Dow AgroSciences Petition (09-349-01p) for Determination of Nonregulated Status of Event DAS-68416-4

OECD Unique Identifier: DAS-68416-4

Draft Environmental Assessment

May 2012

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ACRONYMS

2,4-D	2,4-dichlorophenoxyacetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid
AAD-12	aryloxyalkanoate dioxygenase-12
ae	acid equivalent
a.i.	active ingredient
AIA	advanced informed agreement
ALS	acetolactate synthase
AMS	Agricultural Marketing Services
ANZFS	Australia and New Zealand Food Standards Agency
AOSCA	Association of Official Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
ARS	Agricultural Research Service
ARMS	Agricultural Resource Management Survey
BEE	2,4-D butoxyethyl ester
BRS	Biotechnology Regulatory Services
C	carbon
°C	degrees celsius
CAA	Clean Air Act
$CaCO_3$	calcium carbonate
CBD	Convention on Biological Diversity
CH_4	methane
cp4 epsps	5-enolpyruvylshikimate-3-phosphate synthase
CO	carbon monoxide
CO_2	carbon dioxide
CFR	Code of Federal Regulations
CoE/EPPO	Council of Europe/European and Mediterranean Plant Protection Organization
CWA	Clean Water Act
DAS	Dow AgroSciences
DMAS	dimethylamine salt
DNA	deoxyribonucleic acid
DRT	Drift Reduction Technology
DT_{50}	dissipation time needed for herbicide to degrade to half of its original concentration
EA	environmental assessment
EFSA	European Food Safety Agency
EIS	environmental impact statement
EPA	U.S. Environmental Protection Agency
ERS	Economic Research Service
ESU	evolutionarily significant units
EU-27	European Union 27 member countries
EO	Executive Order
°F	degrees fahrenheit
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act

ACRONYMS

FFP	food,feed, or processing
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fl oz/A	fluid ounces per acre
FQPA	Food Quality Protection Act
FSANZ	Food Standards Australia New Zealand
FR	Federal Register
GE	genetically engineered
GHG	greenhouse gases
IP	Identity Protection
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measure
lb/A	pounds per acre
lb ae/A	acid equivalent pounds per acre
lb ai/A	active ingredient pounds per acre
lb/gallon	pounds per gallon
lb/pint	pounds per pint
LMOs	living modified organisms
LOC	level of concern
MG	maturity group
MOE	Margin of Exposure
MOU	Memorandum of Understanding
MPCA	(4-chloro-2-methylphenoxy) acetic acid
N_2O	nitrous oxide
NAAQS	National Ambient Air Quality Standards
NABI	North American Biotechnology Initiative
NAPO	North American Plant Protection Organization
NASS	National Agricultural Statistics Service
NEPA	National Environmental Policy Act
NH_3	ammonia
NHPA	National Historic Preservation Act
NMFS	National Marine Fisheries Service
NO_2	nitrogen dioxide
NOP	National Organic Program
NPS	nonpoint source pollution
NRC	National Research Council
NRCS	Natural Resources Conservation Service
NRDC	Natural Resources Defense Council
O_3	ozone
OECD	Organisation for Economic Co-operation and Development
OPP	Office of Pesticide Programs (U.S. EPA)
OSTP	Office of Science and Technology Policy
PAT	phosphinothricin acetyltransferase
PAD	population adjusted dose
Pb	lead
PDP	Pesticide Data Program

ACRONYMS

pints/A	pints per acre
PIPs	plant-incorporated protectants
PM	particulate matter
$PM_{2.5}$	particulate matter with aerodynamic diameter of 2.5 micrometer or less
PM_{10}	particulate matter with aerodynamic diameter of 10 micrometer or less
PPE	personal protective equipment
PPRA	Plant Pest Risk Assessment
PRA	Pest Risk Analysis
R2	full flowering stage
RED	reregistration eligibility decision
ROW	rest of the world
RQ	risk quotient
RSPM	Regional Standards for Phytosanitary Measures
SIP	State Implementation Plan
SO_2	sulfur dioxide
SOC	soil organic carbon
SOM	soil organic matter
SSA	Sole Source Aquifer
TSCA	Toxic Substances Control Act
U.S.	United States
U.S.C.	United States Code
USDA	U.S. Department of Agriculture

1 PURPOSE AND NEED

1.1 Regulatory Authority

"Protecting American agriculture" is the basic charge of the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. The agency improves agricultural productivity and competitiveness, and contributes to the national economy and the public health. USDA asserts that all methods of agricultural production (conventional, organic, or the use of genetically engineered varieties) can provide benefits to the environment, consumers, and farm income.

Since 1986, the United States (U.S.) government has regulated genetically engineered (GE) organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 *Federal Register* [FR] 23302, 57 FR 22984). The Coordinated Framework, published by the Office of Science and Technology Policy (OSTP), describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner 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 mandated to exercise oversight of GE organisms only when there is evidence of “unreasonable” risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: the USDA’s APHIS, the U.S. Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA).

APHIS is responsible for regulating GE organisms and plants under the plant pest authorities in the Plant Protection Act of 2000, as amended (7 United States Code (U.S.C.) § 7701 *et seq*.) to ensure that they do not pose a plant pest risk to the environment.

The FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). The FDA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those that are genetically engineered. To help developers of food and feed derived from GE crops comply with their obligations under Federal food safety laws, FDA encourages them to participate in a voluntary consultation process. All food and feed derived from GE crops currently on the market in the U.S. have successfully completed this consultation process. The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the FR on May 29, 1992 (57 FR 22984-23005). Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food.

The EPA regulates plant-incorporated protectants (PIPs) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). EPA also sets tolerance limits for residues of pesticides on and in food and animal feed, or establishes an exemption from the requirement for a tolerance, under the FFDCA and regulates certain biological control organisms under the Toxic Substances Control Act (TSCA). The EPA is responsible for regulating the sale, distribution, and use of pesticides, including pesticides that are produced by an organism through techniques of modern biotechnology.

1.2 Regulated Organisms

The APHIS Biotechnology Regulatory Services' (BRS) mission is to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS regulations at 7 Code of Federal Regulations (CFR) part 340, which were promulgated pursuant to authority granted by the Plant Protection Act, as amended (7 U.S.C. § 7701–7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the Plant Protection Act or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to 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) and is also considered a plant pest. A GE organism is also regulated under Part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have information to determine if the GE organism is unlikely to pose a plant pest risk.

A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR part 340. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to pose a plant pest risk.

1.3 Petition for Determination of Nonregulated Status: DAS-68416-4

Dow AgroSciences, LLC (referred to as DAS, hereafter) of Indianapolis, Indiana submitted a petition (APHIS Number 09-349-01p) to APHIS in 2009 seeking a determination that DAS-68416-4 soybean that expresses the *aad-12* and *pat* genes is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under regulations at Part 340.

DAS-68416-4 soybean was genetically engineered to express the arylalkanoate dioxygenase (AAD-12) protein and the phosphinothricin acetyltransferase (PAT) protein. The *aad-12* gene encodes the AAD-12 protein which makes the plant tolerant to several aryloxyalkanoate-based herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D), (4-chloro-2-methylphenoxy) acetic acid (MCPA), and 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB); and pyridyloxyacetate herbicides, such as triclopyr and fluroxypyr (DAS, 2010, 2012). The *pat* gene encodes the PAT protein that inactivates the herbicide glufosinate (DAS, 2010). If no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act , DAS intends to make DAS-68416-4 soybean commercially available as the first soybean variety with improved tolerance to 2,4-D.

In the event of a determination of nonregulated status, the nonregulated status for DAS-68416-4 soybean would include DAS-68416-4 soybean, any progeny derived from crosses between DAS-68416-4 soybean and conventional soybean, and crosses of DAS-68416-4 soybean with other biotechnology-derived soybean that have been deregulated pursuant to Part 340 and the Plant Protection Act.

DAS-68416-4 soybean is currently regulated under 7 CFR part 340. Interstate movements and field trials of DAS-68416-4 soybean have been conducted under permits issued or notifications acknowledged by APHIS since 2008. Data resulting from these field trials are described in the DAS petition (DAS, 2010).

1.4 Purpose of Product

DAS-68416-4 soybean is a GE soybean line developed to increase tolerance to the herbicides 2,4-D and glufosinate. DAS-68416-4 soybean (*Glycine max* (L.) Merr.) plants have been genetically modified to express the aryloxyalkanoate dioxygenase-12 (AAD-12) and phosphinothricin acetyltransferase (PAT) proteins. DAS-68416-4 soybean incorporates the *aad-12* gene, derived from the common soil bacterium derived from *Delftia acidovorans* (DAS, 2010). The *aad-12* gene in DAS-68416-4 soybean expresses the AAD-12 protein, which degrades 2,4-D into herbicidally inactive 2,4-dichlorophenol (DAS, 2010). Additionally, this same protein has been demonstrated to degrade other phenoxy carboxylic acid herbicides, including (4-chloro-2-methylphenoxy) acetic acid (MCPA) and 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), and pyridine carboxylic acids herbicides, such as triclopyr and fluroxypyr. Of these herbicides, DAS-68416-4 has shown acceptable tolerance to MCPA, but does not exhibit commercially acceptable tolerance to triclopyr and fluroxypyr (DAS, 2010, 2012).

The *pat* gene, also inserted into DAS-68416-4 soybean, encodes the PAT protein that inactivates the herbicide glufosinate. The PAT protein is encoded by the *pat* gene isolated from the soil bacterium *S. viridochromogenes* (DAS, 2010). Although there are commercial varieties of GE glufosinate-tolerant soybean currently available and in production, DAS-68416-4 soybean would be the first food and animal feed soybean with both glufosinate and 2,4-D tolerance.

DAS has developed the DAS-68416-4 soybean as an alternative to currently available herbicide-tolerant soybean varieties (DAS, 2010, 2011a, 2011d). The wide adoption of glyphosate herbicide-tolerant crops in the U.S. has been accompanied by a greater use of glyphosate to control weeds and a decreasing diversity of herbicides used for weed management, leading to selection of glyphosate resistance in some weeds found in soybean acreage (USDA-APHIS, 2012). This new soybean offers an additional option for growers that provides for greater flexibility in the choice of herbicides to control economically important weeds by broadening the application window, and providing an additional mode of action to minimize the development of glyphosate herbicide-resistant weeds (DAS, 2010).

2,4-D is an herbicide used for selective control of broadleaf weeds in use since the mid-1940s in over 600 end-use products that are registered for use on over 300 distinct agricultural and residential sites (US-EPA, 2005c). When applied as an herbicide, 2,4-D causes abnormal plant cell division and growth leading to plant injury and death. Conventional non-GE soybean lines treated with post-emergence applications of 2,4-D exhibited 60% to 93% plant damage, depending on the rate and timing of the herbicide application (DAS, 2010). The DAS-68416-4 soybean, which could be planted in any area currently producing soybean, would enable a grower to apply a specially reformulated 2,4-D herbicide to DAS-68416-4 post-emergence soybean.

Glufosinate is a non-selective foliar herbicide used for pre-plant and post-emergence control of broadleaf plants and annual and perennial grasses (DAS, 2010; US-EPA, 2008b). Glufosinate acts by inhibiting the enzyme glutamine synthetase, which leads to poisoning in plants because of the overproduction of ammonia. Glufosinate was first registered by EPA for use in crops in 2000 as a non-selective foliar herbicide used for pre-plant and post-emergence control of broadleaf weeds (US-EPA, 2008b). It is currently registered for use on many crops including apples, berries, canola, corn, cotton, currants, grapes, grass grown for seed, potatoes, rice, soybeans, sugar beets, and tree nuts and for use in non-crop areas including lawns and residential areas (US-EPA, 2008b).

1.5 APHIS Response to Petition for Nonregulated Status

Under the authority of the plant pest provisions of the Plant Protection Act and 7 CFR part 340, APHIS has issued regulations for the safe development and use of GE organisms. As required by 7 CFR § 340.6, APHIS must respond to petitioners that request a determination of the regulated status of GE organisms, including GE plants such as DAS-68416-4 soybean. When a petition for nonregulated status is submitted, APHIS must make a determination if the GE organism is unlikely to pose a plant pest risk. If APHIS determines based on its Plant Pest Risk Assessment (PPRA) that the GE organism is unlikely to pose a plant pest risk, the GE organism is no longer subject to the plant pest provisions of the Plant Protection Act and 7 CFR part 340.

APHIS has prepared this environmental assessment (EA) to consider the potential environmental effects of an agency determination of nonregulated status consistent with the Council of Environmental Quality's National Environmental Policy Act (NEPA) regulations and the USDA and APHIS NEPA implementing regulations and procedures (40 CFR parts 1500-1508, 7 CFR part 1b, and 7 CFR part 372). This EA has been prepared in order to specifically evaluate the effects on the quality of the human environment[1] that may result from a determination of nonregulated status of DAS-68416-4 soybean.

