predatory birds, etc.) (Colombo et al. 2018). These potential impacts are discussed below.

Table 4-2. Fatty Acid Profile of DHA Canola Oil

		Anchovy Oil	Canola Oil (Expeller-Pressed)	DHA Canola Oil
Fatty Acids		*% Fatty Acids*		
C14:0	Myristic acid	6.6 ±0.000	0.08 ± 0.01	0.077 ± 0.004
C16:0	Palmitic acid	18.2 ±0.11	4.33 ± 0.15	4.503 ± 0.086
C17:0	Margaric acid	1.17 ±0.004	0.06 ± 0.02	0.049 ± 0.004
C18:0	Stearic acid	3.20 ±0.017	2.15 ± 0.14	2.151 ± 0.078
C20:0	Arachidic acid	0.62 ±0.005	0.70 ± 0.01	0.589 ± 0.012
C22:0	Behenic acid	0.18 ±0.000	0.33 ± 0.01	0.253 ± 0.007
C24:0	Lignoceric/ Tetracosanoic	0.08 ±0.001	0.17 ± 0.03	0.094 ± 0.005
C16:1n7	Palmitoleic acid	6.64 ±0.043	0.22 ± 0.00	0.193 ± 0.007
C17:1n7	Heptadecenoic acid	0.02 ±0.001	0.15 ± 0.14	**
C18:1n9t	Elaidic acid	0.14 ±0.000	**	**
C18:1n9c	Oleic acid	15.11 ±0.053	61.67 ± 0.26	42.031 ± 2.429
C20:1n9	Gondoic/Eicosenoic acid	1.17 ±0.006	1.20 ± 0.00	1.185 ± 0.032
C22:1n9	Erucic acid	0.25 ±0.003	0.06 ± 0.04	**
C24:1n9	Nervonic acid	0.56 ±0.003	0.10 ± 0.05	0.059 ± 0.004
C18:2n6t	Linolelaidic acid	0.16 ±0.001	**	**
C18:2n6c	Linoleic acid	1.53 ±0.005	19.94 ± 0.11	8.502 ± 0.237
C18:3n6g	Gamma linolenic acid (GLA)	0.15 ±0.002	0.01 ± 0.00	**
C18:3n3a	α-Linolenic (ALA)	1.11 ±0.004	6.22 ± 0.25	21.04 ± 1.081
C20:2n6	Eicosadienoic acid	0.21 ±0.004	0.06 ± 0.00	0.091 ± 0.004
C20:3n3	Eicosatrienoic acid	0.10 ±0.005	0.30 ± 0.3	**
C20:4n6	Arachidonic acid	0.92 ±0.005	3.17	**
C22:2n6	Docosadienoic acid	0.87 ±0.006	ND-0.1	**
C20:5n3	Eicosapentaenoic acid (EPA)	9.85 ±0.051	ND	0.430 ± 0.04
C22 :5n-3	Docosapentaenoic acid (DPA)	2.53 ±1.500	ND	1.05 ± 0.09
C22:6n3	Docosahexaenoic acid (DHA)	14.67 ±0.108	ND	8.376 ± 0.81

** No data, ND = Not Detected
Source: (Kaya and Turan 2008; Khattab et al. 2012; Nuseed 2017)

Omega-3 fatty acids, to include EPA, and to some extent DHA, serve vital structural and functional purposes in most animal species studied, and are involved in a range of physiological processes such as vision, neurological system function, and cell signaling processes (Swanson et al. 2012; Calder 2014; Twining et al. 2016b). Terrestrial animals must either consume EPA and DHA directly in the diet, or consume their anabolic precursor α-linolenic acid (ALA), and then convert ALA into EPA and DHA. Terrestrial plants, apart from GE canola, contain little to no LC-PUFAs but do contain their precursor, ALA (also in DHA canola), which vertebrates and invertebrates can utilize for metabolic purposes as well as conversion to LC-PUFAs (Twining et al. 2016a; Twining et al. 2016b). Certain species of insects among the orders Coleoptera (flea beetles), Hemiptera (aphids), Orthoptera (grasshoppers), and Lepidoptera (moths, butterflies), which feed on canola seed, and which are prey for birds and small rodents, consume and synthesize a variety of fatty acids, to include those present in DHA canola seed (Turunen 1974; Stanley-Samuelson et al. 1988; Fontaneto et al. 2011; Tzompa-Sosa et al. 2014). For example, linoleic acid (C18:2n6), alpha-linolenic (C18:3(n3), gamma-linolenic acid (C18:3n6), homo-gamma-linolenic acid (C20:3n6), arachidonic acid (C20:4n6), and EPA (C20:5n3) have been identified and their physiological roles evaluated in field cricket (Orthoptera), wax moth (Lepidoptera), mealworm beetle (Coleoptera), cockroach (Blattodea), and mosquitoes (Culicidae) (Table 4-3).

Table 4-3. Fatty Acid Profile of Field Cricket, Wax Moth, Mealworm Beetle, and American Cockroach

		Field Cricket *Teleogryllus commodus* (Orthoptera)			Wax Moth *Galleria Mellonella* (Lepidoptera)			Mealworm Beetle *Tenebrio Molitor* (Coleoptera)			American Cockroach *Periplaneta Americana* (Blattodea)		
		Percent Fatty Acid											
		W	*TG*	*PL*	*W*	*TG*	*PL*	*W*	*TG*	*PL*	*W*	*TG*	*PL*
C18:2n6	Linoleic	37.7	29.7	54.7	12.1	3.2	36.8	16.3	18.2	44.8	34.2	28.1	40.1
C18:3n3	α-Linolenic	3.5	3.6	3.7	0.8	0.7	0.1			0.2			
C18:3n6	γ -Linolenic			0.7	0.1		1.6	1.4		3.0	0.7	0.5	0.6
C20:2	Eicosadienoic		0.1	0.2									
C20:3n6	Homo- γ -Linolenic	0.2	T	0.4	0.3		I.I	0.9	3.3	0.2	I.I	0.3	3.1
C20:4n6	Arachidonic	0.1	T	0.3	0.3	0.2	0.1			0.3	5.0	0.4	7.2
C20:5n3	EPA	0.1		0.2	0.3		I.I			0.2	0.1		0.1
C22:4n6	Docosatetraenoic			T									
C22:5n6	Docosapentaenoic-6				0.2		T			0.3			
C22:5n3	Docosapentaenoic-3									0.4			

W = whole body; PL = phospholipids; TG = triacylglycerols; T = trace level

Source: (Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988)

Studies of two of the more common Lepidoptera pests of canola, the fall armyworm and cabbage looper, have identified various fatty acids that are likewise extant in DHA canola (Table 4-4), save for DHA and homo-γ-linolenic acid. Further examples include studies of yellow mealworm (*Tenebrio molitor*) and lesser mealworm (*Alphitobius diaperinus*) (Coleoptera), house cricket (*Acheta domesticus*) (Orthoptera), and the Dubia cockroach (*Blaptica dubia*) (Blattodea), which characterized ratios of unsaturated fatty acids relative to saturated fatty acids (Tzompa-Sosa et al. 2014).

Table 4-4. Fatty Acid Profile of Fall Armyworm and Cabbage Looper

	Fall Armyworm *Spodoptera frugiperda* Larvae			Cabbage Looper *Trichoplusia ni* Larvae		
Fatty Acids	PL	TG	TTL in Diet	PL	TG	TTL in Diet

C14:0	Myristic	t	t	2.5 (1.0)	t	I.I (0.3)	2.0(0.8)
Cl6:0	Palmitic	13.0(3.0)	32.5 (2.6)	33.5 (1.6)	10.4 (3.7)	27.5 (2.4)	15.6 (0.5)
Cl6:l	Palmitoleic	9.7 (2.8)	17. I (3.4)	t	t	3.3 (0.8)	1.1 (0.1)
Cl8:0	Stearic	5.5 (1.2)	2.1 (0.6)	5.8 (1.6)	6.6(0.9)	3.0(0.5)	3.9 (0.3)
Cl8:l	Oleic	22.7 (2.6)	37.8 (3.0)	23.0 (2.3)	16.1 (5.9)	31.2(1.5)	18.2 (0.9)
Cl8:2	Linoleic	38.0 (3.2)	9.4(1.6)	24.9 (2.9)	39.6 (6.0)	26.8 (2.3)	41.9(2.2)
C20:0	Arachidic	0.5 (0.3)	x	x	0.7 (0.4)	t	x
Cl8:3n6	γ –Linolenic		x	x		t	x
Cl8:3n3	α-Linolenic	8.0(0.5)	x	3.3 (0.6)	21.7(4.3)	5.4 (0.9)	12.9 (0.8)
C20:2n6	Eicosadienoic	x	x	2.9 (0.8)	x	x	0.2 (0.1)
C20:3n6	Homo- γ - Linolenic	x	x	x	x	x	x
C20:4n6	Arachidonic	t	x		0.3 (0.1)	x	x
C20:5n3	EPA	0.6 (0.5)	x	1.4(0.1)	1.1 (0.4)	x	x
C22:0	Behenic	0.6 (0.3)	x	x	1.3 (0.7)	x	x
C22:6n3	DHA	x	x	x	x	x	x

Proportions of fatty acids, as percentage of total fatty acids, in the phospholipid (PL) and triacylglycerol (TG) fractions of total lipid extracts from last instar larvae of *S. frugiperda* and *T. ni*, and from their respective larval diets. Values are mean (standard deviation).
X = not detected
t = trace

Source: (Stanley-Samuelson et al. 1986)

Insects may consume as well as synthesize LC-PUFAs, and certain species synthesize EPA and DHA, specifically. When groups of waxmoth larvae were raised on media that provided increasing levels of dietary C18:3n-3 fatty acid (ALA), analysis of the adult fatty acid compositions showed tissue ALA and 20:5n-3 (EPA) proportions increased with increasing levels of dietary ALA (Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988). This was taken to suggest elongation/desaturation of the parental ALA to EPA. House crickets have been reported to synthesize EPA (Blomquist et al. 1991), as well as DHA (Oonincx et al. 2015). One study detected C22:6n3 (DHA) in house cricket tissues samples, and concluded that, because C20:3n3 and DHA were not present in the diets fed to house crickets, yet present in house cricket tissue, this suggested *de novo* synthesis of DHA (Oonincx et al. 2015). In other studies, both EPA and DHA were consistently detected in various species of mosquito (*Ochlerotatus* spp.) (Sushchik et al. 2013), and DHA in locust (*Locusta migratoria*) (Mohamed 2015).

While the physiological roles of various saturated, monosaturated, and LC-PUFAs have been studied across a wide range of orders of insects, the synthesis and dietary accumulation of DHA by terrestrial insects, specifically, apart from those studies cited here, has not been widely reported in the literature (Stanley-Samuelson et al. 1988; Fontaneto et al. 2011; Tzompa-Sosa et al. 2014). In general, most species of terrestrial insects do not appear to accumulate DHA, or do so at only trace levels. While some studies have reported DHA in beetles and ants, around 26 and 12 mg/100g DHA (C22:6n-3), respectively, other studies in beetles, crickets, and cicadas were unable to detect DHA (Siriamornpun and Thammapat 2008). The lack of detection of DHA in certain insect species may reflect low conversion from ALA and/or a limited dietary supply. Of note, while DHA is not commonly observed in terrestrial insects, similar C22 fatty acids have been reported, such as C22:5n6 (docosapentaenoic-6) and C22:5n3 (docosapentaenoic-3) (see Table 4-3 and Table 4-4).

At the organismal level, C20 and C22 LC-PUFAs comprise a very small proportion of total, whole body, fatty acids, often detectable only via analysis of particular fractions (e.g., phospholipid and triacylglycerol) of specific tissues. This is because various fatty acids, owing to their structural and functional purposes, have distinctly different patterns of occurrence among specific tissue types. For example, C20 fatty acids have been found to comprise less than 1% of total fatty acids in whole body analyses of the butterfly *Deilephila elpenor*, however, EPA (C20:5n3) comprised up to 40% of the retinal fatty acids. Similarly, while of low occurrence in whole body analyses, C20 LC-PUFAs comprise around 5% of fatty acids of the testes of the butterfly *P. brassicae* (Stanley-Samuelson et al. 1988).

In general, LC-PUFAs occur in and are probably physiologically important for all insect species, as LC-PUFAs serve not only structural purposes in maintaining cell membrane integrity, but serve metabolic roles as precursors to prostaglandins and other eicosanoids (Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988). EPA, as well as DHA and similar C22 omega-3 and omega-6 fatty acids, likely serve important functions for some insect species, given their presence in those species studied (Stanley-Samuelson et al. 1988; Blomquist et al. 1991; Sushchik et al. 2013; Mohamed 2015; Oonincx et al. 2015).

While the significance of fatty acids in insect physiology is well recognized (e.g, see (Fraenkel and Blewett 1946; Stanley-Samuelson et al. 1988)), Hixson et al. (2016) reported that, under laboratory conditions using experimental diets, increasing the amount of EPA and DHA in larval cabbage butterfly (*P. rapae*) diets resulted in changes in adult weight and wing morphology. Diets containing increasing amounts of EPA and DHA did not affect developmental phenology, larval or pupal weight, food consumption, nor larval mortality. However, the authors report that addition of EPA and DHA in larval diets resulted in progressively heavier adults with smaller wings ($p < 0.05$), and a higher frequency of wing deformities ($p = 0.001$). The authors concluded that the presence of EPA and DHA in diets of larval *P. rapae* may alter adult mass and wing morphology, and that further research on the impacts of EPA and DHA in GE camelina on terrestrial biota was needed (Hixson et al. 2016). The authors noted that there are challenges associated with using experimental artificial diets as a model and extrapolating results from these conditions to nature (Hixson et al. 2016). This study did not use or evaluate EPA/DHA enriched seed oil from GE camelina, discussed in more detail below. There were no other studies found in the scientific literature describing adverse effects of EPA/DHA on the development of Lepidoptera larva. However, it is noted that Fraenkel and Blewett (1946) described wing deformities in Lepidoptera reared on diets deficient in linoleic acid and vitamin E.

Animal feeding studies relative to DHA canola are limited. One study evaluating a similar product to DHA canola, a GE *Camelina sativa* that likewise has a seed oil enriched in EPA and DHA, investigated effects on Atlantic salmon growth and development (Betancor et al. 2016). Inclusion of EPA/DHA camelina oil in feeds for Atlantic salmon found no detrimental effect on growth or performance after 11 weeks of feeding (Betancor et al. 2016). A similar study employing GE camelina oil high in EPA found salmon grew well and exhibited no adverse effects as to performance, metabolic processes, nor on the nutritional quality of the flesh of the fish (Betancor et al. 2015). A 10 week mouse study evaluating the effects of GE camelina oil, enriched in DHA and EPA, on development and physiology observed no adverse effects in the mice, apart from weight gain in those mice fed camelina oil, as well as those fed fish oils (Tejera et al. 2016).