1.6 Coordinated Framework Review

1.6.1 <u>Environmental Protection Agency</u>

Under FIFRA, EPA regulates the use of herbicides, such as 2,4-D and glufosinate, requiring registration of a pesticide for a specific use prior to distribution or sale of the pesticide for a

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

proposed use pattern. EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, and timing of its use; and storage and disposal practices. Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide will not cause unreasonable adverse effects on humans, the environment, and non-target species when used in accordance with label instructions. EPA must also approve the language used on the pesticide label in accordance with 40 CFR part 158. Once registered, a pesticide may not legally be used unless the use is consistent with the approved directions for use on the pesticide's label. The overall intent of the label is to provide clear directions for effective product performance while minimizing risks to human health and the environment (US-EPA, 2010d).

Currently, 2,4-D amine and ester formulations are registered by EPA for soybean pre-plant (burndown) use, only. DAS has developed a new herbicide formulation containing 2,4-D choline salt (DAS, 2010, 2011a) for additional pre- and post-emergence use with DAS-68416-4 soybean. In support of the new use and formulations, a new proposed label with supporting plant metabolism and residue data was submitted by DAS in early 2011 to EPA for review and approval (DAS, 2010). APHIS will use the draft 2,4-D label (DAS, 2011a) and previous EPA analyses on 2,4-D (e.g., Reregistration Eligibility Decision (RED) for 2,4-D) as the basis for its evaluation of the potential impacts associated with the use of and exposure to 2,4-D. A copy of the draft label for this new 2,4-D formulation (identified as product GF-2654 TS) (DAS, 2011b) is provided in Appendix A. A list of the required supporting data and studies submitted to EPA by DAS in support of the registration of the new 2,4-D use and formulation is provided in Appendix B (DAS, 2011g).

DAS indicates that there will be no change in the use pattern for glufosinate associated with DAS-68416-4 soybean (DAS, 2010); therefore, a petition to EPA for a change in the label for glufosinate has not be submitted. APHIS will use the current glufosinate application rate on soybean and EPA analyses on glufosinate (Bayer CropScience, 2011) as the basis for evaluating the potential impacts associated with the use of and exposure to glufosinate.

The Food Quality Protection Act (FQPA) of 1996 amended FIFRA, enabling EPA to implement periodic registration review of pesticides to ensure they are meeting current scientific and regulatory standards of safety and continue to have no unreasonable adverse effects (US-EPA, 2011h). The registration review for 2,4-D is scheduled to begin in 2013. For glufosinate, a review was initiated in 2008, with a forthcoming final decision scheduled in 2013 (US-EPA, 2008b, 2011e).

The EPA regulates PIPs under FIFRA (7 U.S.C. 136 *et seq.*) and certain biological control organisms under TSCA (15 U.S.C. 53 *et seq*.). Before planting a crop containing a PIP, a company must seek an experimental use permit from EPA. Commercial production of crops containing PIPs for purposes of seed increases and sale requires a FIFRA Section 3 registration with EPA. The AAD-12 protein expressed in DAS-68416-4 soybean has not been approved previously for use in any commercial crops. Since the protein is not a PIP, it is not regulated by EPA under FIFRA or TSCA.

The PAT protein expressed in DAS 68416-4 is similar to PAT found in other commercially-grown glufosinate-tolerant crops. Since the PAT protein has been included as an herbicide tolerance marker in products containing PIPs, it has been reviewed by EPA as a PIP inert ingredient (US-EPA, 2005b). Based on their environmental risk assessment, the EPA determined that the PAT protein presents a low probability of risk to human health and the environment and granted an exemption from the requirement of a tolerance for this PIP inert ingredient (40 CFR 180.1151; 62 FR 17719, Aug. 11, 1997).

1.6.2 Food and Drug Administration

FDA regulates GE organisms under the authority of the FFDCA (21 U.S.C. 301 *et seq.*). The FDA published its policy statement concerning regulation of products derived from new plant varieties, including those GE, in the FR on May 29, 1992 (57 FR 22984). Under this policy, FDA implements a voluntary consultation process to ensure that human food and animal feed safety issues or other regulatory issues, such as labeling, are resolved before commercial distribution of bioengineered food.

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

DAS-68416-4 soybean is within the scope of the FDA policy statement concerning regulation of products derived from new plant varieties, including those produced through genetic engineering. DAS initiated a consultation with the FDA by submitting an early food safety evaluation of the AAD-12 protein expressed in DAS-68416-4 soybean (NPC 000009) on December 15, 2008 (Krieger, 2008). FDA completed its evaluation with no further questions on May 19, 2010 (US-FDA, 2010). DAS submitted a safety and nutritional assessment of food and feed derived from DAS-68416-4 soybean to the FDA on December 22, 2009 (BNF No. 000124) in support of the consultation process with FDA for the commercial distribution of DAS-68416-4 soybean. FDA evaluated the submission and responded to the developer by letter on November 14, 2011. Based on the information DAS submitted, FDA has no further questions regarding DAS-68416-4 soybean (US-FDA, 2011a, 2011c). An FDA biotechnology consultation on soybean lines containing the PAT protein (BNF No. 000055) (US-FDA, 1998b) was submitted April 21, 1998, and completed on May 15, 1998 (US-FDA, 1998a) and also was evaluated as part of the consultation on DAS-68416-4 completed in 2011. A copy of the completed FDA reviews is provided in Appendix C.

1.7 Public Involvement

APHIS routinely seeks public comment on draft EAs prepared in response to petitions requesting determinations of nonregulated status for GE organisms. APHIS does this through a notice published in the FR. The issues discussed in this EA were developed by considering the public concerns as well as issues raised in public comments submitted for other EAs of GE organisms, concerns raised in lawsuits, as well as those issues of concern that have been raised by various stakeholders. These issues, including those regarding the agricultural production of soybean using various production methods and the environmental and food/feed safety of GE plants were addressed to analyze the potential environmental impacts of DAS-68416-4 soybean.

This EA, the petition submitted by DAS, and APHIS's PPRA will be available for public comment for a period of 60 days (7 CFR § 340.6(d)(2)). Comments received by the end of the 60-day period will be analyzed and used to inform APHIS' determination decision of the regulated status of DAS-68416-4 soybean and to assist APHIS in determining whether an Environmental Impact Statement (EIS) is required prior to the determination decision of the regulated status of this soybean variety.

1.8 Issues Considered

The list of resource areas considered in this draft EA were developed by APHIS through experience in considering public concerns and issues raised in public comments submitted for other EAs of GE organisms. The resource areas considered also address concerns raised in previous and unrelated lawsuits, as well as issues that have been raised by various stakeholders in the past. The resource areas considered in this EA can be categorized as follows:

Agricultural Production Considerations:

- Acreage and Areas of Soybean Production
- Agronomic/Cropping Practices
- Soybean Seed Production
- Organic Soybean Production

Environmental Considerations:

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

Human Health Considerations:

- Human Health
- Worker Safety

Livestock Health Considerations:

- Animal Feed

Socioeconomic Considerations:

- Domestic Economic Environment

- Trade Economic Environment

2 AFFECTED ENVIRONMENT

The Affected Environment Section provides a discussion of the current conditions of those aspects of the human environment potentially impacted by a determination of nonregulated status of DAS-68416-4 soybean. For the purposes of this EA, those aspects of the human environment are: soybean production practices, the physical environment, biological resources, public health, animal feed, and socioeconomic issues.

2.1 Agricultural Production of Soybean

Soybean (*Glycine max* (L.) Merr.) is an economically important leguminous crop, providing oil and protein. Soybean plants are grown for their seed, which is further processed to yield oil and meal. Soybean is ranked number one in oil production (58 percent) among the major oil seed crops production in the world (ASA, 2011). Other expanding uses for soybeans in the U.S. include soy biodiesel, animal agriculture, exports, and edible soybean oil (USB, 2011b).

2.1.1 <u>Acreage and Area of Soybean Production</u>

As of 2007, there were about 406 million acres of cropland in the U.S., of which approximately 332 million acres (including harvested, failed crops, and cultivated fallow) were used for crop production (USDA-NASS, 2009b). The remaining cropland was either idle or was used for pasture. From 1991 to 2011, acreage planted with soybean increased from just over 59 million acres to approximately 75 million acres (Figure 1) (USDA-NASS, 2011e, 2011f). The amount of soybeans planted in 2011 was nearly 2.4 million acres or 3% less than grown in 2010 (USDA-NASS, 2011e).

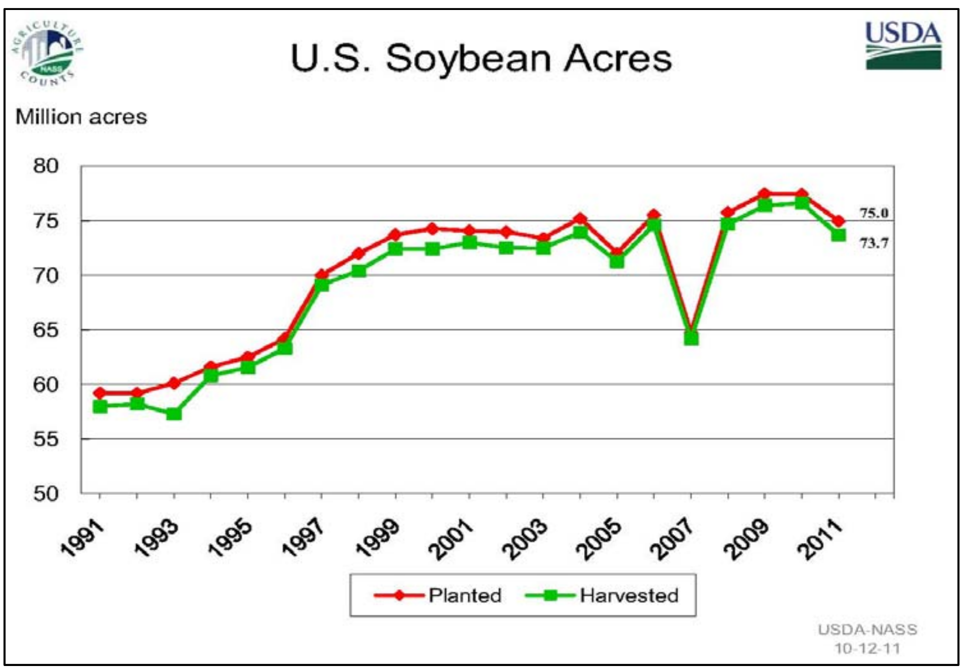


Figure 1. Planted and harvested acreage of soybeans in the U.S. (1991-2011).

Source: USDA-NASS (2011f).

The majority of soybeans produced in the U.S. are grown in 31 states (Figure 2; Table 1). The top producing states are Iowa, Illinois Minnesota, Indiana, and Nebraska, commonly growing soybean in rotation with corn (Soyatech, 2011).

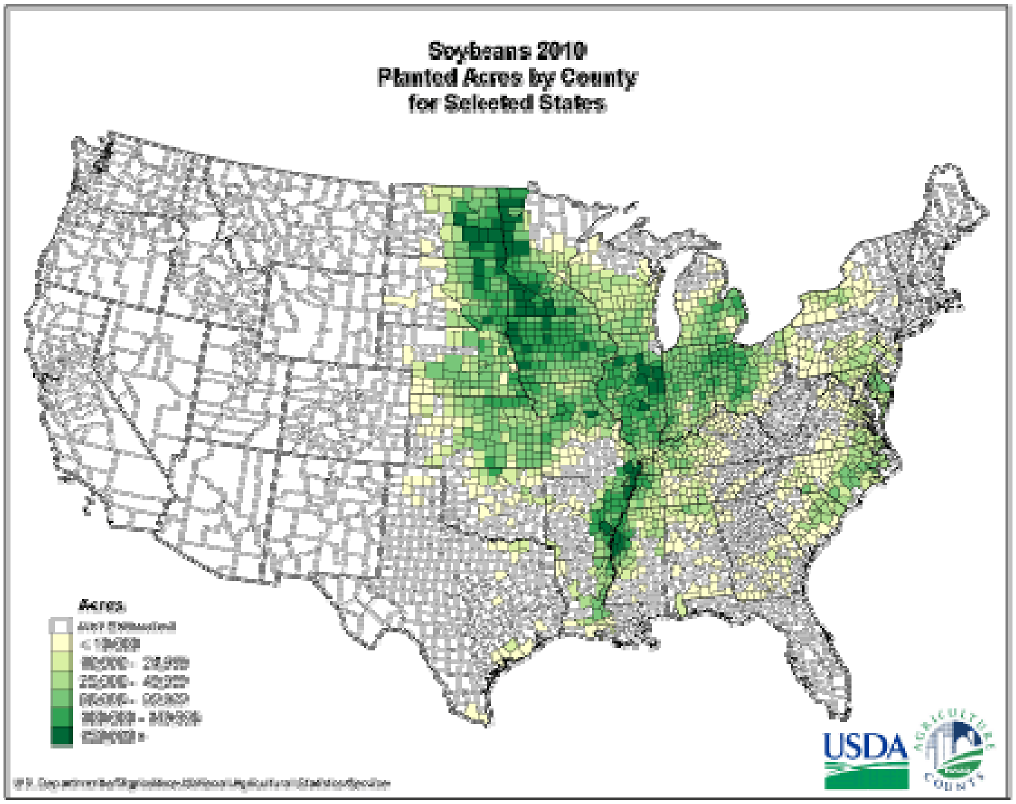


Figure 2. Soybean planted acres by county for selected states, 2010.

Source: USDA-NASS (2011f).

Over the last 20 years, soybean production has increased 35%, from nearly 2.0 billion bushels (43.1 million metric tons) in 1991 to approximately 3.0 billion bushels (64.6 million metric tons) in 2011. Average yield increased during this period approximately 17.6% from 34.2 bushels per acre in 1991 to 41.5 bushels per acre in 2011 (USDA-NASS, 2011e). USDA agricultural projections for 2020 estimate about 3.5 billion bushels (76.3 metric tons) of soybean will be produced, of which approximately 2.0 billion bushels (43.1 million metric tons) will be for domestic consumption and 1.5 billion bushels (33.2 million metric tons) for export in that year (USDA-OCE, 2011).

Large scale field testing of GE crops began in the 1980s, but it was not until ten years later the first generation of GE varieties became commercially available (Fernandez-Cornejo and Caswell, 2006). Since GE soybeans' initial commercial availability in 1996, their use has expanded to 94% of the total U.S. soybean acreage (Table 2) (Fernandez-Cornejo and Caswell, 2006; USDA-ERS, 2011b). Although other varieties are available for selection by growers, the

Table 1. U.S. soybean production, 2010 and 2011.

State	Acres Planted (x 1,000)		Acres Harvested (x 1,000)	
	2010	2011	2010	2011
Alabama	350	310	345	295
Arkansas	3,190	3,250	3,150	3,170
Delaware	175	180	173	178
Florida	25	20	23	18
Georgia	270	170	260	160
Illinois	9,100	8,900	9,050	8,850
Indiana	5,350	5,300	5,330	5,290
Iowa	9,800	9,200	9,730	9,110
Kansas	4,300	3,900	4,250	3,850
Kentucky	1,400	1,520	1,390	1,500
Louisiana	1,030	1,050	1,020	1,000
Maryland	470	455	465	445
Michigan	2,050	1,950	2,040	1,940
Minnesota	7,400	7,200	7,310	7,110
Mississippi	2,000	1,830	1,980	1,780
Missouri	5,150	5,100	5,070	5,050
Nebraska	5,150	4,750	5,100	4,400
New Jersey	94	85	92	93
New York	280	285	279	282
North Carolina	1,580	1,420	1,550	1,390
North Dakota	4,100	4,200	4,070	4,150
Ohio	4,600	4,700	4,590	4,680
Oklahoma	500	460	475	420
Pennsylvania	500	480	495	475
South Carolina	465	400	455	390
South Dakota	4,200	4,300	4,140	4,240
Tennessee	1,450	1,380	1,410	1,340
Texas	205	165	185	145
Virginia	560	570	540	550
West Virginia	20	18	19	17
Wisconsin	1,640	1,660	1,630	1,650
Other States	--	--	--	35
U.S.	77,404	75,208	76,616	74,258

Source: USDA-NASS (2011a)

Table 2. Percentage of soybean acreage planted with GE herbicide-tolerant soybean varieties by state and for the U.S.

State	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Arkansas	43	60	68	84	92	92	92	92	94	94	96	95
Illinois	44	64	71	77	81	81	87	88	87	90	89	92
Indiana	63	78	83	88	87	89	92	94	96	94	95	96
Iowa	59	73	75	84	89	91	91	94	95	94	96	97
Kansas	66	80	83	87	87	90	85	92	95	94	95	96
Michigan	50	59	72	73	75	76	81	87	84	83	85	91
Minnesota	46	63	71	79	82	83	88	92	91	92	93	95
Mississippi	48	63	80	89	93	96	96	96	97	94	98	98
Missouri	62	69	72	83	87	89	93	91	92	89	94	91
Nebraska	72	76	85	86	92	91	90	96	97	96	94	97
North Dakota	22	49	61	74	82	89	90	92	94	94	94	94
Ohio	48	64	73	74	76	77	82	87	89	83	86	85
South Dakota	68	80	89	91	95	95	93	97	97	98	98	98
Wisconsin	51	63	78	84	82	84	85	88	90	85	88	91
Other States[1]	54	64	70	76	82	84	86	86	87	87	90	92
U.S.	54	68	75	81	85	87	89	91	92	91	93	94

Source: USDA-ERS (2011b)
[1]Includes all other states in the soybean estimating program.

Roundup Ready®, glyphosate-tolerant varieties continue to dominate the market (see, e.g., (Tarter, 2011)). Other cultivated herbicide-tolerant soybeans include the LibertyLink® soybean varieties (a GE variety resistant to glufosinate ammonium herbicide first introduced in 1996) and STS (a conventionally bred sulfonylurea-tolerant soybean first introduced in 1993). Additional traits, such as lepidopteran resistance, high oleic acid content, and improved fatty acid profile, have nonregulated status (Table 3) and could potentially be made available for commercial production in the future.

Table 3. GE soybean with nonregulated status.

Petition Number	OECD Unique Identifier	Transformation Event or Line	Transgenic Trait	Date of Determination	Applicant
93-258-01p	**MON-04032-6**	**40-3-2**	Glyphosate tolerant	1994	Monsanto
96-068-01p	ACS-GM001-8	W98	Phosphinothricin[1] tolerant	1996	AgrEvo (Bayer CropScience)
	ACS-GM002-9	W62			
	ACS-GM005-3	**A5547-35**			
	ACS-GM005-3	**A2704-21**			
	ACS-GM005-3	**A2704- 12**			
97-008-01p	DD-026005-3	G94-1, G94-19, G-168	High oleic acid content	1997	Du Pont
98-014-01p\ 96-068-01p	ACS-GM006-4	A5547-127	Phosphinothricin tolerant	1998	AgrEvo (Bayer CropScience)
98-238-01p	ACS-GM003-1	GU262	Phosphinothricin tolerant	1998	AgrEvo (Bayer CropScience)
06-178-01p	**MON-89788-1**	**MON 89788**	Glyphosate tolerant	2007	Monsanto
06-271-01p	DP-356Ø43-5	356043	Glyphosate & acetolactate synthase (ALS) tolerant	2008	Pioneer
06-354-01p	**DP-3Ø5423-1**	**DP-3Ø5423-1**	High oleic acid content	2010	Pioneer
09-082-01p	MON-87701-2	MON 87701	Lepidopteran resistant	2011	Monsanto
09-201-01p	MON-877Ø5-6	MON-877Ø5-6	Improved fatty acid profile	2011	Monsanto

Source: (OECD, 2012; USDA-APHIS, 2011a).
Items in **bold** are currently found in commercial soybean production.
1. Ammonium glufosinate.

2.1.2 Agronomic Practices

"Conventional farming" in this document includes any farming system where synthetic pesticides and fertilizers may be used. Conventional farming covers a broad scope of farming practices, ranging from farmers who only occasionally use synthetic pesticides and fertilizers to those farmers whose harvest depends on regular pesticide and fertilizer inputs. This definition of conventional farming also includes the use of GE varieties that are no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act.

Soybean self-pollinates and is propagated by seed (OECD, 2000). Proper seedbed preparation, appropriate variety selection, appropriate planting dates and plant population, and good integrated pest management practices are important for optimizing the yield potential and economic returns of soybean (Barrentine, 1989; USDA-APHIS, 2010a). The following discussions introduce the basic cultivation requirements of soybean and the agronomic practices commonly employed to produce soybean in the U.S.

2.1.2.1 Cultivation

Soybean (*Glycine max* L.) is a member of the legume family that grows as an erect, bushy herbaceous annual (OECD, 2000). It is a quantitative short-day plant, flowering more quickly under short days (OECD, 2000). As a result, photoperiod and temperature responses are important in determining areas of specific cultivar adaptation. Soybean cultivars are identified based on geographic bands of adaptation that run east-west, determined by latitude and day length. In North America, there are 13 maturity groups (MGs) described, ranging from MG 000 in the north (45 degrees [°] latitude) to MG X near the equator. Within each maturity group, cultivars are described as early, medium, or late maturing (OECD, 2000).

The soybean seed will germinate when the soil temperature reaches approximately 10 degrees Celsius (°C) (50 degrees Fahrenheit [°F]) and, under favorable conditions, seedlings will emerge within a five to seven day period. In new fields of soybean production, an inoculation with *Bradyrhizobium japonicum*, a nitrogen fixing bacterium that develops a symbiotic relationship with soybean, dramatically increases its plant production (Missouri University of Science and Technology, No Date; OMAFRA, 2011; Pedersen, 2007). Inoculation is necessary for optimum efficiency of the nodulates that form on the root system (Berglund and Helms, 2003; Pedersen, 2007).

Soybeans require more moisture to germinate than corn, and seed-to-soil contact is important for good early-season soybean growth. Adequate water supply is most important at planting time, during pod-filling, and seed filling (Hoeft et al., 2000a). Soybeans require approximately 20-25 inches of water during the growing season. In 2008, only 9% of harvested soybean acreage, approximating 12 million acres, were irrigated, primarily in the states of Nebraska, Arkansas, Mississippi, Missouri, and Kansas with 85% of the irrigated acres (USDA-NASS, 2010). Use of irrigation on soybean is discussed further in Section 2.2.2, Water Resources.

Soybean can grow in a diversity of environments, but the optimum soil pH is from 5.8 to 7.0 (NSRL, No Date). Adequate levels of phosphorus, potassium, calcium, and magnesium, as well as other minor nutrients, are required for maximum soybean growth and yield. Given the ability of soybean to fix nitrogen from the air due to its symbiotic relationship with *Bradyrhizobium*, fertilizer nitrogen is not always needed for soybean production. In areas with increased amounts of salt or carbonates, or that have no past history of soybean production, nitrogen amendments prior to or at the time of planting have been shown to increase yield if soil tests reveal levels are not adequate (Berglund and Helms, 2003; Franzen, 1999). A common practice is to fertilize the previous year's corn crop with enough phosphorus and potassium to allow for the subsequent soybean crop to be grown with no supplemental fertilizer (Berglund and Helms, 2003; Ebelhar et al., 2004; Franzen, 1999). Calcium and magnesium are normally present in an adequate supply if the soil is treated with dolomitic limestone and is near the optimum pH (Frank, 2000; Harris, 2011).

2.1.2.2 Crop Rotation

Crop rotation is the successive planting of different crops in the same field over a particular period of years. Crop rotation has the two primary goals of sustaining the productivity of the agricultural system and maximizing economic returns (Hoeft et al., 2000a). Sustaining the agricultural system is achieved by rotating crops that may improve soil health and fertility with more commercially beneficial “cash crops.” Since soybean fixes nitrogen in soil, the yield of some crops following soybean, such as corn or wheat, may increase (Berglund and Helms, 2003). Moreover, the rotation of crops can effectively reduce disease, pest incidence, weediness, and selection pressure for weed resistance to herbicides (Berglund and Helms, 2003; USDA-ERS, 1997b). Crop rotation may also include fallow periods, or sowing with cover crops to prevent soil erosion and to provide livestock forage between cash crops (Hoeft et al., 2000a; USDA-NRCS, 2010a). Maximizing economic returns is realized by rotating crops in a sequence that efficiently produces the most net returns for a producer over a single or multi-year period. Many factors at the individual farm level affect the crop rotation system chosen, including the soil type present in an individual field, the expected commodity price, the need to hire labor, the price of fuel, the availability of funding to buy seed, and the price of agricultural inputs (Duffy, 2011; Hoeft et al., 2000a; Langemeier, 1997).

Soybeans are often rotated with such crops as corn, winter wheat, spring cereals, and dry beans (OECD, 2000), the selection of which varies regionally. As of 2005, approximately 95% of U.S. soybean acres were grown under a rotation system (USDA-ERS, 2005). The same cropland used for soybean production is also used for corn production in many areas, such as Illinois, where over 90% of the cropped area is planted in a two-year corn-soybean rotation (Hoeft et al., 2000a). With the recent high corn prices, many producers are turning to a corn-corn-soybean rotation (Hart, 2006), but returns for producers are variable, dependent upon the price and projected yield of both corn and soybean for an individual operator (Stockton, 2007). Studies have found soybean yield tends to increase under this rotation sequence, attributed to an effective break in the soybean disease and pest cycle (Al-Kaisi, 2011; Nafziger, 2007). Soybean itself may be a cover crop in short rotations for its nitrogen contributions (Hoorman et al., 2009). Continuous soybean production is undertaken, but yield can be reduced the second or later years, and pest and disease incidence may increase (Monsanto, 2010b; Pedersen et al., 2001).

Double-cropping soybeans is also an option to increase returns. Soybean is frequently planted in winter wheat stubble to produce a crop in the same growing season. Double-cropping maximizes profits if high commodity prices can support it, but careful management to achieve uniform stands to sustain high yields is needed: the selection of appropriate varieties, a higher seeding rate, closer row spacing, and adequate moisture for germination are important variables affecting profitability (McMahon, 2011).