In terms of wildlife that may consume DHA canola seed: fatty acids are vital to the normal development and function of all organisms. LC-PUFAs, in particular, are necessary for the health and maintenance of higher organism such as mammals (Sperling et al. 2003; Leonard et al. 2004; Sampedro et al. 2006; Ruess and Chamberlain 2010). All wildlife consume or synthesize, and are comprised of, fatty acids found in DHA canola seed, to include the LC-PUFA EPA, and to some extent DHA (Leonard et al. 2004; Sampedro et al. 2006; Hashimoto et al. 2008; Řezanka and Sigler 2009; Ruess and Chamberlain 2010; Fontaneto et al. 2011; OSU 2017). Fundamentally, the vast majority of fatty acids among eukaryotes and prokaryotes are common across taxa as are biosynthesis pathways (Appendix A). For example, (Hashimoto et al. 2008) investigated 56 eukaryotic genomes and identified 275 desaturase and 265 elongase homologs. Phylogenetic analyses indicated that the desaturases consisted of four functionally distinct subfamilies and the elongases consisted of two subfamilies. Relative to fatty acid biosynthesis in DHA canola seed: Δ12 desaturase and Δ15 desaturases have been identified in lower eukaryotes, plants, and animals (except mammals (Abedi and Sahari 2014; Lee et al. 2016)). Given the lack of Δ12 and Δ15 desaturases and the low levels of conversion of ALA to LC-PUFAs, mammals must consume omega-3 and omega-6 fatty acids in their diet (Abedi and Sahari 2014). Δ4-, Δ5-, and Δ6- desaturases exist in a wide range of organisms such as algae, diatom, fungi, moss, bacteria, and humans (Sperling et al. 2003; Abedi and Sahari 2014; Lee et al. 2016). The genes encoding Δ6-elongase have been cloned from a variety of organisms, including higher plants, algae, mosses, fungi, nematodes, and humans (Lee et al. 2016). Enzymes with Δ5-elongating activity have been identified in marine microalgae, rodents, and humans (Leonard et al. 2004; Meyer et al. 2004).

Based on the vital physiological roles fatty acids serve in vertebrates and invertebrates (e.g., (Swanson et al. 2012; Calder 2014; Napier et al. 2015; Twining et al. 2016a; NIH 2017)), it is unlikely that DHA canola seed presents any risk to wildlife. None of the fatty acids extant in DHA canola seed are novel to terrestrial biota; they are in fact common across almost all taxa. The only difference in DHA canola seed is the presence of DHA, EPA, and DPA, and expected variations in the levels of some other fatty acids, such as ALA. Animals must either consume EPA and DHA directly in the diet, or consume their anabolic precursor ALA, and then convert (biosynthesis) ALA into EPA and DHA. Because animals synthesize EPA and DHA from ALA, as needed, consumption of prey containing EPA and DHA, and trophic transfer of these fatty acids, is unlikely to present any risks to secondary and tertiary consumers. As reviewed above, some species of insects, accumulate EPA, and some DHA, via dietary means. Some insects may also synthesize EPA and DHA. Aside from the intended changes in the fatty acid profile (EPA/DHA), compositional analyses show no significant nutritional differences between the parental AV Jade canola line, commercial varieties, and DHA canola (Nuseed 2017).

While Hixson et al. (2016) reported that, under laboratory conditions, diets fortified with increasing amounts of EPA and DHA resulted in progressively heavier adult butterflies and a higher frequency of wing deformities, this is the first and only report of such adverse effects of DHA and/or EPA on Lepidoptera development that APHIS is aware of. These novel findings may be valid, or they could be anomalous, confounded by experimental conditions. For example, 33% of control larvae in the study exhibited wing deformities (Hixson et al. 2016). At this time the data is not sufficient to draw any conclusions in regard to the potential risk EPA and DHA may present to Lepidoptera insects. In general, the vast majority of current literature finds that LC-PUFAs are of considerable biological significance for insects generally; regular structural components of insect tissues as well the basis for compounds serving vital cell signaling and neurological functions (Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988).

4.5.3 Plant Communities

No Action Alternative: Plant Communities

Under the No Action Alternative, conventional and GE canola production will continue as currently practiced while DHA canola remains a regulated article. Cultivation of other GE and non-GE canola varieties will continue following the trends summarized in Chapter 3. Potential impacts of GE and non-GE canola production on plant communities would be unchanged. Any herbicide used must be registered with the U.S. EPA and applied pursuant to U.S. EPA label requirements.

Preferred Alternative: Plant Communities

Because the agronomic practices and inputs that will be used for DHA canola production would be no different, the potential impacts on vegetation proximate to canola fields are substantially the same under both the Preferred and No Action Alternatives. In the event of a determination of nonregulated status for DHA canola, the risks to communities of wild plants presented by feral canola populations are relatively low (see following discussion in 4.5.5 on gene flow and weediness). Feral populations of DHA canola will likely establish and persist in areas adjacent to DHA canola crops and along transport routes, although feral canola populations can be easily managed via mechanical or chemical means, if management is required.

4.5.4 Gene Flow and Weediness of Canola

No Action Alternative: Gene Flow and Weediness

Both GE and non-GE canola varieties will continue to be cultivated, and gene/transgene flow between commercial GE canola and wild relative *B. napus* and *B. rapa* will likely occur (Legere 2005; Beckie and Warwick 2010; Knispel and McLachlan 2010; Bailleul et al. 2016). Seed dispersal along transport routes, cross-pollination of feral GE HR canola with wild relatives, and development of hybrid populations in areas of seed dispersal and canola crop production is likely. Feral populations of GE HR x wild type hybrids will likely persist in disturbed habitats. Pollen flow from GE canola to sexually compatible wild relative *Brassica* spp. will largely be limited to areas within around 300 feet of crop field edges. The majority of canola pollen disperses within a radius of around 30 feet (10 meters), and hybrid seeds rarely are detected more than 165 feet (50 meters) from the pollen-supplying parent (Myers 2006); however, rare outcrosses have been detected up to 2.4 miles (4 kilometers) away in some circumstances (Myers 2006). Based on current data, it is assumed that interspecific and intraspecific hybridization among GE and wild relative species will occur, although probably at low levels (Warwick et al. 2003; Legere 2005; Myers 2006; Warwick et al. 2008). Gene flow is most likely to occur among *B. napus* crops, and *B. napus* crops and wild relative *B. napus* subspecies and *B. rapa* species occurring in or around crop fields, or where canola seed is spilled during transport (Beckie et al. 2003; Legere 2005; CFIA 2011, 2016).

To date, while feral GE HR canola populations exist worldwide, disruption of wild plant communities, and ecosystems, have not been described in the peer review literature. *B. napus* is not an invasive plant and in this respect not considered of high risk to native plant communities (e.g., see (Katsuta et al. 2015; Belter 2016)). However, the environmental consequences of extant and future GE HR canola and wild type *Brassica* hybrids remain largely unknown, as such consequences have not, to date, been well studied. The persistence of feral GE HR canola and GE HR wild-type hybrids, and any stable incorporation of GE HR traits in wild *Brassica* populations could potentially prove problematic to control of hybrids. For example, GE HR *B. napus* may cross with *B. rapa* in the wild. Hybrid *B. rapa* (wild mustard) may be considered weedy in cultivated fields and disturbed areas, and may displace desirable vegetation if not properly manage (USDA-NRCS 2012). While hybrids may be resistant to one or more herbicide MOAs, there are a variety of herbicide MOAs on the market that could provide control of hybrids, if needed. Mechanical methods are also effective.

Currently, the primary impacts are those associated with GE volunteers (Gulden 2007; Fleury 2015), namely those with multiple HR traits, which can create management problems in rotated crops, and compromise the marketability of certified seed and contaminated crops. The persistence of GE volunteers and gene flow among GE canola and volunteers will likely have agronomic consequences in some areas, requiring adaptation of cultural and chemical management practices.

Under the No Action Alternative, gene/transgene flow between commercial canola varieties, and between commercial canola and wild relatives is not expected to change from what is currently occurring in canola production. Seed dispersal along transport routes, cross-pollination, and development of hybrid populations in areas of seed dispersal and canola crop production is likely to continue. Additionally, weediness potential associated with canola production is not expected to change under the No Action Alternative.

Preferred Alternative: Gene Flow and Weediness

DHA canola, if grown for commercial purposes, would be cultivated as are current canola varieties and present the same potential risk for gene flow, specifically the propensity and frequency of gene flow, as current canola varieties. Accordingly, a determination of nonregulated status for DHA canola and its progeny would not be expected to present more or less risk for gene flow to wild relative species as do current canola varieties.

As discussed above for the No Action Alternative, pollen flow from DHA canola crops to sexually compatible wild relative species is inevitable (Section 3.5.3 – Gene Flow and Weediness of Canola). This is more of a concern, from an environmental perspective, with feral populations of GE canola (e.g., pollination of DHA canola by wild brassica species would present an economic concern for the grower). Feral GE HR canola, to include stacked-trait feral hybrids, are fairly common in areas where canola is commercially grown, and along seed transport routes. Where feral GE canola populations establish, the potential for hybridization with sexually compatible wild relative species exists. For all canola crops (*B. napus*), it is expected that outcrossing to sexually compatible wild plants on the order of 1% to 10% will occur within about a 30ft range (10 meters), and from 0.1% to 0.01% to plants within around 300ft (100 meters) (Myers 2006; EFSA 2013). However, pollen flow via wind has been reported at distances of up to 3 km (1.9 miles) (Warwick et al. 2008), albeit rarely. Introgression of transgenes from GE HR canola into wild populations appears to be limited, with few instances of introgression being documented to date (Warwick et al. 2008; Luijten et al. 2015; Bailleul et al. 2016; Belter 2016; Busi and Powles 2016). Whether this is due to lack of detection, occurrence, or combination of both, has not been well elucidated.

DHA canola can potentially transfer the trait genes governing omega-3 fatty acid biosynthesis to sexually compatible *Brassica* species via intraspecific or interspecific hybridization. Upon pollination of sexually compatible wild *Brassica* species by DHA canola, incorporation of the fatty acid biosynthesis traits would not be expected to confer any competitive advantage or disadvantage to wild *Brassica* species (USDA-APHIS 2018a). The trait genes and elongase and desaturase enzyme products (Appendix A) extant in DHA canola do not result in characteristics commonly observed in weeds (i.e., hardy, prolific, highly competitive, difficult to control). Consequently, it is unlikely that gene introgression from DHA canola to other *Brassica* species with which it can interbreed will increase their weediness (USDA-APHIS 2018a).

Based on DHA canola field studies, pathogen susceptibility and disease resistance characteristics of DHA canola were unchanged when compared to its non-GE parental line. DHA canola, by virtue of its parental line AV Jade, is naturally resistant to blackleg disease (*Leptosphaeria maculans* + *L. biglobosa*), a trait that could confer a completive advantage to wild hybrids lacking blackleg resistance.

Considering these factors – the potential weediness characteristics of DHA canola (lack of), inherent blackleg disease resistance, unaltered pathogen susceptibility, and environmentally benign nature of the elongase and desaturase enzymes governing fatty acid biosynthesis enzymes (e.g., they are ubiquitous among eukaryotes (Hashimoto et al. 2008)) – the risk of environmental harm resulting from a DHA canola and wild-type hybrid is considered very low to negligible.

Because DHA canola will be produced using standard industry identity preserved (IP) practices (see Socioeconomics discussion, 4.8), contamination of other crops or their products, contamination of DHA canola by other crops, or pollination of DHA by sexually compatible wild relative species, is unlikely. If such occurred, these events would be expected to be of low incidence.

4.5.5 Biodiversity

No Action Alternative: Biodiversity

The impacts of commercial canola production on biodiversity (i.e., planting, irrigation, pesticide application, fertilizer applications, use of agriculture equipment) within and surrounding crop fields, whether non-GE or GE varieties, would not change under the No Action Alternative. Life forms typically associated with canola fields will continue to be affected by currently utilized management practices and production activities.

Preferred Alternative: Biodiversity

Commercial production of DHA canola would affect biodiversity in and around DHA canola crops no differently than other canola cropping systems. As discussed in the sections addressing wildlife, and Appendix A, the elongase and desaturase enzymes, and fatty acids present in DHA canola are unlikely to present any risk to plant, animal, fungal, or bacterial communities. Most of the fatty acids present in DHA canola seed are common among both prokaryotic and eukaryotic organisms (Stanley-Samuelson et al. 1988; Leonard et al. 2004; Twining et al. 2016a; Garba et al. 2017; Harwood 2017). The same or functionally similar elongase and desaturase enzymes, and the majority of fatty acids in DHA canola seed, save for EPA and DHA, are ubiquitous among terrestrial plants and microorganisms (Hashimoto et al. 2008; Lee et al. 2016; Garba et al. 2017). EPA and DHA are common among animals, and some insect and plant species.

Considering the lack of risk the elongase and desaturase enzymes, and their fatty acid products, present to biota, cultivation of DHA canola would present the same potential impacts on biodiversity in and around DHA canola cropping systems as do currently cultivated canola varieties, both GE and non-GE – these impacts derived from common agronomic practices and inputs.

As discussed in Chapter 3, while biodiversity will be inherently limited, canola growers recognize that maintaining some degree of biodiversity is essential to sustainable farming (SARE 2012). Bees in particular can have a positive impact on canola production. Although pollinators aren't essential for canola production, several studies have shown that pollination by bees can improve both the productivity and quality of canola seed – in part because bees transfer pollen more efficiently than self-pollination in canola (CCoC 2017). Pollination by honey bees has been shown to increase canola yields from 13% to 46% (Sabbahi et al. 2005). Seed set has also been found to be greater in fields with higher bee abundance. Some species of hover flies (Syrphidae) may also significantly increase seed set and yield in canola (Jauker and Wolters 2008). Guidance for U.S. and Canadian canola production encourages growers to apply insecticides only if needed, and after 8:00 p.m. when possible to do so, when bees have ceased foraging (NDSU 2011; CCoC 2017).

Relative to pollen and nectar, canola is considered a good resource for honey bees (CCoC 2017). Canola flowers produce high amounts of nectar that has a good sugar profile for honey production, and ample pollen that provides a good nutritional balance of amino acids, protein, and fats. Plentiful canola blooms in commercial crop fields also allow bees to feed efficiently without covering large distances. Canola pollen contains on average 23% to 24% crude protein, with levels as high as 27.1%, and a full complement of essential amino acids (Somerville 2001). At 24% protein, canola pollen is one of the most nutritious of all pollens collected by bees.

Although little is known about the nutritional need for fats in honey bee diets, there is some indication that pollens with higher fat/lipid levels are more attractive to honey bees (Somerville 2001). Among 60 pollen producing species evaluated, fat content for pollens collected ranged from 0% (red stringybark) to 11.2% (flatweed), with a mean of 2.52%. Field studies have found that canola pollen (mean of 6% fats), hedge mustard (mean 5.8%), turnip weed (mean 6%) and flatweed (mean 7.2%) are relatively high in fat as compared to other pollens, and all very attractive to bees (Somerville 2001). Because the transgenes and fatty acid biosynthesis enzymes are expressed only in the seed, it is expected that the fatty acid profile of DHA canola pollen and nectar content would not significantly differ from its non-GE parental line (AV Jade). Tissue samples from DHA canola were collected representative of specific growth stages from leaves, roots, pods, and reproductive tissues. None of the transgene enzymes were detected in non-seed tissues of DHA canola (Nuseed 2017).

While there is limited data for U.S. canola production, during the past decade the number of honey bees in Canada has reached near-record levels, with more than 722,000 colonies Canada-wide in 2015, up from 600,000 in 2000 (CCoC 2017). Over 70% of these colonies are in Western Canada, where canola his widely grown. Although honey bees can be an abundant pollinator in canola field, there are also many species of wild bees that can be present. A study in Manitoba, Canada, identified 15 species of bumble bees in canola fields (Turnock et al. 2006). A study from Simon Fraser University (British Columbia) found bee abundance was greatest in canola fields that had uncultivated land within 750 m of field edges (Morandin and Winston 2006). In general, studies to date indicate that canola and pollinators share a mutually beneficial relationship. Because, apart from the fatty acid profile of the seed, DHA canola is agronomically and phenotypically the same as non-GE canola, commercial production of DHA canola would be expected to affect biodiversity in and around DHA canola crops no differently than other canola cropping systems.

4.6 Human Health and Worker Safety

Public health considerations are those related to (1) the safety and nutritional value of DHA canola and (2) the potential health effects of pesticides that may be used in the production of DHA canola. As for food safety, consumer health concerns are in regard to the potential toxicity or allergenicity of the introduced proteins, possibly altered levels of potential allergens in canola, or the expression of new antigenic proteins.

No Action Alternative: Human Health and Worker Safety

Denial of the petition would not be expected to have a significant impact on public health. Conceptually, it could be argued that denial of the petition and ability of the food, feed, and nutraceutical industries to utilize the beneficial nutritional aspects of this canola variety could entail potential public health impacts over the long-term; this is due to limited sources of EPA/DHA from fish stocks (oils) in light of an increasing U.S. and global population in the coming years (discussed in Section 5.5.3 – Fisheries) (Nichols et al. 2010; Tacon and Metian 2013; Salem and Eggersdorfer 2015; Tocher 2015). However, there are current efforts in the development of other alternative sources of EPA and DHA. These include aquaculture, krill, farmed marine microalgae, yeasts, GE camelina, and other varieties of GE canola (Adarme-Vega et al. 2012; Napier et al. 2015; Salem and Eggersdorfer 2015; Xie et al. 2015).

Preferred Alternative: Human Health and Worker Safety

Approval of the petition for DHA canola would provide for use of this variety in the food and feed industries, subject to Nuseed’s consultation with the FDA. In March 2017, Nuseed submitted a safety and nutritional assessment for food and feed derived from DHA canola to the FDA. Compositional analyses (e.g., proteins, carbohydrates, amino acids, vitamins, minerals) demonstrate that, apart from the expected changes in the fatty acid profile in the seed, there are no significant differences between DHA canola, the parent line AV Jade, and other commercial canola varieties (Nuseed 2017). Bioinformatic analyses were conducted to compare the amino acid sequence of the expressed proteins in DHA canola to known allergens using the AllergenOnline.org database and the NCBI Protein database (National Center for Biotechnology Information, U.S. National Library of Medicine). None of the bioinformatic analyses identified any sequence that matched to a protein likely to present any risk of allergenicity or toxicity (Nuseed 2017).

Nuseed’s DHA canola has been evaluated by Food Standards Australia New Zealand (FSANZ) for marketing in these countries. In its safety assessment FSANZ concluded that "... no potential public health and safety concerns have been identified in the assessment of DHA canola. On the basis of the data provided in the present Application [Nuseed’s application], and other available information, food derived from DHA canola is considered to be as safe for human consumption as food derived from conventional canola varieties ..." (FSANZ 2017).

DHA canola also contains the *pat* gene that encodes for the production of the enzyme phosphinothricin N-acetyltransferase (PAT). In some GE crop plants, PAT confers resistance to the herbicide active ingredient glufosinate-ammonium. In DHA canola, PAT is utilized as a genetic marker, it is not intended to be utilized as a glufosinate resistance trait. The pat gene was derived from the naturally occurring soil bacterium *Streptomyces viridochromogenes.* The PAT enzyme is non-pathogenic to humans and does not possess characteristics associated with food allergens (Herouet et al. 2005; OECD 2006). PAT has been extensively reviewed over the years, and approved for human and animal consumption in various countries including the United States, Canada, Australia, Argentina, Japan, South Africa, and the European Union (CERA 2011). Herbicide resistant crop plants containing the PAT protein have been widely grown for over a decade, and canola oil and canola meal derived from these plants widely utilized in the global food and feed markets. There are no known reports of adverse effects on human or animal health associated with products derived from GE canola that expresses PAT. Because the PAT protein is considered to pose negligible risk to human health (Herouet et al. 2005; CERA 2011), PAT and the genetic material encoding for PAT in all plants are exempt from the requirement of a tolerance when used as plant-pesticide inert ingredients in all raw plant agricultural commodities (62 FR 70, Apr. 11, 1997, p. 17719).

The vital physiological roles and health benefits of EPA and DHA continue to be elucidated (Russell and Bürgin-Maunder 2012; Swanson et al. 2012), and it is generally acknowledged that most individuals in the United States do not acquire a recommended dietary intake of EPA and DHA (Swanson et al. 2012; Calder 2014; NIH 2017). Dietary recommendations for EPA and DHA range from 250 to 1000 mg/day for adults and from 40 to 250 mg/day for infants older than six months, children, and adolescents (Weylandt et al. 2015). Many observational studies link higher intakes of fish and other seafood, rich in EPA, DHA, and other omega-3 fatty acids, with improved health outcomes (NIH 2017). Whether the benefits are due to the omega-3 fatty acid content of the seafood (which varies among species), other components in the seafood, the substitution of seafood for other less healthful foods, other healthy behaviors, or a combination of these factors, is not yet clear. However, in general, current research suggests the health benefits of omega-3s, namely EPA and DHA, involve prevention of cardiovascular disease; neurodevelopment in infants; cancer prevention; Alzheimer’s disease and dementia prevention, and cognitive function; age-related macular degeneration prevention; rheumatoid arthritis prevention; and other conditions (NIH 2017).

If APHIS approves the petition, subject to Nuseed’s consultation with the FDA, there would be a vegetable oil comprised of LC-PUFAs (i.e., EPA, DPA) available to the commercial market. DHA canola oil could potentially augment current sources of EPA/DHA, which would be considered of potential benefit to public health, this relative to the use of DHA canola oil by the food and feed industries, and consumers. Conceptually, DHA canola provides an additional source of EPA and DHA to help meet an increasing dietary demand for these nutrients (increasing U.S. and global population).

DHA canola oil could potentially serve as a cooking oil and source ingredient in processed foods. DHA canola oil could also be used, potentially, in dietary supplements and nutraceutical products. Soybean, palm, canola, and sunflower are the primary vegetable oils used in the food/feed industry; they have comparable fatty acid profiles (Gunstone 2011). Soybean, canola, safflower, and sunflower are interchangeable as cooking oils, and these along with palm and cottonseed oil interchangeable in processed foods (Scrimgeour 2003). It follows that DHA canola oil, if adopted in the market, may supplant, in some instances, current food uses of soybean, safflower, and sunflower oil.

These possible outcomes considered, consumer preference for a GE canola oil enriched in long chain omega-3 fatty acids is uncertain. Were the petition approved, the extent to which certain consumers would be amenable to consuming a GE canola oil enriched in EPA and DHA, livestock or farmed fish reared on feed derived from DHA canola seed, or EPA/DHA supplements derived from DHA canola oil, is unclear (Wunderlich and Gatto 2015).

If commercially adopted, worker exposures to agronomic practices and inputs used in DHA canola production would be the same as those currently used. The U.S. EPA regulation of pesticides, and worker protection standards, would be no different than that of the No Action Alternative. Approval of the petition would present no risk to human health, nor any new risk to worker safety.

4.7 Animal Feed

Animal feed derived from canola is primarily in the form of canola meal, which is one of the most widely used protein sources for livestock, poultry, and fish; the second-most widely traded protein ingredient after soybean meal (CCC 2015). Feeds, to include those used in aquaculture, may also be produced using canola oil. Canola can be grazed by livestock, and made into hay or silage (NDSU 2008), although this is not a common practice.

No Action Alternative: Animal Feed

Under the No Action Alternative, DHA canola would remain a regulated article and would not be available for use as an animal feed. Current availability of GE and non-GE canola oil/meal for animal feed would remain unchanged.

Preferred Alternative: Animal Feed

For the reasons discussed for human health above, approval of the petition is unlikely to present any risks to animal health and welfare. The meal component of DHA canola seed is compositionally similar to other canola meal and would be used as feed in a manner similar to conventional canola meal; it contains only trace levels of EPA or DHA (Nuseed, 2017). DHA canola oil and whole seed, enriched in EPA and DHA, would be marketed for use in the production of animal feed, to include feeds for use in the aquaculture industry. Producers of livestock and farmed fish would be expected to utilize DHA canola to the extent they determined it provided, as a dietary component, optimal quality beef, swine, poultry, and farmed fish. As previously noted, because DHA canola is within the scope of the FDA policy statement concerning regulation of products derived from new plant varieties, including those produced through genetic engineering, Nuseed submitted a safety and nutritional assessment of food and feed derived from DHA canola to FDA in March 2017 (Nuseed 2017).

4.8 Socioeconomics

4.8.1 Domestic Economic Environment

No Action Alternative: Domestic Economic Environment

Denial of the petition would have no effect on the U.S. domestic canola oil or meal markets. GE and non-GE canola will continue to be commercially cultivated with most of the production occurring in North Dakota. Demand for canola oil and meal is expected to increase through 2024, with canola remaining as, or more profitable (Westcott and Hansen 2015).

Preferred Alternative: Domestic Economic Environment

Approval of the petition would not be expected to present any significant risks to domestic markets. To the extent DHA canola augmented current marine sources of EPA and DHA and the oil and seed valued commodities in the food and feed industries, benefits to domestic markets would be expected. It is assumed that growers would adopt and produce DHA canola commensurate with market demand for GE vegetable oil and whole seed enriched in DHA and EPA.

An important consideration in the marketing of agricultural commodities is the preservation of crop and crop commodity identity across GE, organic, and conventional production and marketing systems. This is not an environmental safety or health issue per se; rather, it is an economic issue associated with agronomic and industry practices. This is particularly important for the identity-preserved (IP) and organic markets, which are required to maintain the genetic integrity of their crop commodities. The unintended presence of foreign GE plant material in an IP or organic crop product can occur not only as a result of cross-pollination and seed dispersal, but also due to failed crop segregation during harvesting, shipping, and processing. Thus, the maintenance of crop product identity is fundamental to ensuring the sustainability of GE, organic, and conventional crop production systems, maintenance of price premiums in the market, and avoidance of trade barriers for IP and organic crops.

IP is a process for ensuring segregation and channeling of agricultural commodities to respective buyers and markets (e.g., human foods, animal feeds, cosmetics, pharmaceuticals, industrial uses), requiring strict separation be maintained at all times (Sundstrom et al. 2002). IP applies to commodities derived from conventional, organic, and GE crops alike (Sundstrom et al. 2002). Commodities with unique traits such as specialty grains, high-oleic canola, and blue corn require IP programs to channel these commodities to specific markets in order to capture their added value. It should be noted that IP and USDA organic certification are not the same processes. Organic commodities that use an IP program must still be produced according to specific criteria in order to receive price premiums.

Because DHA canola is, in terms of canola commodities, a novel product, the mixing of a canola oil enriched with LC-PUFAs with conventional or high oleic IP canola oil would incur economic losses for producers and processers. As such, the commercial production of DHA canola seed, due to its fatty acid profile, would require particular attention to prevent commingling with other canola commodities, namely whole seed, oil, and meal during post-harvest processing and throughout the supply chain. In recognition of these requirements, Nuseed developed a closed-loop IP system and stewardship plan to facilitate isolation of DHA canola materials and maintenance of product identity throughout production and marketing processes. Stewardship requirements will encompass all participant activities in the supply chain from research to supply oil and meal to the customer (Nuseed 2018a). Nuseed will share testing methodology with the rest of the canola industry to facilitate maintenance of product integrity (Nuseed 2018b). DHA canola will be grown under legal contract and subject to management requirements defined by

Nuseed (Nuseed 2018b). DHA harvest material will be confined to the farm in secured facilities, and the seed processed at specified crushing facilities in combination with testing that ensures the integrity of DHA canola seed (Nuseed 2018b).

Fundamentally, it is in the best interest of the developer, Nuseed, to steward the production and marketing of DHA canola, and ensure compliance with legal requirements and industry standards for the successful, sustainable commercialization of their product.

Considering these factors, adverse impacts on domestic markets are unlikely to derive from approval of the petition. While DHA canola would require strict segregation in supply chains, it would present no more risk for commingling than do other IP products (e.g., canola, corn, cotton).

4.8.2 International Trade

No Action Alternative: Trade Economic Environment

Denial of the petition would have no effect on the international trade of canola oil or meal. The current availability and use of commercially cultivated GE and non-GE canola are expected to remain the same under the No Action Alternative.

Preferred Alternative: Trade Economic Environment

When evaluating market demand among dietary supplement, food and beverage, pet nutrition, infant nutrition, pharmaceuticals, and clinical nutrition sectors, it has been estimated that by 2020 demand for supplemental omega-3 PUFAs such as EPA and DHA, globally, may reach 241 thousand metric tons, at a value of $4.96 billion (R&M 2014). This equates to a compound annual growth rate of almost 10% (R&M 2014). DHA canola would serve the same global uses in provision of canola oil and meal, with the added provision that the oil would be comprised of EPA and DHA. Considering EPA and DHA are almost exclusively derived from finite marine sources such as finfish, krill, squid, and algae, DHA canola, as an alternative/supplemental source, could prove useful to international markets where there were a demand for vegetable oils and whole seed comprised of EPA and DHA.

The trade of DHA canola products would be subject to the laws, regulations, and policy of the importing country, which are impacted by international treaties, agreements, and other arrangements. International trade is facilitated by the World Trade Organization (WTO) and the Organization for Economic Cooperation and Development (OECD 2015; WTO 2015c). Standards and guidelines for the safety evaluation and trade of GE crop commodities are established under international policy and agreements such as the Codex Alimentarius (FAO 2009), the WTO International Plant Protection Convention (WTO 2015a), WTO Sanitary and Phytosanitary Measures (WTO 2015b), WTO Technical Barriers to Trade Agreement (WTO 2015c), and the Cartagena Protocol on Biosafety (CBD 2015a).

As with all GE crop commodities, there exist the potential for low level presence (LLP) occurring in countries importing U.S. agricultural commodities. LLP situations occur in the importing country when there is asynchrony between the authorization of the exporting country and that of the importing country; an issue described as an “asynchronous approval” (AA). LLP is generally described as a situation where there is authorization of a particular GE commodity by one or more exporting countries, but authorization is still pending or has not been requested in the importing country. The issue of AA, and resulting LLP situations, can lead to trade delays, shipment rejection, and costs to traders (FAO 2014). AA can also result in the diversion of shipments to other markets by some exporters, and rejection of agricultural products by importers due to zero tolerance policies for the presence of unauthorized GE materials in shipments (Frisvold 2015; WTO 2015c). Incidents of LLP can lead to income loss for exporters and importers, and consequently for producers. Consumers in importing countries can also, potentially, face higher domestic commodity prices when an import is deterred or directed to another trading partner (Atici 2014b).

In addition to situations arising from AA and LLP, trade can also be impacted by moratoria, or bans on the import or use of GE crops or crop products. These bans can be explicit as a result of legislation, or de facto. De facto bans may occur if a country does not have a GE product decision making framework, or chooses to take no action regardless of its existing decision making framework.