2.1.2.3 Tillage

Prior to planting, the soil must be stripped of weeds that would otherwise compete with the crop for space, water, and nutrients. Tillage in soybean production systems is used to prepare a seedbed, address soil compaction, incorporate fertilizers and herbicides, manage water movement both within and out of a production field, and control weeds (Heatherly et al., 2009). Field preparation is accomplished through a variety of tillage systems, with each system defined by the remaining plant residue on the field. Types of tillage systems utilized include conventional, reduced, conservation (including mulch-till, strip-till, ridge-till, and no-till), and deep. Multiple definitions of these tillage systems are abundant (Heatherly et al., 2009).

In conventional tillage, postharvest crop residue is plowed into the soil using moldboard plows, heavy disks, and chisel plows to prepare a clean seedbed for planting and to reduce the growth of weeds, leaving less than 15% of crop residue on the surface (Heatherly et al., 2009; Towery and Werblow, 2010). Conservation tillage employs tools that disturb soil less and leave more crop residues on the surface (at least 30%), whereas no-till farming only disturbs the soil for planting seed (Towery and Werblow, 2010; USDA-NRCS, 2005). Crop residues are materials left in an agricultural field after the crop has been harvested, including stalks and stubble (stems), leaves and seed pods (USDA-NRCS, 2005). These residues aid in conserving soil moisture and reduce wind and water-induced soil erosion (Heatherly et al., 2009; USDA-ERS, 1997a; USDA-NRCS, 2005). According to USDA Agricultural Resource Management Survey (ARMS) data (USDA-ERS, 2006), conservation tillage ranging from no-till to reduced-till conserving 15-30% of residues was utilized on 88% of planted soybean acres in 2006.

Since 1996, the use of a no-till system has increased more than any other reduced tillage system, with nearly all of the growth in adoption occurring in herbicide-tolerant crop production (i.e., soybean, cotton, canola) (Fawcett and Towery, 2002). In a survey conducted in 1997, it was found that farmers using no-till practices were more likely to adopt herbicide-tolerant soybeans as an effective weed control practice, although the study also found that the commercialization of herbicide-tolerant soybean did not encourage the adoption of no-till practices at that time (Fernandez-Cornejo and McBride, 2002). Another survey conducted between 1996 and 2001 found that producers using herbicide-tolerant seed varieties were more likely to use conservation tillage practices over conventional methods and practice conservation tillage to a greater degree than producers that did not use herbicide-tolerant crops (Fawcett and Towery, 2002). A survey of 1,195 producers conducted by Givens et al. (2009) between November 2005 and January 2006 revealed that 25% of farmers that had been using conventional tillage switched to no-till and 31% switched to reduced-till after adopting glyphosate-tolerant GE crops.

With the increase in production of glyphosate-tolerant soybeans, there has been a corresponding increase in the use of no-till production practices (Carpenter et al., 2002; Sankula, 2006). From the introduction and commercial availability of glyphosate-tolerant soybeans in 1996 to 2004, the use of no-till practices increased by 64% (Sankula, 2006). Utilization of conservation tillage practices by U.S. soybean growers increased from 51% in 1996 to 63% in 2008, equating to an additional 12 million acres (NRC, 2010). No-till soybean production is not suitable for all producers or areas. For example, no-till soybean production is less successful in heavier, cooler soils more typical of northern latitudes (Kok et al., 1997; NRC, 2010).

2.1.2.4 Agronomic Inputs

Agronomic inputs, including water, soil and foliar nutrients, inoculates, fungicides, pesticides, and herbicides, are used in soybean production to maximize yields (Clevenger, 2010; Hoeft et al., 2000a; OECD, 2000; OMAFRA, 2011). Irrigation provides essential water for growth where rainfall is insufficient or erratic. This issue is discussed in detail in Subsection 2.2.2, Water Resources, and the corresponding impacts analysis in Subsection 4.3.2, Soil Quality. Soil and foliar macronutrient applications to soybean primarily include nitrogen, phosphorous (phosphate), potassium (potash), calcium and sulfur, with other micronutrient supplements such as zinc, iron, and magnesium applied as needed (NSRL, No Date; USDA-NASS, 2007a; Whitney, 1997).

Nutrients/Fertilizer

Table 4 presents summary data of the latest available USDA chemical fertilizer usage statistics from a 2006 survey reported by USDA National Agricultural Statistics Service (NASS) (USDA-NASS, 2007a). The survey found that among 19 select states, nitrogen was applied to 18% of the planted soybean acreage in those states at an average rate of 16 pounds per acre (lb/A) per year, and phosphate was applied to 23% of the planted acres at an annual average rate of 46 lb/A. Potash was applied to 25% of the planted acreage at an average annual rate of 80 lb/A, and sulfur was applied to 3% of the planted acres at an average annual rate of 11 lb/A (USDA-NASS, 2007a) (Table 4).

Table 4. Soybeans: total fertilizer primary nutrient applications, 2006[1].

Primary Nutrient	Area Applied (percent)	Applications (number)	Rate per Application (pounds per acre)	Rate per Crop Year (pounds per acre)	Total Applied (million pounds)
Nitrogen	18	1.1	15	16	212.4
Phosphate	23	1.0	45	46	772.8
Potash	25	1.0	79	80	1,454.7
Sulfur	3	1.1	10	11	20.0

Source: USDA-NASS (2007a)

[1] Program states surveyed - Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, South Dakota, Tennessee, Virginia, and Wisconsin; totaling 72.9 million planted acres.

Inoculates

As mentioned above, inoculates of the bacteria *Bradyrhizobium japonicum* can increase soybean yields, estimated at an average of a bushel per acre (Conley and Christmas, 2005). Historically, a nonsterile peat powder applied to the seed at planting had been the carrier for the inoculant into the field. More recently, improvements have been made in inoculant manufacturing, such as the use of sterile carriers, the addition of adhesives for inoculates to stick to seed, the introduction of liquid carriers, the use of concentrated frozen products, the introduction of new organism strains, the use of pre-inoculants, and the introduction of inoculants with extended biofertilizer and biopesticidal properties (Conley and Christmas, 2005). Industry has approximated that about one-third of U.S. soybean acreage was inoculated in 2009 (Seed Today, 2009).

Insecticides

A wide variety of pests can hinder soybean production and many require agricultural pesticidal inputs for their control. Several groups and types of insects can feed on the foliage, seed pods and roots of the soybean plant, and can reduce yield if not adequately controlled (Lorenz et al., 2006; Whitworth et al., 2011). A major pest for soybean producers is the soybean nematode that has no effective pesticidal treatment, especially the soybean cyst nematode (Nelson and Bradley, 2003). Nematodes are microscopic organisms that feed on the roots of various plants, including soybeans. There are several races or different groups of nematodes and their control is difficult. Some soybean varieties have resistance to some of the races, but often these resistant varieties have yielded less than other commercially available soybean varieties. A combination of crop rotation to a non-susceptible host and the use of resistant varieties can help alleviate the problem (Nelson and Bradley, 2003).

Insect infestation thresholds have been established to indicate when insecticide applications are actually necessary (Higgins, 1997). The thresholds are commonly based on number of insects found in field sampling surveys and/or in established standard defoliation thresholds, such as those provided by the National Information System of the Regional Integrated Pest Management Centers in pest management strategic plans (USDA, 2011). Table 5 presents summary data of the latest available USDA-NASS chemical insecticide usage statistics for U.S. soybeans from a 2006 survey (USDA-NASS, 2007a). The survey found that insecticides were applied to 16% of the 72.9 million soybean acres planted in surveyed states in 2006. Of the 12 reported insecticides, the three most common, lambda-cyhalothrin, chlorpyrifos, and esfenvalerate, were applied to 6%, 5%, and 3% of the planted acres, respectively (USDA-NASS, 2007a). Other methods of addressing insect infestations include the introduction of beneficial pests that prey on targeted insects obtained from commercial suppliers, as well as crop rotation and tillage as discussed above.

Table 5. Soybeans: total insecticide applications, 2006[1].

Insecticide	Area Applied (percent)	Applications (number)	Rate per Application (pounds per acre)	Rate per Crop Year (pounds per acre)	Total Applied (million pounds)
Acephate	1	1.3	0.72	0.934	0.546
Benzoic acid	<0.5	1.1	0.051	0.056	0.009
Carbaryl	<0.5	1	0.633	0.633	0.091
Chlorpyrifos	5	1.1	0.454	0.48	1.663
Cyfluthrin	<0.5	1.1	0.028	0.03	0.01
Diflubenzuron	<0.5	1.7	0.037	0.062	0.01
Esfenvalerate	3	1.1	0.035	0.037	0.07
Gamma-cyhalothrin	<0.5	1	0.011	0.011	0.003
Lambda-cyhalothrin	6	1.1	0.02	0.021	0.097
Methyl parathion	<0.5	1.1	0.529	0.565	0.066
Permethrin	<0.5	1	0.065	0.065	0.012
Thiodicarb	<0.5	1	0.32	0.32	0.039

Source: USDA-NASS (2007a)
[1] Program states surveyed - Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, South Dakota, Tennessee, Virginia, and Wisconsin; totaling 72.9 million planted acres.

Fungicides

Several plant diseases can also reduce soybean yield, many of which are addressed by planting disease resistant cultivars, and relatively few that may be treated with fungicides. Diseases that afflict soybean include fungal, bacterial, viral, and nematodes (Jardine, 1997). Diseases of major concern in soybean are *Cercospora* foliar blight, purple seed stain, aerial blight, soybean rust, pod and stem blight, and anthracnose (Padgett et al., 2011). Besides selecting cultivars with resistance to diseases prevalent in a producer's particular region (Hershman, 1997), a healthy soybean crop starts with planting disease-free seed (Jardine, 1997), implementing best management practices such as crop rotation to reduce disease carryover from crop to crop, and providing adequate nutrients and water for growth (Nelson, 2011). Additionally, a grower may also purchase seed treated with various chemicals, such as fungicide, to enhance soybean seed germination success (Jardine, 1997).

When disease does occur in soybean, despite taking such measures, chemical treatment options are fairly limited to those of fungal origin (Jardine, 1997; Padgett et al., 2011). USDA-NASS (2007a) found that the most commonly applied fungicides on soybean (azoxystrobin, propiconazole, pyraclostrobin, tebuconazole, and trifloxystrobin) were applied to only 4% of the planted soybean acreage in the 19 states surveyed in 2006. Of these fungicides, pyraclostrobin and azoxystrobin were the only two applied on more than 0.5% of the planted acres. Pyraclostrobin was applied to 2% of the planted acres at an average rate of 0.112 lb/A per year, whereas azoxystrobin was applied to 1% of planted acres at an average rate of 0.106 lb/A per year (USDA-NASS, 2007a).

Herbicides

The presence of weeds in soybean fields is a primary detriment to soybean productivity. Weeds have been estimated to cause a potential yield loss of 37% in world-wide soybean production (Heatherly et al., 2009). Weeds compete with soybean for light, nutrients, and soil moisture; can harbor insects and diseases; and can also interfere with harvest, causing extra wear on harvest equipment (Loux et al., 2008). In addition to weed density, the time period that weeds compete with the soybean crop influences the level of yield loss. The later the weeds emerge, the less impact they will have on yield. Soybean plants withstand early-season weed competition longer than corn, as the soybean canopy closes earlier (Boerboom, 2000). The extent of canopy closure restricts the light available for weeds and other plants growing below the soybean. In addition, canopy closure occurs more quickly when soybean is drilled or planted in narrow rows (Boerboom, 1999); however, in some studies it has also been observed that, depending on factors such as weed species, environmental conditions (i.e., rainfall amounts), and soybean cultivar, soybeans are able to compete with weeds with no resulting yield reduction (Krausz et al., 2001). Place et al. (2011) have determined that larger soybean seeds produce a larger canopy more quickly and are, therefore, more successful at outcompeting weeds.

Herbicides have been the primary tactic used to manage weed communities in soybeans since the mid-1960s and will continue to be an important feature of row crop weed management for the foreseeable future. One study looked at aggregagte data on crop yield losses and herbicide use and estimated that even if additional tillage and hand weeding labor replaced the use of herbicides U.S. crop production would decline by 20% with a $16 billion loss in value if herbicides were not used (Gianessi and Reigner, 2007).

In selecting an herbicide, a grower must consider, among other factors, whether an herbicide can be used on the crop (herbicides are registered by the EPA for specific uses/crops), the potential adverse effects on the crop, residual effects that can limit crops that can be grown in rotation, effectiveness on expected weeds, and cost. Herbicides have different ways of acting on plant physiology (i.e., modes of action) to affect the health of a given plant. Some common modes of herbicide action include auxin growth regulators like 2,4-D; amino acid inhibitors such as glyphosate; chlorophyll pigment inhibitors such as atrazine; lipid biosynthesis inhibitors like quizalofop; and glutamine synthase inhibitors such as glufosinate (UW-NPMP, No Date). Applications of herbicides to a crop may occur pre-plant (i.e., burndown), pre-emergence, or post-emergence. Herbicide use is not regulated by APHIS but regulated by EPA under FIFRA and its amendments (Schneider and Strittmatter, 2003).