Nuseed states in their petition that they are or will be seeking approvals for marketing of DHA canola with U.S. trading partners. Nuseed submitted an application for authorization of foods derived from DHA canola to Food Standards Australia New Zealand in February of 2017. Submissions for food, feed, and environmental approval will be made to Health Canada and the Canadian Food Inspection Agency. Submissions will also be made for import approvals in Mexico, Japan, South Korea, China, European Union, and other countries as required. Nuseed states they are committed to product stewardship prior to and after all relevant authorizations are granted (Nuseed 2017).

In general, developers have various legal, quality control, and marketing incentives to implement rigorous stewardship measures to ensure IP, prevent commingling, and avoid AA and LLP. By necessity, all international regulatory and industry standards and requirements must be met for marketing of DHA canola commodities. As discussed for potential impacts on domestic markets, Nuseed developed a closed-loop IP system and stewardship plan to ensure that all DHA canola materials are isolated to maintain product identity throughout production and marketing processes. Based on these factors, it is assumed that there will be implementation of and strict adherence to stewardship requirements to maintain the integrity DHA canola crop commodities, reduce legal exposure, and potential loss of standing in the market.

5 CUMULATIVE IMPACTS

CEQ NEPA implementing regulations (40 CFR 1508.7) define a cumulative impact as an "impact on the environment which results from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency [federal or non-federal] or person undertakes such other actions." Emissions of air pollutants from a multitude of individual sources, and impairment of air quality, is an example of a cumulative environmental impact.

5.1 Assumptions and Uncertainties

If there are no direct or indirect impacts associated with those aspects of the human environment discussed in Chapter 4 APHIS assumes there can be no cumulative impacts. Further assumptions and uncertainties that are part of evaluation of potential cumulative impacts are summarized as follows.

Nuseed's studies of DHA canola demonstrated that, except for the fatty acid profile in the seed, DHA canola is agronomically, phenotypically, phenologically, and compositionally the same as the parental AV Jade line and other conventionally bred canola varieties. APHIS assumes that DHA canola only differs, as it pertains to the genetic modification, in that it is comprised of the described enzymes and fatty acids in the seed (Appendix A).

5.1.1 Potential Future Uses of DHA Canola

In February 2017, Nuseed submitted data required for safety and nutritional assessments for food and feed derived from DHA canola to the Food Standards Australia New Zealand. In March 2017, Nuseed initiated consultation with the U.S. FDA for evaluation of the safety of food and feed derived from DHA canola (Nuseed 2017). It also intends to submit for food, feed, and environmental approvals with Health Canada and the Canadian Food Inspection Agency, and for import approvals in Mexico, Japan, South Korea, China, the European Union, and other countries as required (Nuseed 2017).

If APHIS approves the petition it is assumed that DHA canola will be commercially marketed for food and feed purposes in the United States and abroad. It is recognized that consumer preference for a GE canola oil enriched in omega-3 fatty acids is uncertain, and that many consumers may not prefer DHA canola oil. Meta-analyses of consumer behavior still show that consumers as a whole are willing to pay more for non-GE food products, with a willingness to pay an extra 29% – 45% more to avoid GE foods (Wunderlich and Gatto 2015). Overall, 40% of consumers report avoiding GE foods in their diet, 71% of whom were worried about health repercussions and 48% wanting to know "exactly what goes into the food [they] eat"(Wunderlich and Gatto 2015). While consumer preference trends toward non-GE foods, APHIS assumes there will be marginal use of DHA canola in the food and feed industries (subject to FDA consultation), with initial use likely greater in the animal feed industry.

DHA canola and any progeny derived from it could also be combined with other nonregulated GE and non-GE canola varieties through traditional breeding techniques. For example, DHA canola could be crossbred with other nonregulated disease resistant canola varieties that protect against yield loss from sclerotinia stem rot (fungal pathogen) and damping-off (wirestem), as well as HR varieties. It is assumed that these types of stacked-trait varieties would be produced only as a result of their potential utility; to expand grower choice and production efficiencies in the management of plant pests, pathogens, and agricultural weeds.

Whether DHA canola or progeny will be stacked with traits from any particular nonregulated GE canola variety, or non-GE cultivar, is uncertain. The adoption level of crossbred progeny of DHA canola would depend on the extent to which producers valued the traits offered by such stacked-trait DHA canola varieties over other stacked-trait canola varieties, and the pricing and production efficiencies of such stacked-trait DHA canola varieties relative to other canola varieties (of which there are a substantial number). Consumer preference, or lack thereof, for GE canola oil comprised of EPA and DHA, derived from any future crossbred progeny, as well as feed manufacturer preference, would also determine the market viability of such progeny.

5.2 Cumulative Impacts: Acreage and Areas of Canola Production

It is possible that other canola varietals with enhanced LC-PUFA profiles may be developed and commercialized. Apart from Nuseed, Cargill, in partnership with BASF, is working on developing lines of canola containing EPA and DHA. Dow Agrosciences has likewise developed an EPA/DHA producing canola (Walsh et al. 2016). It is plausible that other oilseed crops with enhanced fatty acid profiles, such as sunflower, safflower, or flax, may also be developed and commercialized. For example, a GE camelina that produces EPA and DHA has been developed, although not commercially available in the United States (Ruiz-Lopez et al. 2014). If other types of omega-3 fatty acid producing oilseed varieties are developed and introduced into commerce, how these, in conjunction with DHA canola, may contribute in a cumulative manner to an increase in acreage used for oilseed crop production is uncertain. As the oil, whole seed, and meal would be similar if not the same commodities, EPA/DHA producing oilseed varieties would be expected to compete with each other for market share and planted in areas currently utilized for canola, sunflower, safflower, and flax production.

Apart from other plant based sources of DHA and EPA that may enter commercial production, such as GE camelina (Napier et al. 2015), engineered yeasts are being explored as an industrial-scale source (Xie et al. 2015), as well as large scale farming of marine microalgae (Adarme-Vega et al. 2012). These sources would serve to limit canola acreage.

Another factor potentially contributing to cumulative effects on canola acreage is the use of canola as a source of biodiesel. The primary feedstocks used to produce biodiesel and renewable diesel in the United States have been vegetable oils (namely soybean, corn, and canola oils) and waste fats and greases. According to U.S. Energy Information Administration data 4,906 million pounds of soybean oil and 1,044 million pounds of corn oil were used to produce biodiesel in the United States in 2015. Other significant sources of feedstock were yellow grease (1,232 million pounds), canola oil (745 million pounds), white grease (588 million pounds), tallow (429 million pounds), and poultry fat (190 million pounds).[30] While additional supplies of feedstocks could be produced by increasing the planted acres of oilseed crops (soy, canola, etc.), with the exception of palm oil, most vegetable oils are produced as a coproduct along with seed meal used for

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[30] EIA Biodiesel Production Report Available at http://www.eia.gov/biofuels/ biodiesel/production/archive/2015/2015_12/ biodiesel.pdf.

animal feed. Consequently, increased demand for vegetable oil feedstocks would not be expected to result in a significant increase in oilseed crop planting absent increased demand for the meal for animal feed.[31] In general, significant increases in the planted acres of oilseed crops for biodiesel are expected to be limited by competition for arable land from other higher value crops and demand for the animal feed co-products produced by most oilseed crops.

Considering these factors, and that consumer acceptance/preference of GE oilseeds remains to be seen, providing a reasonably accurate estimate of the potential cumulative effects of DHA/EPA producing oilseed crops on acreage, beyond those upper bound estimates provided in Section 4.2 – Acreage and Areas of Canola Production, is not possible. In general, total production (acreage) will be relative to market demand for conventional canola oil and meal, modified oilseeds comprised of LC-PUFAs, and biodiesel. Growers will elect to produce the GE oilseed varietal that supplies demand and provides them optimal net returns. Any cumulative impact on total oilseed crop acreage that may derive from the availability of DHA canola and other varietals of EPA/DHA producing oilseed crops is expected to be negligible, as total acreage will be limited by market demand for EPA/DHA enriched vegetable oils, and such varietals, to the degree they are accepted by consumers, will compete for market share (acreage).

5.3 Cumulative Impacts: Agronomic Practices and Inputs

Agronomic practices and inputs used for DHA canola production would be no different than those currently used, consequently, the types of potential cumulative impacts that derive from these practices and inputs, namely those on physical and biological resources, are the same under both alternatives. The only difference between the alternatives would be relative to any increase in acreage for DHA canola crop production, discussed above, and how this may expand the range of potential cumulative impacts. Potential cumulative impacts on physical and biological resources related to agronomic practices and inputs are discussed in more detail in the following sections.

5.3.1 Pest and Weed Resistance Management

By its very nature, the development of pest and weed resistance is a cumulative effect, particularly when adjacent cropping systems do not implement recommended IWM/IPM programs. As summarized in Chapter 4, to date, HR weeds in canola have not emerged as a significant problem in the United States. No HR weeds have been reported for canola crops in North Dakota. During the last 15 years, the only reports for U.S. canola crops have been HR Italian ryegrass in Idaho and HR green foxtail in Montana – both of these reported in 2005 (Heap, 2017). DHA canola cropping systems, to include progeny, will however have the potential to contribute in a cumulative manner to the emergence of HR weed populations, insecticide resistant pest populations, and resistant pathogen populations, just as all other cropping systems that use pest and weed controls do.

Approval nor denial of the petition would not have any effect on grower options or choices in the management of pests, diseases, and weeds over the coming years. As discussed in Sections 3.3 and 4.3, academia, weed and pest specialists, and the U.S. EPA through further refining of IWM

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[31] Renewable Fuel Standard Program: Standards for 2017 and Biomass-
Based Diesel Volume for 2018 (EPA–HQ–OAR–2016–0004). Federal Register / Vol. 81, No. 238 / Monday, December 12, 2016 / Rules and Regulations, https://www.gpo.gov/fdsys/pkg/FR-2016-12-12/pdf/2016-28879.pdf]

and IPM strategies, are addressing resistance management. In 2017, the U.S. EPA issued PR Notice 2017-2, *Guidance for Herbicide-Resistance Management, Labeling, Education, Training and Stewardship* (US-EPA 2017c). The U.S. EPA guidance is part of a more holistic, proactive approach involving crop consultants, agricultural commodity organizations, professional /scientific societies, researchers, and the pesticide registrants themselves. PRN 2017-2 provides 11 elements focused on labeling, education, training, and stewardship strategies. The U.S. EPA also issued PRN 2017-1, *Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling* (US-EPA 2017e). PRN 2017-1 is aimed at improving information on pesticide labels about how pesticide users can minimize and manage pest resistance.

It is assumed that the majority of growers who adopt DHA canola will increasingly employ management practices recommended by the U.S. EPA, WSSA, and university extension services to help deter the development of HR weeds, and development of insect and pathogen resistance, as there are economic and practical incentives for doing so (Fernandez-Cornejo et al. 2014; Fernandez-Cornejo and Osteen 2015; Livingston et al. 2015).

5.4 Cumulative Impacts: Physical Environment

DHA canola production would entail the use of pesticides and fertilizers, and to some extent tillage, which will contribute to potential cumulative impacts on water, soil, and air quality, as does production of current canola varieties. For water, these impacts would be relative to the proximity of surface waters to DHA canola crops and depth of the water table. For air, there would be a contribution to the cumulative emissions of NAAQS pollutants and greenhouse gases. Because the agronomic practices and inputs used in the cultivation of DHA canola would be no different from current canola varieties, the potential cumulative impacts on water, soil, and air quality would be no different than that already occurring with current canola production. Any use of pesticides would be subject to U.S. EPA as well as state requirements.

However, if total U.S. canola acreage increases due to DHA canola adoption in the market, there would be a commensurate increase in the contribution to total agricultural inputs (pesticides and fertilizers) as well as NAAQS emissions, relative to the amount of increased acreage. As discussed above for Acreage and Areas of Canola Production, and in Section 4.2, due to the uncertainty involved in the market viability of DHA canola, and other EPA/DHA sources in development (e.g., GE plant, yeast, and marine microalgae), provision of reasonably accurate quantitative data per potential cumulative effects on agricultural inputs and NAAQS emissions is not possible. A maximum increase in acreage would likely be less than 400,000 acres (Section 4.2), and this accumulating over a period of years, perhaps a decade or more. In general, if DHA canola is accepted by consumers, there may be a marginal increase in canola acreage, with commensurate cumulative effects on total agricultural inputs and NAAQS emissions. Attempting to quantify any potential cumulative increase in agricultural inputs and emissions in light of the uncertainties discussed, and how these may present environmental and human health risks, would be an exhaustive exercise beyond the scope of this EA. Agricultural inputs vary widely depending on the fertilizer (e.g., N,P, K,S) and soil type, as well as among the various pesticides used (e.g., lbs/active ingredient/acre, and inherent toxicity of the active ingredient to biota). In regard to any increase in total pesticide use, it is not the mass of the pesticide used that is of salient concern, it is the potential toxicity of the pesticide, which can vary widely. NAAQS emission data is not available for cropland because NAAQS emissions on croplands are not monitored as regulated emissions.

On the other hand, because commercial production of DHA canola may also, to some degree, alleviate pressure on fisheries as a source of EPA/DHA (discussed following in Subsection 5.5.3), there could be commensurate reductions, to some degree, in NAAQS emissions associated with extraction of EPA/DHA from commercial fisheries. Similarly, in concept, direct emissions from canola production operations utilizing fossil fuels are potentially offset by use of canola for biodiesel production. In 2016, approximately 1.1. billion lbs of canola oil was used as biodiesel feedstock (EIA 2017b). U.S. EPA analyses have determined that use of biodiesel (as B100 or a B20 blend) instead of conventional diesel results in reductions in hydrocarbons, particulate matter, and carbon monoxide emissions, although increases nitrogen oxide emissions (US-EPA 2002). While such potential offsets in emissions exists, due to the value added nature of DHA canola and its intended market, it is unlikely DHA canola would be used for biodiesel production purposes.

As discussed in Chapter 3 and 4, the potential cumulative impacts of agricultural activities on air, water, and soil quality are well recognized and several federal and state cooperative initiatives address these issues with the purpose of mitigating cumulative impacts. In 2012, the USDA NRCS launched NWQI, in collaboration with the U.S. EPA and state water quality agencies, to reduce fertilizer and sediment run-off in small high-priority watersheds in each state (US-EPA 2017i). These priority watersheds have been selected by NRCS State Conservationists in consultation with state water quality agencies and NRCS State Technical Committees where targeted on-farm conservation investments will deliver the greatest water quality benefits. NWQI provides a means to accelerate voluntary, private lands conservation investments to improve water quality with dedicated financial assistance through NRCS's Environmental Quality Incentives Program and Clean Water Act Section 319 or other funds to focus state water quality monitoring and assessment efforts where they are most needed to track change. A key part of the NWQI targeting effort includes the implementation of conservation systems that avoid, trap, and control run-off in watersheds.

In the 2006 Particulate Matter (PM) NAAQS and 2008 Ozone NAAQS preambles, the U.S. EPA recommended that in areas where agricultural activities have been identified as a contributor to a violation of the NAAQS, when properly implemented to control airborne emissions of the desired NAAQS pollutant, USDA-approved conservation systems and activities may be implemented to achieve reasonably available control measures and best available control measures. The USDA and the U.S. EPA provide guidance for regional, state, and local regulatory agencies on how to manage agricultural air emissions (USDA-EPA 2012). These measures allow stakeholders the flexibility in choosing which measures are best suited for their specific situations/conditions and desired purposes.

Considering these factors, approval of the petition is not anticipated to result in any significant cumulative impacts on water quality or use, or soil or air quality, relative to the No Action Alternative. APHIS has not identified any changes in the agronomic practices used for cultivation of DHA canola, or its progeny, that would present any novel risks to the physical environment.

5.5 Cumulative Impacts: Biological Resources

As discussed in Chapter 4, it is highly unlikely the transgenes, gene products, and fatty acids in DHA canola present any risk to wildlife, to include plant pests. Because the agronomic practices and inputs for DHA canola are the same as for other canola varietals, approval of the petition would not present any novel risks to biological resources with respect to chemical inputs. Cultivation of DHA canola would not be expected to directly, indirectly, or cumulatively impact biological resources any differently than cultivation of current canola cultivars.

5.6 Gene Flow and Weediness

DHA canola will likely contribute in a cumulative manner to the diversity of stacked-trait feral canola populations in proximity to canola crop fields and along transport routes. As discussed in Subsection 3.5.3 - Gene Flow and Weediness of Canola, feral GE canola is fairly widespread and persists in North Dakota as populations are founded by seed spills along transport routes, from the continuous recruitment of seed from feral soil seedbanks, and pollen flow (Devos et al. 2012). Populations of wild *Brassica* species commonly occur throughout the United States and may hybridize with feral GE canola to produce novel genotypes (Knispel et al. 2008; Schafer et al. 2011). Some populations of feral GE HR canola and wild type *Brassica* hybrids have exhibited multiple GE traits (Warwick et al. 2008; Devos et al. 2012).

Based on the PPRA, APHIS concluded that introgression from DHA canola to certain species of wild *Brassica* spp. is possible; this would apply to any progeny derived from DHA canola (USDA-APHIS 2018a). Pollen and seed from DHA canola and its progeny would likely be distributed to areas adjacent to commercial crop fields, and seed distributed along transport routes, both contributing to the development of feral populations of GE canola, and GE canola x wild type *Brassica* hybrids. Currently, the only other GE canola varieties produced commercially are those that are herbicide resistant. DHA canola would, over time, likely add to the mix of traits in feral hybrid populations; these from extant GE HR and conventional varieties, and those GE HR and conventional varieties that will be developed and commercially produced in the future.

While the biosynthesis pathways in DHA canola have been modified using genes from microalga and fungi, there is no reason to believe the desaturase and elongase enzymes, as well as the fatty acids these synthesize, present any risk to brassica species with which DHA canola may hybridize. Plants synthesize a wide variety of fatty acids, and various desaturase and elongase enzymes and fatty acid biosynthesis pathways are common among the plant kingdom (Harwood 2017). Long-chain fatty acids are synthesized de novo via acetyl-CoA carboxylase and fatty acid synthase. The end products of this synthesis are usually the saturated fatty acids palmitate and stearate (Harwood 2017). Once the long-chain fatty acids have been produced they can be subject to elongation, desaturation, and further modifications. As discussed in 4.5.2 – Wildlife Communities, the Δ12 desaturase, omega-3-/Δ15 desaturase, Δ6 desaturase , Δ6 elongase, Δ5 desaturase, Δ5 elongase, and Δ4 desaturase share homology with other desaturase and elongase enzymes isolated from plants. Apart from the fatty acid profile in the seed, DHA canola is phenotypically and phenologically the same as its parental line (AV Jade) and other non-GE canola varieties (Nuseed 2017).

Currently, feral GE canola populations have not proven to be a particular control problem; canola is not an invasive plant and feral GE populations are largely limited to disturbed sites (e.g., disturbed lands adjacent to commercial canola fields and transport routes) (Katsuta et al. 2015; Belter 2016). Canola is generally regarded as an opportunistic species, not as an invasive species of ecological concern. In undisturbed natural habitats, canola lacks the characteristics that provide for establishment of stable populations, and once established, feral GE populations, in the absence of seed dispersal, trend toward extinction over a period of years (Warwick et al. 2008; Devos et al. 2012; Belter 2016). For GE feral canola and wild type hybrids, where feral and/or hybrid populations need control or removal, activities involving the use of synthetic chemicals and mechanical means to control or remove feral or hybrid populations could adversely affect biota in these areas, albeit temporarily. Management with herbicides and hand pulling of feral plants along roadsides has resulted in effective control, if not eradication, of feral populations (Munier et al. 2012). While control or removal of feral hybrid populations may be warranted in some instances, and could adversely impact biota in these areas, such impacts are expected to be transient in nature, with little influence on the long-term integrity of plant and animal communities. In the event that DHA canola becomes feral or hybridizes with wild type canola, it is unlikely that this would lead to management issues different from what is occurring with current canola varieties.

The persistence or recurrence of a feral GE canola populations and associated hybrids in a given location will depend on the frequency of seed spills and pollen flow (Warwick et al. 2008; Devos et al. 2012). Seed dispersal via transportation has largely contributed to the distribution of feral GE canola populations in North Dakota, although re-seeding by fertile hybrid plants further contributes to population persistence (Schafer et al. 2011). The same would apply to DHA canola.

In summary, DHA canola will likely contribute in a cumulative manner to the diversity of stacked-trait feral canola populations near canola crop fields and along transport routes. However, because the DHA canola trait genes and gene products are unlikely to confer any competitive advantage or disadvantage to *Brassica* species with which it can hybridize, nor are the introduced biosynthesis enzymes considered a risk to plant viability, the potential for DHA canola to contribute to adverse cumulative impacts on plant an animal communities via gene flow are considered unlikely.

5.7 Fisheries

Of consideration is a potential benefit from market adoption of DHA canola to alleviation/reduction in pressure on commercial fish stocks. This is more a theoretical outcome, the benefits of which may be only nominal, but nonetheless a possibility (Racine and Deckelbaum 2007; Nichols et al. 2010; Salem and Eggersdorfer 2015; Tocher 2015). Fish are the primary dietary source of EPA and DHA. The majority of fisheries, globally, operate at maximum withdraws per annum to supply fish for human consumption, as well as supplying feed for industrial fish farms and fish oil for supplements (FAO 2016). Based on the Food and Agriculture Organization’s analysis of assessed commercial fish stocks, the share of fish stocks within biologically sustainable levels decreased from 90 % in 1974 to 68.6 % in 2013 (FAO 2016). Thus, 31.4 % of fish stocks are estimated as overfished. Of the total number of stocks assessed in 2013, fully fished stocks accounted for 58.1 % and under-fished stocks only 10.5 %

(FAO 2016). To sustain fisheries, the National Oceanic and Atmospheric Administration (NOAA) imposes catch limits on all 46 of major U.S. fisheries (NOAA 2017).

In general, the capture of wild fish peaked in the early 1990s and has been fairly constant since at about 90 million metric tons per year. The annual yield of fish via aquaculture has grown substantially since the early 1990s and is now comparable to that the wild fisheries. The primary fish sourced for EPA/DHA is anchovy, with most of this obtained from the Peruvian fisheries. Menhaden, cod, whiting, carp, mackerel, tuna, salmon, pollock, capelin, and sardine, in roughly that order, are the other primary species of fish harvested (Salem and Eggersdorfer 2015).

In order to conserve fish stocks and provide the dietary needs of EPA/DHA for an increasing global population, it is argued that alternative sources for LC-PUFAs will be required (Lenihan-Geels et al. 2013; Salem and Eggersdorfer 2015). Plants high in omega-3 PUFA, such as linseed, primrose, echium, and hempseed, contain only shorter-chain omega-3 PUFAs and no, or low levels of EPA and DHA (Salem and Eggersdorfer 2015). Currently explored alternatives include GE plant sources (canola, camelina), yeast, and farming of marine microalgae (Lenihan-Geels et al. 2013). EPA/DHA production via farmed marine microalgae can in the future be increased, although this is likely to remain more expensive than fish and terrestrial plant derived oils. Cost, extraction, and purification methods are currently limiting the potential of using microalgae at an industrial-scale (Lenihan-Geels et al. 2013).

While the United States has no official Recommended Daily Allowance for EPA or DHA, there is a general consensus among health professionals that a daily individual intake (recommended intake - RI) of > 500 mg/day of EPA/DHA is required for optimal health and disease prevention (Calder 2014). Dietary recommendations for EPA and DHA range from 250 to 1000 mg/day for adults and from 40 to 250 mg/day for infants older than six months, children, and adolescents (Weylandt et al. 2015). In April 2016, the Global Organization for EPA and DHA Omega-3s (GOED 2018), a United States-based trade association, endorsed a daily recommendation of 500 milligrams, in line with the Academy of Nutrition and Dietetics (Vannice and Rasmussen 2014). Assuming daily intakes of 500 mg/day; for about 7.6 billion people (global population as of 2017) this equates to a daily demand of about 3,800 metric tons, and annual demand of about 1.3 million metric tons of EPA/DHA (Salem and Eggersdorfer 2015).

Globally, most individuals do not consume the RI. For example, in the United States, consumption of DHA and EPA from foods contributes a very small amount to total daily omega-3 intake (about 40 mg in children and teens and about 90 mg in adults) (NIH 2017). Data from the 2012 National Health Interview Survey indicate that 7.8% of U.S. adults and 1.1% of U.S. children use supplements containing fish oil, omega-3s, and/or DHA or EPA (NIH 2017). Based on the National Health and Nutrition Examination Survey, use of fish oil supplements only adds about 100 mg to mean daily ALA intakes, 10 mg to mean DHA intakes, and 20 mg to mean EPA intakes in adults (NIH 2017).

Estimates of the present global fish supply of omega-3 LC-PUFAs range from about 0.2 to 0.8 million metric tons (200 kilograms) on an annual basis (Salem and Eggersdorfer 2015; Tocher 2015). Given an annual demand of about 1.3 million metric tons of EPA/DHA, this is a general shortfall in the global supply of RI of about 0.5 to 1.1 million metric tons per annum, relative to a RI of 500 mg/day. The majority of EPA/DHA supply (almost 90%) is from wild fisheries, with relatively small amounts derived from aquaculture and algal sources (Tocher 2015). With wild fish stocks from which EPA/DHA are obtained finite in supply and under pressure, there is a general consensus among many scientists that alternate sources of dietary EPA/DHA and other LC–PUFAs need to be secured to ensure sufficient supplies can be sustained (Racine and Deckelbaum 2007; Nichols et al. 2010; Salem and Eggersdorfer 2015; Tocher 2015). It is possible that a plant based source such as DHA canola could help address a growing global demand for EPA/DHA and other omega-3 and omega-6 LC-PUFAs, and relieve, to some degree, pressure on wild fish stocks, as well as farmed fish sources. For example, 1 hectare (2.47 acres) of DHA canola has the potential to provide the omega-3 fatty acid oil yield comparable to 10,000 kilograms (10 metric tons) of fish (Sprague et al. 2017). To what degree utilization of DHA canola could help reduce pressure on finite marine fisheries, the extraction from which is limited through fish reproductive capacities and regulated catch quotas, is highly uncertain, as this will depend on consumer acceptance of GE vegetable oils in the coming years.

5.8 Cumulative Impacts: Human and Animal Health

5.8.1 Public Health

There are no adverse cumulative impacts on human health associated with a decision to deny the petition. Approval of the petition would result in the potential provision to commercial markets of a vegetable oil comprised of EPA and DHA. To the extent DHA canola oil contributes to augmenting long-term sustainable and cost effective sources of EPA and DHA, there could be some cumulative benefits to public health. Various other EPA/DHA sources are being sought, to include large scale marine microalgae farming, GE camelina, other GE canola varieties, and yeast.[32] For example, a GE camelina that produces EPA and DHA has already been developed (Ruiz-Lopez et al. 2014), as well as an EPA and DHA producing yeast, although these are not yet commercially available. Farming of microalgae for production of EPA and DHA is expected to increase. Collectively, these sources would be expected to be of value to domestic and international markets, public health, and could serve to alleviate pressure on fisheries for provision of EPA/DHA.

5.8.2 Worker Safety

There are no reasonably foreseeable cumulative impacts on worker safety associated with production of DHA canola, nor any progeny that would derive from DHA canola. The agronomic practices an inputs used for DHA canola production would be no different than those for other canola varieties, consequently, potential impacts on worker safety would be the same.

5.8.3 Animal Feed

As discussed for human health, to the extent DHA canola oil contributes to augmenting long-term sustainable and cost effective sources of EPA and DHA, there may be cumulative benefits to the animal feed industry. Producers of livestock, poultry, and farmed fish would be expected to utilize DHA canola oil and perhaps whole seed to the extent they determined these provided,

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[32] https://www.nutraingredients-usa.com/Article/2010/09/16/Omega-3-from-GM-yeast-comparable-to-GRAS-fish-oil-DuPont-study

as a dietary component, optimal quality beef, swine, poultry, and fish. No potential cumulative impacts on animal health and welfare have been identified relative to denial of the petition.

5.9 Cumulative Impacts: Socioeconomics

5.9.1 Cumulative Impacts: Domestic Economic Environment

If the Nuseed petition is approved there could be indirect benefits of this decision on domestic markets – to the extent DHA canola was accepted by consumers and valued in the food and feed industries. Potential cumulative impacts on domestic markets are discussed below.

5.9.1.1 Organic, Non-GE, and Non-GMO Canola Production

As discussed in Section 4.8 – Socioeconomics, one of the challenges among organic, GE, and conventional crop production systems is preventing the accidental comingling among commodities derived from these cropping systems in order to protect product identity and price premiums. Potential adverse impacts to non-GE crop producers are those related to cross-pollination and commingling of GE crop material with non-GE crops or crop products, leading to instances of unintended presence. This is particularly important for identity preserved (IP) and organic crop commodities.

As of 2016, there were only 4 USDA certified organic canola farms in the United States, 2 in Pennsylvania, 1 in Indiana, and 1 in Iowa (USDA-NASS 2017d). Acreage and economic value data is not available in the USDA's census data; it was withheld by growers to avoid disclosing data for individual farms. Currently, data on organic canola production in the United States is limited.

Similar to the organic canola market, there is an expanding non-GMO canola market. These are products verified to contain GE trait material below an established threshold (e.g, food < 0.9% GE material by weight, feed < 5%),[33] but are not necessarily USDA certified organic products – non-GMO canola may be produced via conventional or organic means. The non-GMO verified market has expanded rapidly since 2007. According to the Non-GMO Project, Non-GMO Project Verified is the fastest growing label in the natural products industry, with more than 3,000 verified brands representing around 43,000 products, and annual sales of around $19.2 billion.[34] There are over 10 brands of canola oil bearing the "Non-GMO" verified label.[35] It should be noted that "Non-GMO" verified does not necessarily mean it was produced via an IP program.