Table 6 presents the most commonly applied herbicides to U.S. soybeans in 1995, 2001, and 2006 (the latest year with available national statistics) and the corresponding percent of acres treated (USDA-NASS, 2007b). Figure 3 graphs the usage trends of the top 10 herbicides in terms of percent planted acres treated. Glyphosate has become the most often-used herbicide on U.S. soybean, while the use of other herbices has decreased. In 2006, nearly 92 million pounds of glyphosate were applied on 92% of the planted acres, compared to 21% in 1995 (Table 6). Prior to 1995, glyphosate was primarily used for pre-plant weed control in soybean (Young, 2006). After 1995, annual glyphosate usage increased due to post-emergence application on Monsanto's Roundup Ready® Soybean (GTS 40-3-2) which became commercially available to growers in 1996.

Table 6. Percent of U.S. soybean acres[1] treated with herbicides in 1995, 2001 and 2006.

Herbicide	Percent Soybean Acres Treated			Herbicide	Percent Soybean Acres Treated		
	1995	2001	2006		1995	2001	2006
2,4-D[2]	10	4	3	Fomesafen	4	7	2
2,4-DB	1	--[3]	--	Glyphosate	21	73	92
Acetamide	--	<1	<1	Imazamox	--	5	<1
Acetic acid (2,4-D)	--	<1	7	Imazaquin	15	2	1
Acifluorfen	--	3	<1	Imazethapyr	44	9	3
Alachlor	4	<1	<1	Lactofen	5	1	<1
Bentazon	12	1	<1	Linuron	2	--	--
Butoxy ester 2,4-D	--	--	<1	Metolachlor	7	<1	--
Carfentrazone	--	--	<1	Metribuzin	11	2	2
Chlorimuron	16	5	4	Paraquat	2	--	1
Clethodim	5	4	3	Pendimethalin	26	10	3

Table 6. Percent of U.S. soybean acres[1] treated with herbicides in 1995, 2001 and 2006 (continued).

Herbicide	Percent Soybean Acres Treated			Herbicide	Percent Soybean Acres Treated		
	1995	2001	2006		1995	2001	2006
Clomazone	4	<1	--	Quizalofop	6	--	--
Cloransulam	--	5	1	S-Metolachlor	--	<1	1
Dimethenamid	1	--	--	Sethoxydim	7	1	<1
Ethalfluralin	1	--	--	Sulfentrazone	--	5	1
Fenoxaprop	6	3	<1	Sulfosate	--	3	1
Fluazifop	10	3	1	Thifensulfuron	12	2	1
Flumetsulam	2	<1	<1	Tribenuron	--	--	1
Flumiclorac	--	<1	1	Trifluralin	20	7	2

Source USDA-NASS, (2007b).
[1] Survey states:
1995: Arkansas, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, Ohio, and Tennessee.
2001: Arkansas, Illinois, Indiana, Iowa, Minnesota, Missouri, Nebraska, and Ohio.
2006: Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, South Dakota, Tennessee, Virginia, and Wisconsin.
[2] Dimethylamine salt formulation of 2,4-D
[3] -- = No value

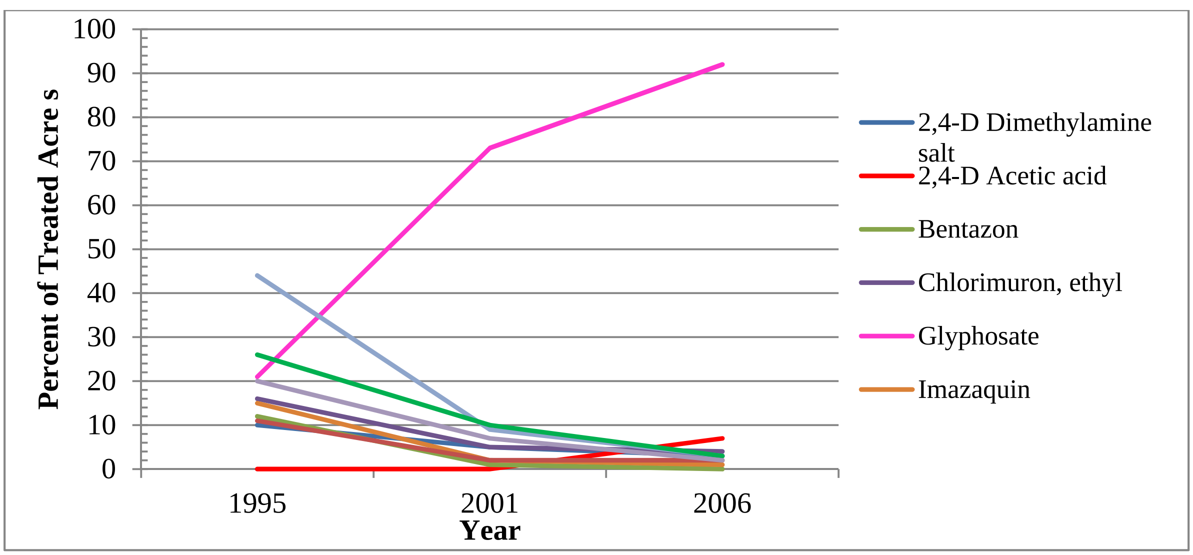


Figure 3. Percent of U.S. soybean acres treated with the 10 most used herbicides: 1995, 2001 and 2006.[1]

Source: USDA-NASS (2007b).
[1] Survey states:
1995: Arkansas, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, Ohio, and Tennessee.
2001: Arkansas, Illinois, Indiana, Iowa, Minnesota, Missouri, Nebraska, and Ohio.
2006: Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, South Dakota, Tennessee, Virginia, and Wisconsin.

The second most applied herbicide to soybean acres, as shown in Tables 6 and 7 and Figure 3, was acetic acid 2,4-D, with just over 3.5 million pounds applied to 7% of the planted soybean acres (USDA-NASS, 2007a). 2,4-D is approved for preplant use as a burndown herbicide for soybean. Figure 3 also shows that, while glyphosate-applied acres increased during the 12-year period, the number of acres that other herbicides were applied to significantly declined (NRC, 2010; Young, 2006). In 2006, based on soybean farmers surveyed in selected states, it was estimated that 98 percent of the planted soybeans were treated with at least one type of herbicide, ranging from 0.004 to 1.931 pounds of product per acre (lbs/A) per crop year (Table 7) (USDA-NASS, 2007a).

Herbicide usage trends since the adoption of GE crops are the subject of much debate, with initial assessments indicating a decline in herbicide use in the early years of herbicide-tolerant crop production (Carpenter et al., 2002) that some argue was then followed by an increase in the volume of herbicide usage as the technology spread (Benbrook, 2009). Others report a continuing decline in herbicide use with the adoption of GE crops (Fernandez-Cornejo and Caswell, 2006), or relative stability in the amount of herbicide active ingredients applied to soybeans (Brookes and Barfoot, 2010). The contradictory findings have been attributed to the different measurement approaches used by researchers, how different factors affecting pesticide use such as weather or cropping patterns were controlled for, and how the collected data was statistically analyzed (NRC, 2010).

Table 7. Soybeans: total herbicide applications, 2006[1].

Herbicide	Area Applied (percent)	Applications (number)	Rate per Application (pounds per acre)	Rate per Crop Year (pounds per acre)	Total Applied (thousand pounds)
2,4-D, 2-EHE	7	1.0	0.493	0.503	2,505
2,4-D, BEE	<0.5	1.1	0.426	0.459	68
2,4-D, dimeth. salt	3	1.0	0.462	0.475	953
Acifluorfen, sodium	<0.5	1.0	0.287	0.296	47
Alachlor	<0.5	1.0	1.931	1.931	485
Bentazon	<0.5	1.0	0.687	0.687	70
Carfentrazone-ethyl	<0.5	1.2	0.038	0.046	10
Chlorimuron-ethyl	4	1.0	0.017	0.017	52
Clethodim	3	1.1	0.096	0.102	190
Cloransulam-methyl	1	1.0	0.019	0.019	17
Dicamba, digly salt	<0.5	1.0	0.250	0.250	16
Fenoxaprop	<0.5	1.0	0.031	0.031	9
Fluazifop-P-butyl	1	1.0	0.099	0.099	43
Flufenacet	<0.5	1.0	0.265	0.265	80
Flumetsulam	<0.5	1.0	0.048	0.048	8
Flumiclorac-pentyl	1	1.4	0.020	0.028	17
Flumioxazin	3	1.0	0.066	0.066	138
Fomesafen	2	1.2	0.190	0.233	330

Table 7. Soybeans: total herbicide applications, 2006[1] (continued).

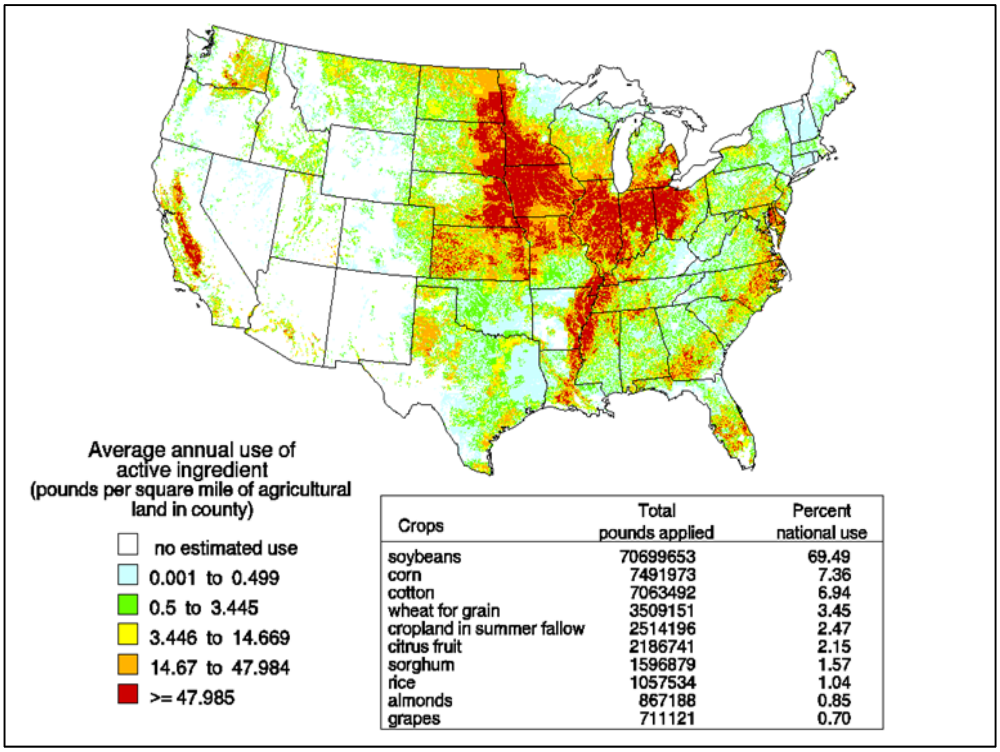
Herbicide	Area Applied (percent)	Applications (number)	Rate per Application (pounds per acre)	Rate per Crop Year (pounds per acre)	Total Applied (thousand pounds)
Glyphosate	4	1.5	0.630	1.044	2,841
Glyphosate amm. salt	<0.5	1.7	0.489	0.745	142
Glyphosate isop.salt	92	1.5	0.802	1.330	88,903
Imazamox	<0.5	1.0	0.030	0.030	9
Imazaquin	1	1.0	0.061	0.062	66
Imazethapyr	3	1.0	0.053	0.053	100
Imazethapyr, ammon	<0.5	1.0	0.048	0.048	5
Lactofen	<0.5	1.0	0.110	0.110	23
Metribuzin	2	1.0	0.255	0.260	437
Paraquat	1	1.0	0.492	0.511	335
Pendimethalin	3	1.0	0.920	0.926	1,894
Quizalofop-P-ethyl	<0.5	1.1	0.038	0.041	14
S-Metolachlor	1	1.0	1.023	1.023	837
Sethoxydim	<0.5	1.0	0.153	0.153	10
Sulfentrazone	1	1.0	0.087	0.091	70
Sulfosate	1	1.8	0.967	1.701	970
Thifensulfuron	1	1.1	0.004	0.004	3
Tribenuron-methyl	1	1.0	0.008	0.008	5
Trifluralin	2	1.0	0.818	0.818	1,454

Source: USDA-NASS (2007a)

[1] Program states surveyed - Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, South Dakota, Tennessee, Virginia, and Wisconsin; totaling 72.9 million planted acres.

As noted, above, glyphosate is the most used herbicide on U.S. soybean. Glyphosate (N-phosphonomethyl-glycine), a nonselective herbicide, was first introduced under the trade name of Roundup® by Monsanto in 1974. Glyphosate is a systemic herbicide used on both agricultural and nonagricultural sites (Cerdeira and Duke, 2006). The CP4 EPSPS protein confers tolerance to glyphosate and has been used in many Roundup Ready® crops (e.g., canola, corn, cotton, soybean, and sugar beet). Glyphosate may be used premergent, preplant incorporated, or postemergent with Roundup Ready® crops. Annual agricurlural usage of glyphosate in the U.S., based on data collected from 1999 to 2004, is shown is Figure 4. The highest estimated agricultural use of glyphosate, approximately 69 percent, is on soybean (United States Geological Survey, No Date-c)

At normal temperatures, glyphosate is a white crystalline substance that is not volatile (is not likely to vaporize at atmospheric pressure) and is highly soluble in water. Glyphosate salts serve as the source of the active ingredient (a.i.) *N*-(phosphonomethyl) glycine. To improve handling, performance, and concentration, the glyphosate acid is formulated as a salt compound. Several salts of glyphosate are currently marketed. The term acid equivalent (a.e.) refers to the weight of the glyphosate acid, which is herbicidally active, while a.i. is the weight of the glyphosate acid plus the salt. As listed on the Roundup® herbicide labels, Roundup Original MAX®, Roundup WeatherMAX®, and Roundup PowerMAX® products contain 48.8 percent of the potassium salt of glyphosate, equivalent to 4.5 lb of glyphosate a.e. per gallon (540 g glyphosate per L). The product is to be applied over-the-top (e.g., spot treatment, broadcast ground application) for preplant, preemergence, and postemergence weed control (Monsanto, 2007, 2009, 2010c).



Crops	Total pounds applied	Percent national use
soybeans	70699653	69.49
corn	7491973	7.36
cotton	7063492	6.94
wheat for grain	3509151	3.45
cropland in summer fallow	2514196	2.47
citrus fruit	2186741	2.15
sorghum	1596879	1.57
rice	1057534	1.04
almonds	867188	0.85
grapes	711121	0.70

Figure 4. Estimated annual agricultural use of glyphosate in the U.S.

Source: (United States Geological Survey, No Date-c).

Notes: Map represents average annual pesticide use intensity expressed as average weight (in pounds) of a pesticide applied to each square mile of agricultural land and typical use patterns over the five year period of 1999 through 2004.

There are several reasons for the success of glyphosate in the market and the corresponding market sector penetration of glyphosate-tolerant crops since their introduction in the mid-late 1990s. Glyphosate: 1) works non-selectively on a wide range of plant species; 2) is a relatively low-cost herbicide; 3) enhances 'no-till' farming practices; and 4) has minimal animal toxicological and environmental impact (Duke and Powles, 2009; Owen, 2008). The widespread adoption of glyphosate-tolerant soybean, in combination with an increased reliance on glyphosate, has been related to the ability to grow no-till soybean cultivation while effectively controlling weeds, simplification in weed control compared to past practices, reduced input and labor costs associated with the cultivar and glyphosate use, and flexibility in glyphosate application timing to tolerant soybean (Young, 2006).

Not unlike other agronomic practices, herbicide use may impart selection pressures on weed communities resulting in shifts in the weed community that favor those weeds that do not respond to the herbicide used (Owen, 2008). The shift to herbicide resistance in plants is largely a function of the natural selection of herbicide-resistant traits and is strongly related to the repeated use of one or a limited number of herbicides (Duke, 2005; Durgan and Gunsolus, 2003). Both the increased selection pressure resulting from the extensive use of glyphosate associated with glyphosate-tolerant crops with subsequent reductions in the use of other herbicides and changes in weed management practices (i.e., conservation tillage or no-till) have resulted in weed population shifts and increasing glyphosate resistance among some weed populations (Duke and Powles, 2009; Owen, 2008). Glyphosate-resistant crops themselves do not influence weeds any more than non-transgenic crops. It is the weed control tactics chosen by growers that create selection pressure that ultimately over time change these weed communities and may result in the evolution of herbicide-resistant weeds (Owen, 2008). Herbicide resistant weeds are discussed in more detail in Section 2.3.2, Plant Communities.

Currently, internationally there are 372 herbicide-resistant weed individuals or biotypes described which are represented in 200 plant families (Heap, 2011). The first herbicide-resistant biotypes were described in the 1950s, but the number of weeds resistant to herbicides increased dramatically in the 1980s and 1990s, and currently evolved resistance to 21 different herbicide modes of action is identified throughout the world (Heap, 2011). An estimated 6 percent of the total planted corn, soybean, and cotton acres in the U.S. have some level of weeds that are resistant to glyphosate (WSSA, 2010).

To combat this trend and to avoid decreased crop yields resulting from weed competition, growers continually adapt weed management strategies, including the use of herbicides with alternative modes of action (DAS, 2010). Alternative modes of action in this case refer to herbicides which are different with respect to how they act on the plant physiology. Some common modes of herbicide action include auxin growth regulators, amino acid inhibitors, chlorophyll pigment inhibitors, or lipid biosynthesis inhibitors (Ross and Childs, 2011). The practice of using herbicides with alternative modes of action could potentially diminish the populations of glyphosate-tolerant weeds and reduce the likelihood of the development of new herbicide-resistant weed populations (Dill et al., 2008; Duke and Powles, 2009; Owen, 2008).

A variety of strategies have been proposed to help farmers deal with glyphosate-resistant weeds (Beckie, 2006; Boerboom, 1999; Frisvold et al., 2009; Sammons et al., 2007), including:

- The rotation of herbicides with different modes of action;
- Site specific herbicide applications;
- Use of full labeled application rates;
- Crop rotation;
- Use of tillage for supplemental weed control;
- Cleaning equipment between fields;
- Controlling weed escapes;
- Controlling weeds early; and
- Scouting for weeds before and after herbicide applications.

DAS-68416-4 soybean, with its tolerance to two herbicides, 2,4-D and the glufosinate, was developed to provide growers with alternative herbicides to use in soybean (DAS, 2010). The following paragraphs present a summary of the current uses and registrations of these two herbicides.

2,4-D is in the phenoxy or phenoxyacetic acid family and is listed as an herbicide, a plant growth regulator, and a fungicide. Its main use is as a selective post-emergence herbicide for controlling broadleaf weed species. In 2002, 2,4-D was ranked as the third most used herbicide by active ingredient in the U.S. for all purposes (~40 million pounds), behind glyphosate (~102 million pounds) and atrazine (~77 million pounds) (Gianessi and Reigner, 2006). The herbicide is approved for use on a wide variety of crops, and has more than 600 registered end-use products for use on more than 300 distinct agricultural and residential sites, including terrestrial and aquatic settings (US-EPA, 2005c). Of the approximately 46 million pounds of 2,4-D used annually in the U.S., 30 million pounds (66%) were used by agriculture (Figure 5) and 16 million pounds (34%) were used in non-agriculture settings such as pasture/rangeland and lawn/garden (US-EPA, 2005a). As can be seen in Figure 5, 2,4-D is used predominantly in the Midwest, Great Plains, and Northwestern U.S. Agriculturally, it is used on a variety of crops including corn, rice, sorghum, sugar cane, wheat, rangeland, and pasture. In addition, 2,4-D is used to control unwanted vegetative growth on utility corridors, rights-of-way, roadsides, non-crop areas, managed forest, and lawn and turf areas. It is also used to control aquatic and nuisance weeds, e.g., purple loosestrife (Industry Task Force II, 2005). 2,4-D controls many broadleaf weeds including carpetweed, dandelion, cocklebur, horseweed, morning glory, pigweed sp., lambsquarters, ragweed spp., shepherd's-purse, and velvetleaf. It has little to no effective activity on grasses, including wheat, corn, and rice (Industry Task Force II, 2005).

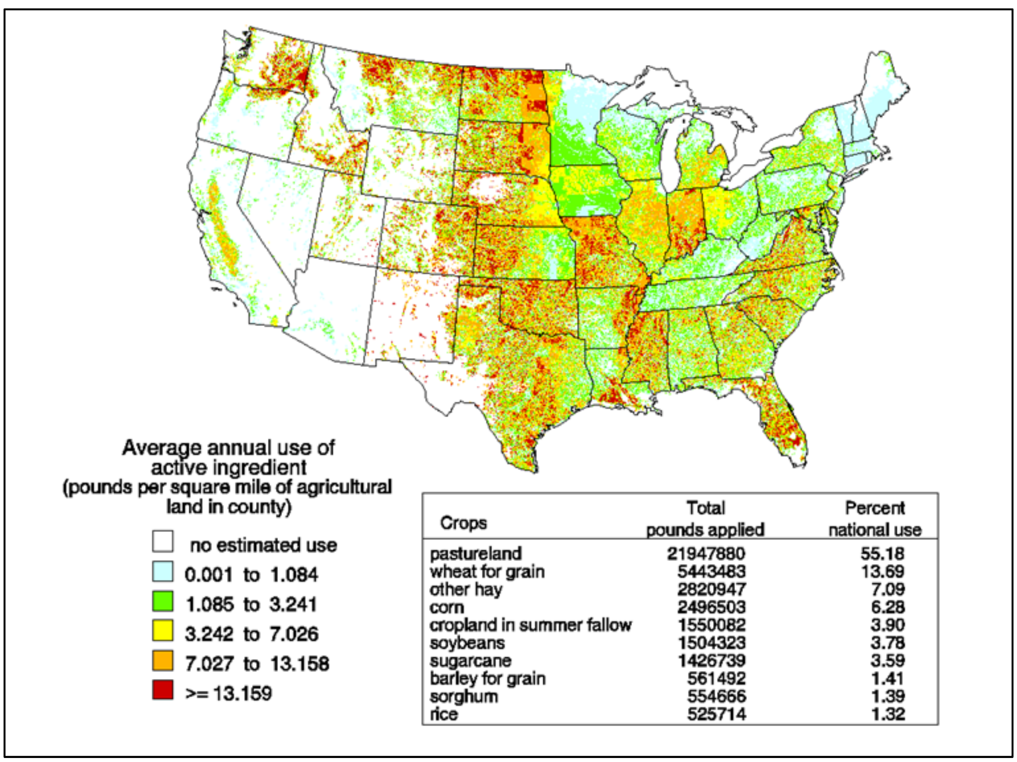


Figure 5. Estimated annual agricultural use of 2,4-D in the U.S.

Source: (United States Geological Survey, No Date-a).

Note: Map represents average annual pesticide use intensity expressed as average weight (in pounds) of a pesticide applied to each square mile of agricultural land and typical use patterns over the five year period of 1999 through 2004.

In terms of total pounds of usage, 2,4-D is mainly applied to pasture/rangeland (24%), lawn by homeowners with fertilizer (12%), spring wheat (8%), winter wheat (7%), lawn/garden by lawn care operators/landscape maintenance contractors (7%), lawn by homeowners alone (without fertilizer) (6%), field corn (6%), soybeans (4%), summer fallow (3%), hay other than alfalfa (3%), and roadways (3%) (US-EPA, 2005a). It is the second most used herbicide for soybean production (Table 7).

The mode of action of 2,4-D is described as an "auxin mimic," meaning that it kills the target weed by mimicking auxin plant growth hormones, like indole acetic acid (IAA) (Tu et al., 2001). Auxins and synthetic auxinic herbicides regulate virtually every aspect of plant growth and development; at low doses, auxinic herbicides possess similar hormonal properties to natural auxin (Kelley and Riechers, 2007). However, as rates increase, they can cause various plant growth abnormalities in sensitive dicots (Tu et al., 2001). Observable plant responses to 2,4-D can include epinasty, root growth inhibition, meristematic proliferation/callusing, leaf cupping/narrowing, stem cracking, adventitious root formation, senescence, and chlorosis. This uncontrolled and disorganized plant growth eventually leads to plant death when applied at effective doses (Tu et al., 2001).

The herbicide 2,4-D is currently available in several formulations, including 2,4-D acid, 2,4-D sodium salt, 2,4-D diethylamine, 2,4-D dimethylamine salt, 2,4-D isopropyl acid, 2,4-D triisopropyl acid, 2,4-D butoxyethyl ester (BEE), 2,4-D ethylhexyl ester, and 2,4-D isopropyl ester (US-EPA, 2005a). 2,4-D is formulated primarily as an amine salt in an aqueous solution or as an ester in an emulsifiable concentrate (US-EPA, 2005c). The 2,4-D mode of action as a synthetic auxin is not changed by these formulations, but the chemical and physical properties of each formulation influence the selection of equipment, mitigation measures adopted in the field to minimize off-target impacts, and formulation-specific safety measures. For a majority of uses, 2,4-D is combined with other herbicides because it economically enhances the weed control spectrum of many other herbicides such as glyphosate, dicamba, mecoprop, and acetolactate synthase (ALS) herbicides (US-EPA, 2005c).