Because DHA canola is agronomically and phenotypically (apart from the fatty acid profile) similar to existing GE and non-GE canola cultivars, it would present the same potential risks for cross-pollination and commingling with organic and conventional canola crops as current GE and non-GE canola varieties. If DHA canola were to cross-pollinate a canola produced for the organic or non-GMO markets, or commingle with commodities derived from these crops, it would reduce the value of that crop commodity Whether these contaminated commodities could still be sold to buyers of GE canola is uncertain. DHA canola potentially adds to the number and variety of GE traits in commerce that need to be segregated among GE, organic, and

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[33] The Non-GMO Project: http://www.nongmoproject.org/product-verification/

[34] https://www.nongmoproject.org/product-verification/

[35] https://www.nongmoproject.org/find-non-gmo/verified-products/results/?keyword=canola

conventional post-harvest processing chains. In this sense, there could be an additive effect on commercial canola and oilseed markets – potential costs incurred for segregation of DHA canola commodities from other canola and oilseed supply chains. For example, apart from Nuseed, Cargill, in partnership with BASF, is working on developing lines of canola containing EPA and DHA, as well as Dow Agrosciences (Walsh et al. 2016). It is possible that other oilseed crops with enhanced fatty acid profiles, such as sunflower, safflower, or flax, may also be commercialized. A GE camelina that produces EPA and DHA has likewise been developed, although not yet commercially available in the United States (Ruiz-Lopez et al. 2014). An increase in development and adoption of new varieties of GE crops would necessitate maintaining segregation of GE crop products from those produced via conventional, organic, "non-GMO" and identity preserved cropping systems and supply chains.

These factors considered, the availability of DHA canola, or its progeny, to commercial producers is not expected to contribute, in a cumulative manner, to adverse impacts on organic/non-GMO canola markets. Current and future canola producers targeting IP, organic, and non-GMO markets are expected to use a variety of measures to preserve the integrity of their production systems and commodities, to include those required by USDA organic standards; measures that would be unaffected by approval of the petition. DHA canola is expected to be produced using standard industry practices for IP crops (Nuseed 2018b, a), which segregates the harvesting and post-processing food chains to ensure integrity of the crop product (Sundstrom et al. 2002). Crop varieties with unique product quality traits, such as high oleic sunflowers, low linolenic canola, or high oil corn, require IP programs to channel these commodities to specific markets to capture their added value (Sundstrom et al. 2002). Similarly, organic commodities must be produced according to specific criteria and segregated in the marketplace in order to receive price premiums. Because DHA canola will be produced using standard industry IP practices (Nuseed 2018b, a), contamination of other crops or their products (unintended presence), and contamination of DHA canola products by other crops, is no more likely than that which exists among current canola commodity supply chains. If such occurred, these events would be expected to be of low incidence.

The economic impact to growers of organic, non-GMO, and IP commodities from such unintended presence would depend on the price premium affected. For instance, organic commodities can receive a significant price premium in the food and personal care products markets (e.g., from 30% to 500%) relative to the price of commodities derived from conventionally grown crops. Because "organic" and "non-GMO" commodities can, in most instances, be sold as "conventional" commodities, it is the price premium above the conventional price that represents a measure of the value affected by the unintended presence of GE plant material.

5.9.2 Cumulative Impacts: Trade Economic Environment

If APHIS approves the petition and DHA canola is not approved for import by other countries this could theoretically present the opportunity for low level presence (LLP) incidents.[36]

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[36] During canola harvesting, transport, storage, and processing trace amounts of seed or meal may become mixed with other canola varieties, despite the use of best management practices by industry. As a result, a GE canola that has not yet been approved by an importing country may unintentionally be present, at low levels, in shipments exported to that country. This is termed low level presence (LLP).

However, adverse cumulative impacts on U.S. exports via LLP under this scenario is considered unlikely. To preclude LLP events in other countries, subsequent to any approval of the petition, Nuseed will need to seek import approval for DHA canola. Approvals for the commercial production or import of DHA canola are being sought by Nuseed in Australia, New Zealand, Canada, Mexico, Japan, South Korea, China, the European Union, and other countries as required (Nuseed 2017). It is assumed that developers of future canola varieties derived from DHA progeny would consult with foreign regulatory authorities if they intended to market food and feed to international markets.

As with domestic markets, DHA canola could prove valuable to international markets where there were a demand for vegetable oil and animal feed comprised of omega-3 fatty acids. Growers will cultivate DHA canola and its progeny, in lieu of or in addition to other GE canola options, as well as conventional cultivars, to the extent it can meet global demand, and it provides growers benefits in the way of yields, production efficiencies, and net-returns. Considering these factors, there are no reasonably foreseeable cumulative effects on international trade that would likely derive from entry of DHA canola into commercial markets.

6 THREATENED AND ENDANGERED SPECIES

The Endangered Species Act (ESA) of 1973, as amended, is a far-reaching wildlife conservation law. The purpose of the ESA is to prevent extinctions of fish, wildlife, and plant species by conserving endangered and threatened species and the ecosystems upon which they depend. To implement the ESA, the U.S. Fish & Wildlife Service (USFWS) works in cooperation with the National Marine Fisheries Service (NMFS), together "the Services," as well as other Federal, State, and local agencies, Tribes, non-governmental organizations, and private citizens. Before a plant or animal species can receive the protection under ESA, it must be added to the Federal list of threatened and endangered wildlife and plants. Threatened and endangered (T&E) species are those plants and animals recognized for being at risk of becoming extinct throughout all or part of their geographic range (endangered species) or species likely to become endangered in the foreseeable future throughout all or a significant portion of their ranges (threatened species).

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

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

Once a species is added to the list, protective measures apply to the species and its habitat. These measures include protection from adverse effects of federal activities.

6.1 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. This 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 that request determination of nonregulated status of GE 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 under Section 7 of the ESA for biotechnology regulatory actions.

APHIS regulatory authority over GE organisms is limited to those GE organisms for which it has reason to believe might be a plant pest or those for which APHIS does not have sufficient information to determine that the GE organism is unlikely to pose a plant pest risk (7 CFR §340.1). In this case, Nuseed requests that the USDA APHIS consider that DHA canola is not a plant pest as defined by the PPA. After completing a PPRA, if APHIS determines that DHA canola seeds, plants, or parts thereof do not pose a plant pest risk, then this article would no longer be subject to the plant pest provisions of the PPA or to the regulatory requirements of 7 CFR part 340, and therefore, APHIS must reach a determination that this article is no longer regulated. As part of its EA, APHIS analyzed the potential effects of DHA canola on the environment including any potential effects to T&E species and critical habitat. As part of this process, APHIS thoroughly reviews GE product information and data related to the GE organism to inform the ESA effects analysis and, if necessary, the biological assessment. For each transgene/transgenic plant the following information, data, and questions are considered by APHIS:

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

In following this review process, APHIS, as described below, has evaluated the potential effects that a determination of nonregulated status for DHA canola may have, if any, on Federally-listed T&E species and species proposed for listing, as well as designated critical habitat and habitat proposed for designation.

6.2 Potential Effects of DHA Canola on T&E Species

As discussed in further detail elsewhere in this EA and in the petition (Nuseed 2017), DHA canola contains inserted genes that express seven fatty acid desaturases and elongases that convert oleic acid to EPA and DHA. The seven fall into 3 groups, two yeast acyl-CoA type fatty acid desaturases (Lackl-Δ12D and Picpa-ω3D); two microalgae fatty acid elongases (Pyrco-Δ5E and Pyrco-Δ6E); and three algae front-end desaturases (Micpu-Δ6D, Pavsa-Δ5D and Pavsa-Δ4D). In addition, DHA canola also contains the *pat* gene from *Streptomyces viridochromogenes* that expresses the herbicide selection marker protein phosphinothricin N-acetyltransferase (PAT) that confers glufosinate tolerance. The PAT coding sequence was only used as a selection marker during the transformation stage and was not used for selection during the breeding process. The PAT protein is present in many commercial biotechnology-derived crops and has an extensive history of safe use. *Streptomyces viridochromogenes* is a widespread saprophytic, soil-borne bacteria with no known safety issues (US-FDA 2017).

Nuseed has presented results of field trials comparing DHA canola and reference varieties at ten sites in major canola growing regions of Canada and Australia (Nuseed 2017). These regions are typical of the areas where most of the U.S. canola crop is grown, in particular North Dakota (USDA-APHIS 2018a). The reference lines represent a diverse range of natural variability of cultivars typically grown. Based on the information submitted by the applicant and reviewed by APHIS, DHA canola is agronomically similar to other canola varieties currently grown (Nuseed 2017; USDA-APHIS 2018a). The common agricultural practices that would be carried out in the cultivation of DHA canola are not expected to deviate from current practices, including the use of U.S. EPA-registered pesticides. It is possible that additional acreage may be utilized for DHA canola production. However, as discussed in Subsection 4.2, Acreage and Areas of Canola Production, any increased acreage allotted to DHA canola production is expected to be limited. DHA canola, if adopted, would likely be planted in areas currently utilized for canola production (North Dakota), safflower (Montana, South Dakota), sunflower (North Dakota, South Dakota), and flaxseed (North Dakota).

Canola production is largely concentrated in the Northern Great Plains where a cooler climate is more amenable to production. As of 2012 (latest census data) there were 3,995 canola farms across 34 states (USDA-NASS 2014b). In 2017, the area of canola acreage planted and harvested totaled about 2.0 million acres (USDA-NASS 2018a). While canola is produced in many states, around 90% of U.S. production occurs in North Dakota, with significantly less production occurring in Oklahoma, Montana, and other states (see Subsection 3.2.2 – U.S. Production: Conventional, GE, and Organic Canola). Considering that seed derived from DHA canola is not expected to expand production to areas beyond where canola is currently grown, the effects analysis could be limited to geographic areas within the 34 states where canola is currently grown. APHIS considered this limitation, but rejected it because of the difficulty of compiling a species list solely for specific canola growing areas; the likelihood that the analysis may not identify any stressors that could affect species or habitat; the issue that canola may naturalize in the environment; and the potential for *Brassica napus* to cross with wild relatives. Instead it was decided to consider effects on all listed and proposed species and all designated and proposed critical habitat in all 50 states. APHIS obtained and reviewed the USFWS list of T&E species (listed and proposed) for all 50 states from the USFWS Environmental Conservation Online System (USFWS 2018).

For its analysis on T&E plants and critical habitat, APHIS focused on the agronomic differences between the regulated article and canola 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 T&E animals, APHIS focused on the implications of exposure to the seven fatty acid desaturases and elongases, and the PAT protein expressed in DHA canola as a result of the transformation (Nuseed 2017), and the ability of the plants to serve as a host for a T&E species.

6.2.1 Threatened and Endangered Plant Species and Critical Habitat

Taxonomic proximity increases the likelihood of plants' ability to cross with one another. In order to determine if any federally protected species could potentially be at risk from nearby DHA canola one would need to identify protected species that are closely related to *Brassica napus*. Using the USFWS Environmental Conservation Online System, all species from the plant family *Brassicaceae* that are federally listed as Endangered or Threatened, as well as all those species that have been proposed for listing and candidate species for listing were isolated (Table 6-1). None of the federally protected, proposed, or candidate species fall under the genus *Brassica* (USFWS 2018). All species currently fall within other genera and are therefore relatively unlikely to hybridize with *Brassica*. Plant taxonomists group plant genera into Tribes within a plant family. Among the plant tribes within the family *Brassicaceae*, some are more closely related to the *Brassiceae*, the tribe that *Brassica* falls within, and some are less related. While hybridization is common among closely related species of *Brassicaceae*, there is no evidence that divergent groups hybridize (Bailey et al. 2006; Franzke et al. 2011).

Table 6-1. Federally Listed Threatened and Endangered Species (plus Proposed and Candidate Species) of the Family Brassicaceae in the Lower 48 U.S. States, Hawaii and Alaska

Scientific Name	Common Name	Tribe	Federal Listing Status	States Occurring
Arabis georgiana	Georgia rockcress	Arabideae	Threatened	AL, GA
Arabis hoffmannii	Hoffmann's rock-cress	Arabideae	Endangered	CA
Arabis macdonaldiana	McDonald's rock-cress	Arabideae	Endangered	CA, OR
Arabis perstellata	Braun's rock-cress	Arabideae	Endangered	KY, TN
Arabis serotina	Shale barren rock cress	Arabideae	Endangered	VA, WV
Boechera pusilla	Rockcress, Fremont County	Boechereae	Candidate	WY
Cardamine micranthera	Small-anthered bittercress	Cardamineae	Endangered	NC, VA
Caulanthus californicus	California jewel flower	Schizopetaleae	Endangered	CA
Erysimum capitatum var. *angustatum*	Contra Costa wallflower	Camelieae	Endangered	CA
Erysimum menziesii	Menzies' wallflower	Camelieae	Endangered	CA
Erysimum teretifolium	Ben Lomond wallflower	Camelieae	Endangered	CA
Eutrema penlandii	Penland alpine fen mustard	Eutremeae	Threatened	CO
Leavenworthia crassa	Fleshy-fruit gladecress	Cardamineae	Endangered	AL
Leavenworthia exigua laciniata	Kentucky glade cress	Cardamineae	Threatened	KY
Leavenworthia texana	Texas golden Gladecress	Cardamineae	Endangered	TX
Lepidium arbuscula	`Anaunau	Lepideaea	Endangered	HI
Lepidium barnebyanum	Barneby ridge-cress	Lepideaea	Endangered	UT

Lepidium orbiculare	Round pepperweed	Lepideaea	Proposed Endangered	HI
Lepidium ostleri	Peppergrass, Ostler's	Lepidieae	Candidate	UT
Lepidium papilliferum	Slickspot peppergrass	Lepideaea	Threatened	ID
Lesquerella congesta	Dudley Bluffs bladderpod	Physarieae	Threatened	CO
Lesquerella kingii ssp. *bernardina*	San Bernardino Mountains bladderpod	Physarieae	Endangered	CA
Lesquerella lyrata	Lyrate bladderpod	Physarieae	Threatened	AL
Lesquerella pallida	White bladderpod	Physarieae	Endangered	TX
Lesquerella perforata	Spring Creek bladderpod	Physarieae	Endangered	TN
Lesquerella thamnophila	Zapata bladderpod	Physarieae	Endangered	TX
Lesquerella tumulosa	Kodachrome bladderpod	Physarieae	Endangered	UT
Physaria douglasii ssp. *tuplashensis*	White Bluffs bladderpod	Physarieae	Threatened	WA
Physaria filiformis	Missouri bladderpod	Physarieae	Threatened	AR, MO
Physaria globosa	Short's bladderpod	Physarieae	Endangered	IN, KY, TN
Physaria obcordata	Dudley Bluffs twinpod	Physarieae	Threatened	CO
Rorippa gambellii	Gambel's watercress	Cardamineae	Endangered	CA
Schoenocrambe argillacea	Clay reed-mustard	Schizopetaleae	Threatened	UT
Schoenocrambe barnebyi	Barneby reed-mustard	Schizopetaleae	Endangered	UT
Schoenocrambe suffrutescens	Shrubby reed-mustard	Schizopetaleae	Endangered	UT
Sibara filifolia	Santa Cruz Island rockcress	Schizopetaleae	Endangered	CA
Streptanthus albidus ssp. *albidus*	Metcalf Canyon jewelflower	Schizopetaleae	Endangered	CA
Streptanthus bracteatus	Twistflower, bracted	Schizopetaleae	Candidate	TX
Streptanthus niger	Tiburon jewelflower	Schizopetaleae	Endangered	CA
Thelypodium howellii spectabilis	Howell's spectacular thelypody	Schizopetaleae	Threatened	OR
Thelypodium stenopetalum	Slender-petaled mustard	Schizopetaleae	Endangered	CA
Thlaspi californicum	Kneeland Prairie penny-cress	Noccaeeae (alt. Lepideaea or Thlaspidae)	Endangered	CA
Thysanocarpus conchuliferus	Santa Cruz Island fringepod	Schizopetaleae	Endangered	CA

Warea amplexifolia	Wide-leaf warea	Schizopetaleae	Endangered	FL
Warea carteri	Carter's mustard	Schizopetaleae	Endangered	FL

Source: (Bailey et al. 2006; FitzJohn et al. 2007; Armstrong et al. 2012; Al-Shehbaz 2014; Kaneko 2014)

A review of the listed and proposed T&E plants indicates that none of them are classified in the same genus as that of the mustard varieties from which canola is derived (i.e., *Brassica rapa*, *B. napa*, *B. campestris* or *B. juncea*) (USFWS 2018). The review also indicates that there are no listed or proposed T&E plants that are sexually compatible with *Brassica* spp., so transgenic canola will not cross-pollinate with any T&E plant species. Therefore, there is no evidence indicating that DHA canola would directly affect any T&E plant species.

In conclusion, DHA canola and lines derived from it will require the same inputs and the same agronomic practices as used for canola varieties currently grown; the potential for increased weediness is no different than for other canola varieties; and there is no difference in the potential for gene movement to native plants, listed species, and species proposed for listing. In addition, there is no more likelihood for DHA canola or lines derived from it to naturalize in the environment, including designated critical habitat. Based on the analysis in the PPRA and this EA, APHIS has concluded that approval of a petition for nonregulated status for DHA canola, will have no effect on listed T&E plant species or species proposed for listing, and will not affect designated habitat or habitat proposed for designation.

6.2.2 Threatened and Endangered Animal Species

As discussed in Section 3.5.2.1 – Animals, the types and numbers of species found in and around commercial crop fields are less diverse as compared to unmanaged areas. Canola fields, however, can provide both food and habitat for some species of wildlife, including a variety of birds as well as large and small mammals.

Geese and blackbirds, for example, feed on canola seeds, while horned larks feed on emerging winter canola (Boyles et al. 2012; Schillinger and Werner 2016). Horned larks feed on the cotyledons of emerging canola, and typically do not eat the stem or seed (Schillinger and Werner 2016). Most animals that use canola fields are ground-foraging omnivores that feed on the remaining plant matter and associated biota following harvest. Small mammals of the Northern Great Plains that may be associated with canola fields are sagebrush voles (*Lemmiscus curtatus*), meadow voles (*Microtus pennsylvanicus*), shrews (Soricidae family), and deer mice (*Peromyscus maniculatus*) (Heisler et al. 2013; Heisler et al. 2014). Of the listed and proposed animal species in the main growing regions of North Dakota, Montana, and Oklahoma, it is conceivable that whooping cranes (*Grus americana*) may be exposed to DHA canola. In North Dakota, whooping cranes have short stops statewide during migration in the spring (late April to mid-June) and fall (late September to mid-October). During these migratory periods, whooping cranes reside in North Dakota for only a few weeks (USFWS 2018).

To assess any potential metabolite alteration as a result of the expression of the inserted genes, Nuseed analyzed the composition of DHA canola grown at eight field sites in major canola growing regions in Australia, in comparison with the parental variety, AV Jade, and commercial reference varieties representing a range of natural variability (Nuseed 2017). The compositional analysis included the following analytes: protein, fat, acid detergent fiber, neutral detergent fiber, crude fiber, ash, carbohydrates, FAs, AAs, vitamins, minerals, phytosterols and key anti-nutrients (Nuseed 2017).

The analytes for compositional assessment were selected considering the OECD revised consensus document (OECD 2011). Among the numerous compositional analyses that were carried out, concentrations of most analytes were not significantly different between DHA canola and control canola. Statistically significant differences were noted for concentrations of oleic and linolenic fatty acids; delta- and total tocopherols; magnesium; the glucosinolates progoitrin; and cholesterol (Nuseed 2017). The magnitudes of the differences were small, however, and in every case the ranges of values were all within the respective tolerance interval established using commercial canola varieties (OECD 2011). Overall, no consistent patterns emerged to suggest that biologically significant changes in composition or nutritive value of the seed had occurred as an unexpected result of the transformation process (Nuseed 2017). Based on the OECD guidelines for compositional equivalence, Nuseed has concluded that DHA canola was compositionally comparable to conventional canola except for the intentional production of the omega-3 fatty acids. There are no observed or anticipated unintended metabolic composition changes in the DHA canola that could impart any new plant pest or disease risk than non-GE canola varieties (USDA-APHIS 2018a).

Nuseed has provided data in its petition indicating that DHA canola is agronomically similar to canola varieties currently grown (Nuseed 2017). The phenotypic change, the alteration of the fatty acid profile, should have no effect on T&E animal species. As discussed in Sections 4.5.2 Wildlife Communities, 4.5.5 Biodiversity; and Appendix A, the elongase and desaturase enzymes, and fatty acids present in DHA canola are unlikely to present any risk to plant, animal, fungal, or bacterial communities. The same or functionally similar elongase and desaturase enzymes, and their fatty acid products, are ubiquitous among plants, animals, and microorganisms (Hashimoto et al. 2008; Lee et al. 2016; Garba et al. 2017). There are no toxins or allergens associated with DHA canola, as discussed in Section 4.6 – Human Health. The *pat* gene in DHA canola encodes for expression of phosphinothricin acetyltransferase (PAT), which confers resistance to the herbicide glufosinate-ammonium, but in this case at low levels for use as a selectable marker (Nuseed 2017). The *pat* gene was isolated from the soil bacterium *Streptomyces viridochromogenes*, which occurs in soils worldwide. Thus, the *pat* gene and its product, PAT, are naturally present in the environment, and humans and wildlife are potentially exposed to the *pat* gene and PAT on a daily basis. GE corn, soybean, and cotton plants expressing PAT have been widely grown in the United States, and globally, for over a decade, with no evidence of adverse environmental effects (Herouet et al. 2005). The safety of the PAT proteins has been previously established (Herouet et al. 2005; ILSI 2011). The safety of PAT in existing commercial transgenic crop products is supported by a permanent exemption from food and feed tolerances in all crops in the United States (US-EPA 2005).

APHIS considered the possibility that DHA canola could serve as a host plant for a threatened or endangered species (i.e., a listed insect or other organism that may use the canola plant to complete its lifecycle). A review of the species list reveals that there are no members of the genus *Brassica* that serve as a host plant for any threatened or endangered species (USFWS 2018).

Considering the similarity between DHA canola and other varieties currently grown and the lack of toxicity and allergenicity of the elongase and desaturase enzymes, their fatty acid products, and the PAT protein, APHIS has concluded that exposure and consumption of DHA canola would have no effect on threatened or endangered animal species, including whooping cranes that may come in contact with DHA canola.

6.3 Summary

After reviewing the possible effects of a determination of nonregulated status for DHA canola, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed T&E species or species proposed for listing any differently than canola varieties currently grown. Therefore, a detailed species by species analysis of effects is not necessary. APHIS also considered the potential effect of a determination of nonregulated status of DHA canola on designated critical habitat or habitat proposed for designation, and could identify no differences from effects that would occur from the production of other canola varieties. Canola is neither sexually compatible with, nor serves as a host species for any listed T&E species or species proposed for listing.

DHA canola and lines derived from it will require the same inputs and the same agronomic practices as used for canola varieties currently grown; the potential for weediness is no different than for other canola varieties; and there is no difference in the potential for gene movement to native plants, listed species, and species proposed for listing. In addition, compared to canola varieties currently grown, there is no more likelihood for DHA canola or lines derived from it to naturalize in the environment, including designated critical habitat. Consumption of DHA canola by any listed species or species proposed for listing will not result in a toxic or allergic reaction. Based on these factors, APHIS has concluded that a determination of nonregulated status for DHA canola, and the corresponding environmental release of this canola 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 ESA or the concurrences of the USFWS or NMFS are not required.

7 CONSIDERATION OF FEDERAL AND STATE LAWS AND REGULATIONS, EXECUTIVE ORDERS, STANDARDS, AND TREATIES

7.1 Federal Laws and Regulations

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

7.1.1 National Environmental Policy Act (NEPA)

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

7.1.2 Clean Air Act (CAA), Clean Water Act (CWA), and Safe Drinking Water Act (SDWA)

The CAA, CWA, and SDWA authorize the U.S. EPA to regulate air and water quality in the United States. Because DHA canola is agronomically equivalent to currently utilized canola varieties, the potential sources of adverse impacts on air and water quality are the same under the both No Action and Preferred Alternatives. DHA canola production would entail the use of pesticides and fertilizers, and to some extent tillage, which will contribute to potential cumulative impacts on air quality, and potentially water quality. However, the sources and degree of potential impacts would not be significantly different than that already occurring with current canola production. APHIS assumes use of pesticides on DHA canola will be compliant with U.S. EPA registration and label use requirements. The bar transgene in DHA canola occurs naturally in the bacteria *Streptomyces hygroscopicus*, found in soils worldwide. The desaturase and elongase transgenes and respective proteins occur naturally in soil bacteria, yeasts, and microalgae; hence, are widespread in the environment. The transgenes and gene products present no risk to water or air quality. Considering these factors, approval nor denial of the petition would lead to circumstances that resulted in non-compliance with the requirements of the CWA, CAA, and SDWA.

7.1.3 National Historic Preservation Act (NHPA)

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. Where DHA canola is cultivated there may be the potential for increased noise during the operation of machinery and other equipment, however, crop production activities would have only temporary effects on historic sites in the way of noise, with no consistent long-term effects on the enjoyment of a historical site.

7.2 Executive Orders Related to Domestic Issues

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

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

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

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

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

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

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

The No Action and Preferred Alternatives have been evaluated with respect to EO 12898, EO 13045, and EO 13175. Neither alternative is expected to have disproportionate adverse impacts on minorities, low-income populations, or children, or adversely affect tribal entities. As reviewed in Chapter 4, the trait genes, enzymes, and resultant fatty acids in DHA canola presents no risks to human health, nor animal health and welfare. DHA canola would be cultivated as are all other canola varieties, using the same agronomic practices and inputs.

Tribal entities are recognized as independent governments and agricultural activities on tribal lands would only be conducted if approved by the tribe. Tribes would have control over any potential conflict with cultural resources on tribal properties. Approval nor denial of the petition is not expected to have any effect on Indian tribal self-governance or sovereignty, tribal treaties, or other rights. APHIS notified tribal leaders via letter that Nuseed was seeking a determination of nonregulated status for DHA canola. APHIS provided notification of the petition for tribal review to assist the Agency in identifying potentially significant impacts to tribal lands or resources. There were no impacts to tribal resources identified in association with deregulation of DHA canola.

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

  Invasive species are a significant issue in the United States causing both adverse economic and environmental impacts. This EO directs actions to continue coordinated federal prevention and control efforts related to invasive species. This order maintains the National Invasive Species Council (Council) and the Invasive Species Advisory Committee; expands the membership of the Council; clarifies the operations of the Council; incorporates considerations of human and environmental health, climate change, technological innovation, and other emerging priorities into federal efforts to address invasive species; and strengthens coordinated, cost-efficient federal action.

Based on data submitted by Nuseed and reviewed by APHIS, DHA canola is similar in phenotypic characteristics as compared to other canola varieties currently grown, and is not expected to become more weedy or invasive than conventional canola varieties (USDA-APHIS 2018a). *Brassica napus* is not listed in the United States as a noxious weed species by the federal government (USDA-NRCS 2016), nor is it listed as an invasive species by USDA’s invasive species database (USDA-NAL 2017). APHIS has evaluated the potential for enhanced weediness in DHA canola and concluded that it is unlikely that DHA canola will become weedy or invasive (USDA-APHIS 2018a).

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

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

Agricultural crops can provide habitat for migratory birds in the northern Great Plains of North America during and before migration periods (Hagy et al. 2010). Migratory birds may transit canola fields and forage on canola seed, which provide a valuable source of nutrition to migratory and other birds (Schillinger and Werner 2016). As reviewed in Section 4.5 – Biological Resources, Section 4.6 – Human Health, and Section 4.7 – Animal Feed, it is highly unlikely the trait genes, their protein products, and the fatty acids present in DHA canola seed present any risk to migratory birds. Because migratory birds that forage on DHA canola are unlikely to be adversely affected by ingesting the seed or other plant parts, it is unlikely that approval of the petition would have a negative impact on migratory bird populations.

7.3 Executive Orders on International Issues

- **EO 12114 - Environmental Effects Abroad of Major Federal Actions**

  This Order requires federal officials to take into consideration any potential environmental effects that may occur outside the United States, its territories, and possessions, that may result from actions being taken.

The United States is a member of the World Trade Organization (WTO), which facilitates harmonizing the global rules of trade between nations. The Agreement on the Application of Sanitary and Phytosanitary Measures (the "SPS Agreement"), entered into force with the establishment of the WTO on January 1, 1995, sets out the basic rules for food safety and animal and plant health standards. The SPS agreement recognizes three international organizations/frameworks that have established standards and guidelines related to SPS measures (WTO 2015b). These are: the Codex Alimentarius Commission (Codex), the World Organization for Animal Health (OIE), and the International Plant Protection Convention (IPPC). Any international trade of DHA canola or products derived from it following a determination of nonregulated status would be subject to national phytosanitary requirements and be in accordance with international SPS standards, inclusive of the Codex (food safety) and IPPC (plant pests and disease). Approvals for the commercial use of DHA canola are being sought by Nuseed in Australia, New Zealand, Canada, Mexico, Japan, South Korea, China, European Union, and other countries as required (Nuseed 2017).

All crop production can potentially have adverse impacts on soils, and air and water quality. Any cultivation of DHA canola outside of the United States, its territories, or possessions would utilize the same (or similar) agronomic practices and inputs as those utilized in the United States. Consequently, the sources and degree of environmental impacts that derive from crop production would not differ from those described for United States, as discussed in this EA. In the event APHIS approves the petition for DHA canola, significant adverse environmental impacts outside the United States as a result of cultivation of this canola variety are unlikely.

7.4 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 GE 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 GE organisms within their jurisdiction. For example, South Dakota simply authorizes holders of a federal permit issued under 7 CFR part 340 to use it within the state (SD Stat § 38-12A-31 (2015)). Minnesota issues state permits for release of GE 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 GE organisms within state boundaries.

States with an organic program generally adopt 7 CFR part 205 by reference and may codify provisions. For example, Iowa (Iowa Code 190C.1-190C.26), Puerto Rico (5 L.P.R.A. §§ 131 to 141 (2013)), Oklahoma (Okla. Admin. Code §§ 35:37-15-1 to 35:37-15-11), Texas (Texas Agric. Code Ann. § 18 (2015)), and Utah (Utah Admin. Code r. R68-20 (2016)). When a state adopts the NOP prohibitions on excluded methods, then organic producers cannot use GE seed unless an exception in 7 CFR § 205.204 applies.

Neither of the alternatives considered would affect APHIS partnerships with states in the oversight of GE organisms, specifically in regulation of interstate movement and 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.

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Appendix A: DHA Canola

[OECD Unique Identifier: NS-B5ØØ27-4]

Overview

Fatty acids are basic components of the cell membranes of all living organisms serving various structural and functional purposes. Fatty acids are comprised of a chain of carbon and hydrogen atoms with a carboxylic acid moiety at one end (COOH). They are the basic building blocks of fats. Fatty acids are generally classified by the number of carbon atoms and number of double bonds between carbons. Saturated fatty acids have no double bonds between carbons, monounsaturated one double bond, and polyunsaturated fatty acids (PUFA) two or more double bonds. For PUFAs, they are further classified as long-chain polyunsaturated fatty acids (LC-PUFA, 20 to 24 carbon atoms) and very-long chain polyunsaturated fatty acids (VLC-PUFA, 25 or more carbon atoms) (FAO 2010). The "omega" classification refers to the location of the double bond nearest the end (omega) of the carbon chain (e.g., on the 3rd or 6th carbon).