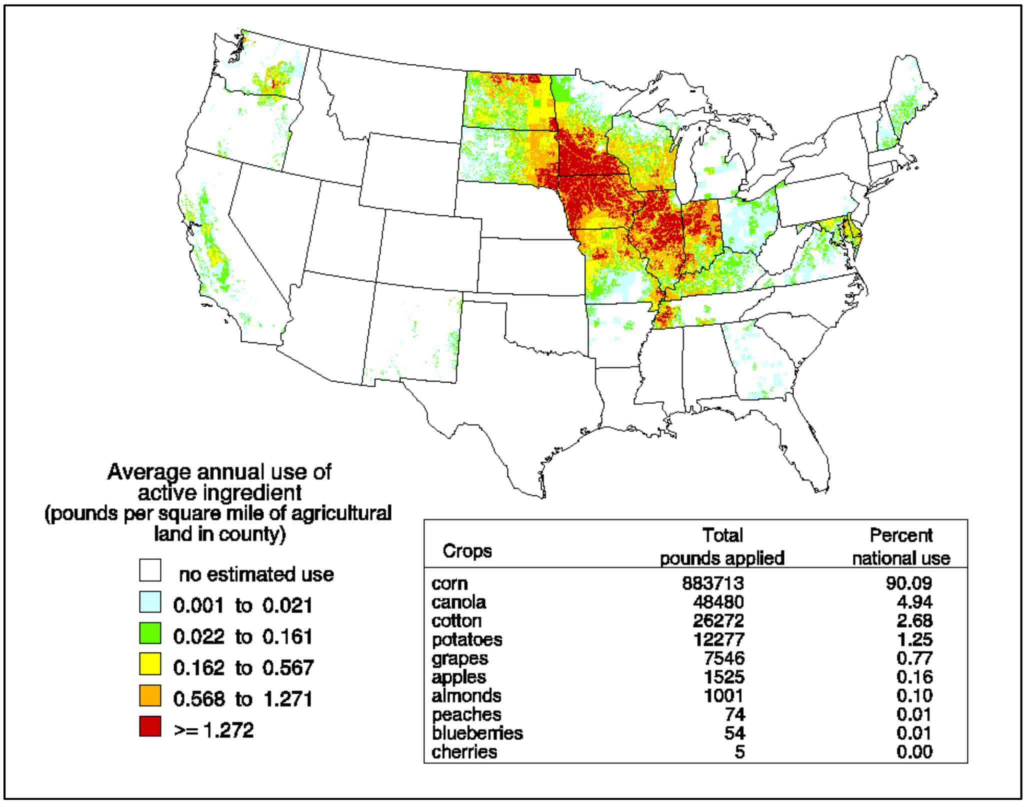
For 2,4-D, rates per application and rates per year are generally less than 1.5 pounds acid equivalent (ae) per acre (lbs ae/A) and 2.0 lb ae/A per year, respectively. Maximum rates are 4.0 lbs ae/A per year for asparagus, forestry uses, and non-cropland uses, among others. The maximum rate for aquatic uses is 10.8 lbs ae/acre foot for submerged aquatic plants. Typically, one to three applications are made per growing season. 2,4-D is currently registered in the U.S. for use on corn. The currently approved application rates for field corn and popcorn are a maximum per-year application rate of 3 lbs/acre and a maximum single application rate of 1.5 lbs/acre. 2,4-D is approved for use on soybean only for pre-plant burndown application. Application rates on soybean are 0.5 or 1.0 lbs ae/A per application or 1.0 lbs ae/A per crop or year (US-EPA, 2005c). It may not be applied any later than 7-15 days (0.5 - 1.0 lb ae/A of ester formulations) or 15-30 days (0.5 - 1.0 lb ae/A of amine formulations) prior to planting due to the potential for crop injury (DAS, 2010).

Glufosinate herbicides contain the active ingredient phosphinothricin and are in the phosphinic acid family of herbicides. The herbicide acts by blocking the plant enzyme glutamine synthetase, which is responsible for nitrogen metabolism and for detoxifying ammonia, a by-product of plant metabolism. The exposed plant dies by the overproduction of ammonia (US-EPA, 2008b). It is a non-selective foliar herbicide that is used for the control of broadleaf and grass weeds in a variety of crops and non-crop areas. First registered with the EPA in 1993, initial glufosinate end-use products were for home owner, light industrial non-food, and farmstead weed control (OSTP, 2001). Glufosinate, a water soluble herbicide, is approved for use on apples, berries, canola, corn, cotton, currants, grapes, grass grown for seed, potatoes, rice, soybeans, sugar beets, and tree nuts. Non-crop areas where glufosinate is registered for use on include residential lawns and industrial and public areas. Products include Rely®, Remove™, AEH®, Derringer® and Finale® (US-EPA, 2008b). Ignite®/Liberty® glufosinate products are registered exclusively for selective over-the-top use on GE LibertyLink® corn, cotton, canola, rice, and soybean.

In 2002, it was estimated that glufosinate use in the U.S. for all purposes was 982,324 lb a.i. (Gianessi and Reigner, 2006). Estimates of annual applications of glufosinate in the U.S. (Figure 6) indicate that approximately 1,000,000 lb a.i. were applied to agricultural land, with the highest percentage (90 percent) used on corn (United States Geological Survey, No Date-b). Based on somewhat newer data from 2001 through 2006, EPA estimated that the highest agricultural uses of glufosinate are in corn (900,000 lb a.i./yr), cotton (300,000 lb a.i./yr), canola (60,000 lb a.i./yr), almonds (30,000 lb a.i./yr), and grapes (20,000 lb a.i./yr). Glufosinate use on potatoes, rice, and soybean is 10,000 lbs a.i./yr for each crop, with less than 1 percent of the crop treated (US-EPA, 2007d). With the commercial availability of glufosinate-tolerant LibertyLink® soybean beginning in 2009, glufosinate use on soybeans has increased slightly. Glufosinate-tolerant soybean accounted for less than 1 percent of soybean acreage planted in the U.S. in 2009 with approximately 72,000 lb a.i. glufosinate applied. In 2011, the planted acreage of glufosinate-tolerant soybeans increased to 1.3 percent and glufosinate use rose to approximately 550,000 lb (DAS, 2011h).

Application rates of glufosinate range signifantly by use pattern, with the highest rate allowed for broadcast (ground) spray applications, 1.5 lbs a.i./A, on orchard nuts and fruits, grapes, grasses grown for seed, and golf course turf. On the low end of application rates, labeled uses of glufosinate on turf and patio are at 0.03 lbs a.i./A. Multiple applications are allowed by most labels, although the interval is not generally specified (US-EPA, 2008b). The EPA-registered use of glufosinate on LibertyLink® (i.e., glufosinate-tolerant) soybean includes an intial application of glufosinate no higher than 0.66 lb a.i./A (36 fl oz/A) with a minimum of 0.40 lb a.i./A (22 fl oz/A). A single second application of glufosinate up to 0.53 lb a.i./A (29 fl oz/A) is the approved on LibertyLink® soybeans, with a seasonal maximum rate of 1.2 lb a.i./A (65 fl oz/A) permitted. Glufosinate applications on LibertyLink® soybean should be made from emergence up to but not including the bloom growth stage and within 70 days of harvesting soybean (Bayer CropScience, 2011).

The implications of the potential use of 2,4-D and glufosinate for soybean cultivation associated with a determination of nonregulated status of DAS-68416-4 soybean are discussed in Section 4.



Crops	Total pounds applied	Percent national use
corn	883713	90.09
canola	48480	4.94
cotton	26272	2.68
potatoes	12277	1.25
grapes	7546	0.77
apples	1525	0.16
almonds	1001	0.10
peaches	74	0.01
blueberries	54	0.01
cherries	5	0.00

Figure 6. Estimated annual agricultural use of glufosinate in the U.S.

Source: (United States Geological Survey, No Date-b).

Note: Map represents average annual pesticide use intensity expressed as average weight (in pounds) of a pesticide applied to each square mile of agricultural land and typical use patterns over the five year period of 1999 through 2004.

2.1.3 Soybean Seed Production

In 2011, nearly 75 million acres of soybean required seed for planting in the U.S. (USDA-NASS, 2011e). Several factors influence optimal planting rate for soybean such as row spacing, seed germination rate, soil conditions, climate, disease and pest pressure, past tillage practices and crop rotation (Robinson and Conley, 2007). Seeding rate is also determined by the plant population desired by the grower. In Iowa, the recommended planting rate for soybean ranges from 150,000 to 200,000 seeds per acre or between 37.5 and 100 pounds of seed per acre, depending on seed size (Whigham, 1998). Seed sizes range from 2,000 to 4,000 seeds per pound (Whigham, 1998). Growers may plant certified soybean seed, uncertified seed, and "bin-run" soybean seed that is grown and stored on individual farms (Oplinger and Amberson, 1986). Since 94% of the soybean acres planted in the U.S. in 2011 were GE varieties (USDA-ERS, 2011b), at least 70.5 million acres were planted with certified seeds. Using a conservative planting rate of 150,000 seeds per acre, an estimated 1.3 to 2.6 million tons of certified soybean planting seeds were required in 2011.

Seed quality includes a variety of attributes, including genetic purity, vigor, weed seed content, seed borne diseases, and the presence of foreign material such as dirt or chaff (Bradford, 2006). The genetic purity of the seed must be maintained to maximize the value of the new variety or cultivar (Sundstrom et al., 2002). Genetic purity in the production of commercial soybean seed is regulated through a system of seed certification which ensures the desired traits in that particular seed remain within purity standards (Bradford, 2006).

The U.S. Federal Seed Act of 1939 recognizes seed certification and official certifying agencies. Implementing regulations further recognize land history, field isolation, and varietal purity standards for seed. States have developed laws to regulate the quality of seed available to farmers (Bradford, 2006). Most of the laws are similar in nature and have general guidelines for providing information on the label for the following:

- Commonly accepted name of agricultural seed;
- Approximate total percentage by weight of purity;
- Approximate total percentage of weight of weed seeds;
- Name and approximate number per pound of each kind of noxious weed seeds;
- Approximate percentage of germination of the seed; and
- Month and year the seed was tested.

Various seed associations have standards to help maintain the quality of soybean seed. The Association of Official Seed Certifying Agencies (AOSCA) (AOSCA, No Date) defines the classes of seed as follows:

- *Breeder* seed is directly controlled by the plant breeder that developed the variety.
- *Foundation* seed is the progeny of Breeder or Foundation seed that is handled to most nearly maintain specific genetic identity and purity.
- *Registered* seed is a progeny of Breeder or Foundation seed that is so handled as to maintain satisfactory genetic identity and purity.
- *Certified* seed is the progeny of Breeder, Foundation, or Registered seed that is so handled as to maintain satisfactory genetic identity and purity.

Seed certification systems should be distinguished from Identity Preservation (IP) systems for certain agricultural commodities. IP refers to a system of production, handling, and marketing practices used in order to maintain the integrity and purity of crop products throughout the food supply chain (Sundstrom et al., 2002). IP systems are utilized to meet the demands for specialized grains products, including those from crops with output-specific traits (e.g., high oleic oil), without specific traits or attributes (e.g., non-GE crops), grown under specific production methods (e.g., organic crops), and requiring rigorous safeguards and confinements practices (e.g., pharmaceutical and industrial crops) (Elbehri, 2007).

Soybean is self-pollinated, propagated commercially by seed (Hoeft et al., 2000a; OECD, 2000). In the U.S. there are no *Glycine* species found outside of cultivation, and the potential for outcrossing is minimal (OECD, 2000). Additionally, Minimum Land, Isolation, Field, and Seed Standards (7 CFR part 201.76) specify that isolation distances for the production of Foundation, Registered and Certified soybean seeds from any potential contaminating source must be adequate to prevent mechanical mixing.

2.1.4 Organic Soybean Production

In the U.S., only products produced using specific methods and certified under the USDA's Agricultural Marketing Service (AMS) National Organic Program (NOP) definition of organic farming can be marketed and labeled as "organic" (USDA-AMS, 2008). Organic certification is a process-based certification, not a certification of the end product; the certification process specifies and audits the methods and procedures by which the product is produced.

In accordance with NOP, an accredited organic certifying agent conducts an annual review of the certified operation's organic system plan and makes on-site inspections of the certified operation and its records. Organic growers must maintain records to show that production and handling procedures comply with USDA organic standards.

The NOP regulations preclude the use of excluded methods. The NOP provides the following guidance under 7 CFR § 205.105:

> …to be sold or labeled as "100 percent organic", "organic" or "made with organic (specified ingredients or group(s))," the product must be produced and handled without the use of:…
>
> (a) Synthetic substances and ingredients,…
>
> (e) Excluded methods,…

Excluded methods are then defined at 7 CFR § 205.2 as:

> A variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes and are not considered compatible with organic production. Such methods include cell fusion, microencapsulation and macroencapsulation, and recombinant deoxyribonucleic acid (DNA) technology (including gene deletion, gene doubling, introducing a foreign gene, and changing the positions of genes when achieved by recombinant DNA technology). Such methods do not include the use of traditional breeding, conjugation, fermentation, hybridization, in vitro fertilization, or tissue culture.

Organic farming operations, as described by the NOP, are required to have distinct, defined boundaries and buffer zones to prevent unintended contact with excluded methods from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods (USDA-AMS, 2008).

Common practices organic growers may use to exclude GE products include planting only organic seed, planting earlier or later than neighboring farmers who may be using GE crops so that the crops will flower at different times, and employing adequate isolation distances between the organic fields and the fields of neighbors to minimize the chance that pollen will be carried between the fields (NCAT, 2003). Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards (USDA-AMS, 2008). The current NOP regulations do not specify an acceptable threshold level for the adventitious presence of GE materials in an organic-labeled product. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan (Ronald and Fouche, 2006; USDA-AMS, 2008).