Generally, the major PUFAs in our diet are terrestrial plant based linoleic acid (LA, C18:2n-6) and alpha-linolenic acid (ALA, C18:3n-3), and depending on seafood intake, a variable but relatively lower proportion of LC-PUFA such as arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) (FAO 2010).

The potential health benefits of consuming the omega-3 fatty acids EPA and DHA, in particular, is an area of significant scientific research (NIH 2017). Many observational studies link higher intakes of fish and other seafood, high in EPA, DHA, and other omega-3 fatty acids, with improved health outcomes. Clinical studies have found that omega-3 fatty acids, to include EPA and DHA are associated with optimal function of vertebrate cardiovascular, neurological, immune, and inflammatory response systems (Swanson et al. 2012; Domenichiello et al. 2015; Weylandt et al. 2015). Because humans and other animals have limited ability to synthesize EPA and DHA, direct dietary intake is recommended (NIH 2017). Finfish and mollusks are the primary dietary source of EPA and DHA for humans. Some foods, such as certain brands of eggs, yogurt, juices, milk, and soy beverages, are fortified with DHA and other omega-3s, and are also sources, as well as over the counter fish oil supplements. In general, current dietary sources of EPA and DHA are almost exclusively limited to finfish and mollusks, or fish oil supplements.

While omega-6 and omega-3 fatty acids are vital to animal (to include human) health, vertebrates are unable to synthesize (or rather, not very well) all the omega-6 and omega-3 fatty acids required to sustain health, and need to acquire certain fatty acids through the diet. For example, because LA and ALA are the parent fatty acids of various omega-6 and omega-3 fatty acids, respectively, and humans can synthesize neither, they must be consumed in the diet (Warude et al. 2006). Once consumed, LA and ALA are elongated to form various LC-PUFAs. Finfish and mollusks are the primary dietary source of EPA and DHA for humans. As with all animals, finfish and mollusks cannot efficiently synthesize EPA and DHA. Mollusks acquire and accumulate EPA and DHA in fatty tissues via direct consumption of marine microalgae, which produce high levels of EPA and DHA. Finfish acquire and accumulate EPA and DHA indirectly via consumption of smaller fish or other prey species, such as crustaceans that have also fed on microalgae – a process known as bioaccumulation (Monroig et al. 2013).[37] Some foods, such as certain brands of eggs, yogurt, juices, milk, and soy beverages, are fortified with DHA and other omega-3s, and are also sources, as well as over the counter fish oil supplements.

Marine microalgae, and consequently finfish and mollusks, contain high concentrations of EPA and DHA, while a shorter chain (18 carbon) omega-3 fatty acid, α-linolenic acid (ALA), is predominant in terrestrial plants (i.e., seed oils of flax, canola, walnut, and soybean) and a primary PUFA in diets based on terrestrial plants and animals. Mammals and fish can convert dietary ALA to EPA and DHA via endogenous enlongase and desaturase enzymes, however, only small amounts of EPA and DHA are biologically synthesized by this process (Monroig et al. 2013). For example, most studies indicate that, in humans, ~2% to 10% of dietary ALA is converted to EPA or DHA (Swanson et al. 2012; Domenichiello et al. 2015). Consequently, while dietary intake of the shorter chain ALA is important and provides a source for EPA and DHA, direct intake of > 500 mg/day of EPA and DHA is recommended for optimal health maintenance (Swanson et al. 2012; Petrie et al. 2014; OSU 2017).

Fatty Acid Biosynthesis Pathways

Figure A1 illustrates PUFA biosynthesis pathways via diverse fatty acid desaturases and elongases in eukaryotic systems (Lee et al. 2016). LA and ALA, from which omega-6 and omega-3 fatty acids are synthesized, respectively, are essential dietary fatty acids for humans (Lee et al. 2016).

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[37] The American Heart Association recommends that all adults eat fish, particularly oily fish, at least twice weekly, to acquire sufficient dietary omega-3 fatty acids; EPA and DHA.

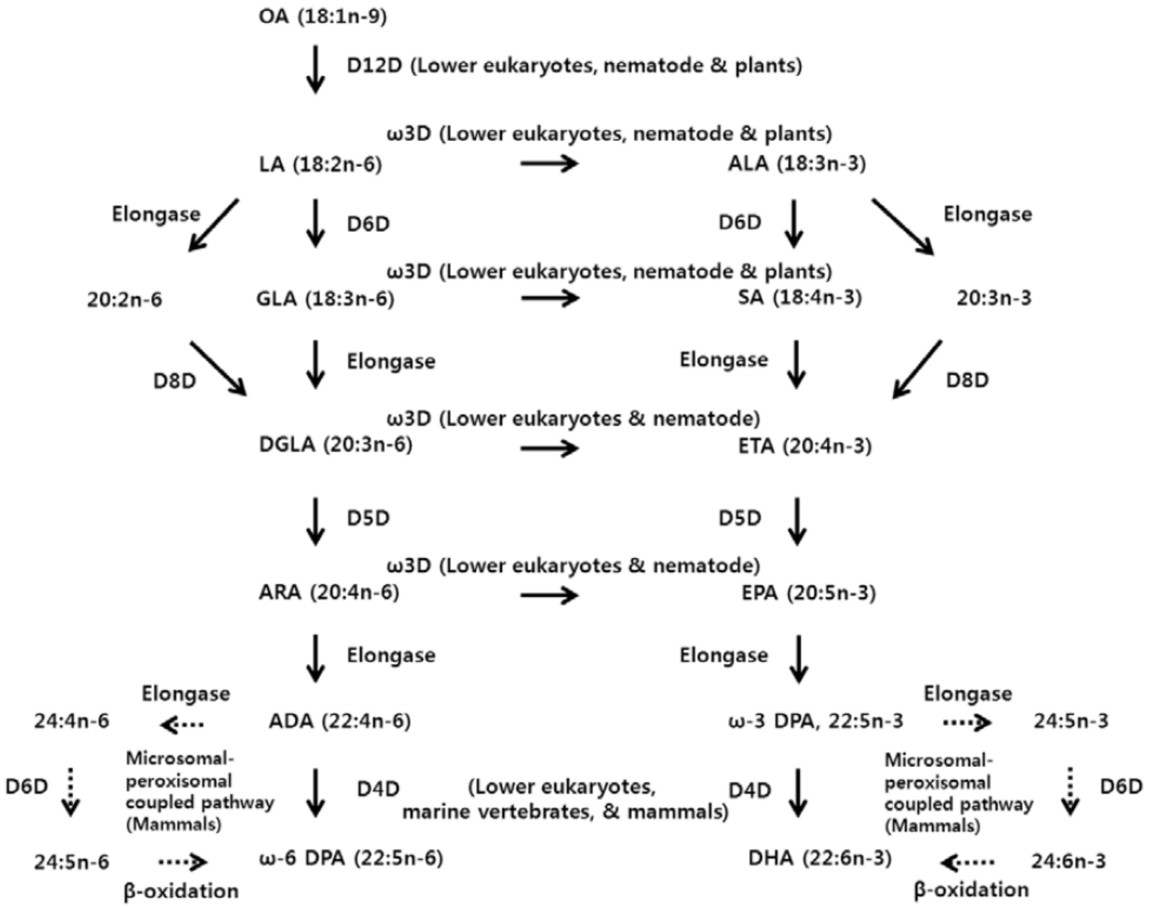

Figure A1. PUFA Biosynthetic Pathways in Eukaryotic Organisms

Source: (Lee et al. 2016)

Transgenes and Regulatory Sequences in DHA Canola

DHA canola was developed using a parent line, AV Jade canola. AV Jade was developed by the Department of Primary Industries Victoria (Oilseeds Breeding Programs, Grains Innovation Park, Horsham, Victoria), and the Grains Research and Development Corporation, as part of the National Brassica Improvement Program. AV Jade was created from canola line ‘RR013’, which was derived in 1998 from a cross between two Victorian breeding lines, ‘RM30’ and ‘RM17’. Over the years RR013 was crossed and selected for multiple beneficial characteristics, including blackleg resistance – a disease that commonly affects canola crops. DHA canola was transformed with *Agrobacterium tumefaciens*, using one vector encoding seven enzymes (desaturases and elongases) involved in the biosynthesis of DHA, EPA, and other fatty acids. These enzymes are comprised of two fatty acid desaturases from yeast (blue), two elongases from marine microalgae (purple), and three desaturases from marine microalgae (green)(Figure

A2). Each fatty acid biosynthesis gene in the T-DNA was incorporated into its own expression cassette that included seed-specific expression.

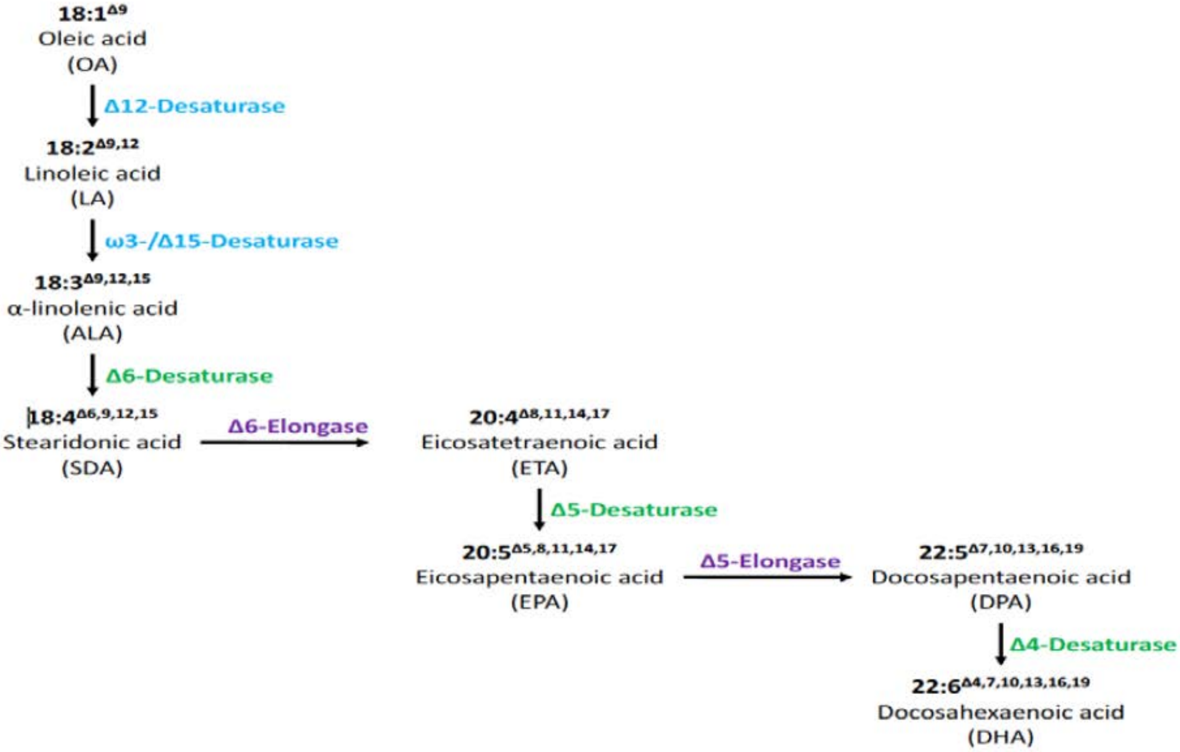

Figure A2. EPA, DPA, and DHA Biosynthesis in DHA Canola

Source: (Nuseed 2017)

The seven transgenes in DHA canola utilized for omega-3 fatty acid biosynthesis, summarized below, are from organisms that are prevalent in the environment.

1. Δ12 Desaturase

The Lackl-Δ12D gene used in DHA canola was cloned from the yeast *Lachancea kluyveri*, formerly called as *Saccharomyces kluyveri*. *L. kluyveri* is widespread in the environment with strains isolated from insects, soil, and trees in North America, Europe, and India (Génolevures-Consortium 2009). Lackl-Δ12D shares homology (33-79%) with other Δ12-desaturases found in yeasts, fungi, and various crop plants, such as canola, rice, soybean, flax, sunflower and sesame (Nuseed 2017). The yeast strain *L. kluyveri* is widely used in Emmental, Roquefort, Damietta, and Greek cheeses, and fermented milk.

2. ω3- Desaturase

The ω3-desaturase gene used in DHA canola was cloned from the yeast *Pichia pastoris*, previously called *Komagataella pastoris*. *P. pastoris* is used for production of proteins used in the pharmaceutical and food and feed industries (Nielsen 2013). ω3/Δ15-desaturases have been cloned from a wide range of organisms. The Picpa–ω3D shares homology (22-72%) to other ω3/Δ15-desaturase proteins from yeasts, fungi and various crop plants, such as canola, soybean, flax and sesame (Nuseed 2017).

3. Δ6 Desaturase

The Micpu-Δ6D gene was cloned from the marine microalga *Micromonas pusilla*. Δ6 desaturases have been described in fungi, invertebrates, and vertebrates (López Alonso et al. 2003; Sperling et al. 2003). The Micpu-Δ6D protein shares about 17-61% sequence identity to other Δ6-desaturase proteins from fungi, salmon, evening primrose, and canola (Goodman 2016; Nuseed 2017).

4. Δ6 Elongase

The Pyrco-Δ6E gene was cloned from the marine microalga *Pyramimonas cordata*. Pyrco-Δ6E shares amino acid sequence identities (19-64%) to elongases present in food and animal feeds (Nuseed 2017). Several human PUFA elongases have been isolated, including Δ6-elongases (Leonard et al. 2004).

5. Δ5-Desaturase

The Pavsa-Δ5D gene was cloned from the marine microalga *Pavlova salina*. Δ5 desaturases have been identified in fungi, invertebrates, and vertebrates (López Alonso et al. 2003; Sperling et al. 2003). *P. salina* is used in mariculture as oyster and clam feed.

6. Δ5 Elongase

The Pyrco-Δ5E gene was cloned from the marine microalga *Pyramimonas cordata.* Enzymes with Δ5-elongating activity have been identified in marine microalgae, rodents, and humans (Leonard et al. 2004; Meyer et al. 2004).

7. Δ4 Desaturase

The Δ4-desaturase gene used in DHA canola was cloned from the marine microalga *Pavlova salina*. Δ4- desaturases have been identified in exist in marine microalgae and fish (Tonon et al. 2003; Fonseca-Madrigal et al. 2014).

Enzyme Expression levels

All seven enzymes were detected in developing and/or mature seeds of DHA canola, although at very low levels (20-740 ng/mg total protein). None of the proteins have been detected in the non-seed tissues of DHA canola (Nuseed 2017).

Analyses of 593 seeds from generations T3 to T7, sequencing of 12 transgenic lines from generations T3 to T5, oil profiling, and inheritance analysis of F2 populations demonstrated that the genetic stability and trait purity of DHA canola were well maintained from one generation to the next, and from AV Jade to different genetic backgrounds in different years and locations (Nuseed 2017).

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