Organic soybean production practices include crop rotation, use of cover crops, green and animal manures, application of rock minerals such as lime, other soil additives, mechanical weed control, biological control of pests, and disease control primarily through management practices (Heatherly et al., 2009; Kuepper, 2003; USDA-AMS, 2011). Utilizing 2006 ARMS data, McBride and Greene (2008) determined that more than 90% of organic soybean producers planted in standard rows, as compared to 60% of other soybean producers. Further, organic soybean operations rotated crops more often, and 40% of the farmers incorporated a one-year fallow into their organic soybean rotation.

Weed control in organic systems is accomplished with delayed seeding to avoid spring weeds, applying fertilizer to growing plants to outcompete weeds, increasing seeding rates, sowing cover crops, crop rotation, intercropping, flame weeding, hand weeding, and mechanical means (e.g., tillage) (Heatherly et al., 2009; Kuepper, 2003; Place et al., 2011). Organic crop production historically employed mulch and ridge tillage practices (NCAT, 2003); however, no-till may be unsustainable in some long-term organic systems because of increasingly poor weed control (Teasdale et al., 2007). The latter cited study conducted field evaluations of several tillage systems over nine years, finding that in the organic system evaluated, factors contributing to poor weed control included uneven seeding beds produced by chisel-tilling in a cover crop and animal manure, variable ground cover occurring in mowed cover crop residue, insufficient disruption of weed roots by sweep-type cultivators, and the short grain crop rotation system used was unsuitable for maintaining a low weed seedbank (Teasdale et al., 2007).

Pest control in organic systems is accomplished with application of natural pesticides, integrated pest management techniques such as introduction of beneficial organisms in the form of soil predator and parasitic organisms, and some of the practices described for weed control, such as crop rotation, intercropping, and use of cover crops (NCAT, 2003).

Diseases are primarily controlled in organic systems by planting disease-resistant varieties and with management practices that promote healthy soil, rotating crops, diligently removing diseased plant material, and plant canopy management (NCAT, 2003). When physical, mechanical, or biological controls are not sufficient for controlling weeds, pests, or disease, only a biological, botanical or synthetic substance approved on the national list may be used (USDA-AMS, 2011).

USDA-Economic Research Service (ERS) recently reported the organic crop production data collected in 2008 (USDA-ERS, 2010b). In that year, 125,621 acres of organic soybeans in 28 states were harvested (Table 8), compared to approximately 74.5 million harvested acres of conventionally produced soybean (USDA-NASS, 2011e). In 2008, organic soybean production consisted of about 0.13% of total U.S. soybean production and was valued at approximately $50.2 million, capturing roughly 0.17% of the overall soybean crop value for that year (USDA-NASS, 2009a, 2011e). Organic soybean producers generally harvest lower yields than other producers (Heatherly et al., 2009; McBride and Greene, 2008). McBride and Greene (2008) also found total operating costs averaged $30 more per acre and capital costs averaged $60 per acre higher for organic soybean producers than for other conventional soybean producers.

Table 8. U.S. certified organic soybean acres by state, 2008.

State	Soybeans (acres)		State	Soybeans (acres)	
	2007	2008		2007	2008
Arizona	241	241	Missouri	7,893	6,441
Arkansas	8,374	11,172	Nebraska	5,672	8,825
Colorado	488	3,502	New York	1,324	5,038
Connecticut	9	9	North Carolina	165	94
Delaware	25	25	North Dakota	3,308	3,773
Idaho	1	1	Ohio	3,665	3,951
Illinois	6,277	7,225	Oklahoma	80	165
Indiana	888	1,104	Oregon	--	141
Iowa	6,989	19,913	Pennsylvania	1,589	1,753
Kansas	639	2,141	South Dakota	4,531	4,786
Maine	144	194	Texas	2,093	2,141
Maryland	416	437	Vermont	--	337
Michigan	11,320	11,251	Virginia	360	363
Minnesota	25,518	21,229	Wisconsin	8,381	9,369
U.S. Total				100,390	125,621

Source: USDA-ERS (2010b)

2.2 Physical Environment

2.2.1 Soil Quality

Soil consists of solids (minerals and organic matter), liquids, and gases. This body of inorganic and organic matter is home to a wide variety of fungi, bacteria, and arthropods, as well as the growth medium for terrestrial plant life (USDA-NRCS, 2004). Soil is characterized by its layers that can be distinguished from the initial parent material due to additions, losses, transfers, and transformations of energy and matter (USDA-NRCS, 1999b). It is further distinguished by its ability to support rooted plants in a natural environment. Soil plays a key role in determining the capacity of a site for biomass vigor and production in terms of physical support, air, water, temperature moderation, protection from toxins, and nutrient availability. Soils also determine a site’s susceptibility to erosion by wind and water, and flood attenuation capacity.

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

Soybeans are normally grown in managed agricultural fields for crop production and are best suited to fertile, well-drained medium-textured loam soils, yet can be produced in a wide range of soil types (Berglund and Helms, 2003; NSRL, No Date). Soybeans need a variety of macronutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, at various levels (NSRL, No Date). They also require smaller amounts of micronutrients such as iron, zinc, copper, boron, manganese, molybdenum, cobalt, and chlorine. These micronutrients may be deficient in poor, weathered soils, sandy soils, alkaline soils, or soils excessively high in organic matter. As with proper nutrient levels, soil pH is critical for soybean development. Soybeans grow best in soil that is slightly acidic (pH 5.8 to 7.0); soil with a pH that is too high (7.3 or greater) negatively affects yield (Cox et al., 2003; NSRL, No Date). Similarly, soils that are high in clay and low in humus may impede plant emergence and development (NSRL, No Date). Soils with some clay content may increase moisture availability during periods of low precipitation (Cox et al., 2003).

Land management practices for soybean cultivation can affect soil quality. While practices such as tillage, fertilization, the use of pesticides and other management tools can improve soil health, they can also cause substantial damage if not properly used. Several concerns relating to agricultural practices include increased erosion, soil compaction, degradation of soil structure, nutrient loss, increased salinity, change in pH, and reduced biological activity (USDA-NRCS, 2001).

As discussed in Subsection 2.1.2, Agronomic Practices, conventional and conservation tillage may be used for the cultivation of soybean. Reducing excessive tillage through practices such as conservation tillage minimizes the loss of organic matter and protects the soil surface by leaving plant residue on the surface. Management of crop residue is one of the most effective conservation methods to reduce wind and water erosion, and also benefits air and water quality and wildlife (USDA-NRCS, 2006a). Residue management that uses intensive tillage and leaves low amounts of crop residue on the surface results in greater losses of soil organic matter (SOM). Intensive tillage turns the soil over and buries the majority of the residue, stimulating microbial activity and increasing the rate of residue breakdown (USDA-NRCS, 1996). The residues left after conservation tillage increase organic matter and improve infiltration, soil stability and structure, and soil microorganism habitat (Fawcett and Caruana, 2001; USDA-NRCS, 2006c). Organic matter is probably the most vital component in maintaining quality soil; it is instrumental in maintaining soil stability and structure, reduces the potential for erosion, provides energy for microorganisms, improves infiltration and water holding capacity, and is important in nutrient cycling, cation exchange capacity[2], and the breakdown of pesticides (USDA-NRCS, 1996).

The residue left over from conservation tillage practices increases SOM in the top three inches of the soil and protects the surface from erosion while maintaining water-conducting pores. Soil aggregates in conservation tillage systems are more stable than those of conventional tillage due to the products of SOM decomposition and the presence of soil bacteria and fungal hyphae (filamentous structures that composes the main growth) that bind aggregates and soil particles together (USDA-NRCS, 1996). Although soil erosion rates are dependent on numerous local conditions such as soil texture and crop, a comparison of 39 studies contrasting conventional and no-till practices illustrates that, on average, no-till practices reduce erosion 488 times over conventional tillage (Montgomery, 2007). This reduction is enough to bring soil production more in line with losses from erosion. From 1982 through 2003, erosion on U.S. cropland dropped from 3.1 billion tons per year to 1.7 billion tons per year (USDA-NRCS, 2006a). This can partially be attributed to the increased effectiveness of weed control through the use of herbicides and the corresponding reduction in the need for mechanical weed control (Carpenter et al., 2002). Conservation tillage also minimizes soil compaction due to the reduced number of tillage trips.

While conservation tillage does have several benefits for soil health, some management concerns are associated with its use. Under no-till practices, soil compaction may become a problem as tillage is useful for breaking up compacted areas (USDA-NRCS, 1996). Likewise, not all soils (such as wet and heavy clay soils) are suited for no-till. Also, no-till practices may lead to increased pest occurrences that conventional tillage is better suited to managing (NRC, 2010).

Other methods to improve soil quality include careful management of fertilizers and pesticides; use of cover crops to increase plant diversity and limit the time soil is exposed to wind and rain; and, increased landscape diversity with buffer strips, contour strips, wind breaks, crop rotations, and varying tillage practices (USDA-NRCS, 2006c).

There are a multitude of organisms associated with soils, ranging from microorganisms to larger organisms, such as worms and insects. The microorganisms that make up the soil community include bacteria, fungi, protozoa, and nematodes. These organisms are responsible for a wide range of activities that impact soil health and plant growth. Decomposers, such as bacteria, actinomycetes (filamentous bacteria), and saprophytic fungi, degrade plant and animal remains, organic materials, and some pesticides (USDA-NRCS, 2004). Other organisms, such as protozoa, mites and nematodes, will consume the decomposer microbes and release macro- and micronutrients, making them available for plant usage. Another important group of soil microorganisms are the mutualists. These are the mycorrhizal fungi, nitrogen-fixing bacteria, and some free-living microbes that have co-evolved with plants that supply nutrients to and obtain food from their plant hosts (USDA-NRCS, 2004). The *Bradyrhizobium japonicum* bacteria associated with soybeans is a nitrogen-fixing rhizobium bacteria found in plant root

[2] Cation exchange capacity is the ability of soil anions (negatively charged clay, organic matter and inorganic minerals such as phosphate, sulfate, and nitrate) to adsorb and store soil cation nutrients (positively charged ions such as potassium, calcium, and ammonium).

nodules (Franzen, 1999). Since neither soybean nor *B. japonicum* is native to North America, if a field has not been planted with soybean within three to five years, either the seed or seed zone must be inoculated with the rhizobium bacteria prior to soybean planting (Berglund and Helms, 2003; Pedersen, 2007).

Pesticide use has the potential to affect soil quality due to the impact to the soil microbial community and is discussed further in Subsection 2.3.4, Microorganisms. The potential effects of using 2,4-D and glufosinate on the soil environment have been well studied. The length of persistence of herbicides in the environment is dependent on the concentration and rate of degradation by biotic and abiotic processes (Carpenter et al., 2002). Persistence is measured by the half-life or dissipation time (DT_{50}), which equates to the length of time needed for the herbicide to degrade to half of its original concentration. The 2,4-D amine salts are non- to moderately persistent under most environmental conditions, including those related to agricultural conditions (US-EPA, 2004). The degradation of 2,4-D appears to be dependent on mineralization by microbes in soil, photodegradation in water, and leaching (US-EPA, 2005c). In soil, it is strongly influenced by moisture, temperature, organic matter content and pH (FAO, 1997; Senseman, 2007). Under most environmental conditions 2,4-D amine salts dissociate in less than three minutes; however, since analytical methods cannot separate and identify 2,4-D dimethylamine salt (DMAS) from 2,4-D, conservative half-life estimates range from 1.1 to 30.5 days with a median half-life of 5.6 days (US-EPA, 2005c).

Glufosinate is weakly absorbed to and is highly mobile in soil, undergoes rapid microbial degradation in soil, and has a short soil residual half-life of seven days (Senseman, 2007). Glufosinate has high leaching potential in soil; however, it degrades rapidly and, therefore, is typically found no deeper than 15 centimeters (approximately 6 inches) in soil (Senseman, 2007).

2.2.2 Water Resources

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

Surface water in rivers, streams, creeks, lakes, and reservoirs supports everyday life through the provision of water for drinking and other public uses, irrigation, and industry. Surface runoff from rain, snowmelt, or irrigation water can affect surface water quality by depositing sediment, minerals, or contaminants into surface water bodies. Surface runoff is influenced by meteorological factors such as rainfall intensity and duration, and physical factors such as vegetation, soil type, and topography.

Groundwater is the water that flows underground and is stored in natural geologic formations called aquifers. It sustains ecosystems by releasing a constant supply of water into wetlands and contributes a sizeable amount of flow to permanent streams and rivers. Based on 2005 data, the largest use of groundwater in the U.S. is irrigation, representing approximately 67.2% of all the groundwater pumped each day (McCray, 2009). In the U.S., approximately 47% of the population depends on groundwater for its drinking water supply. The EPA defines a sole source aquifer (SSA) as an aquifer that supplies at least 50% of the drinking water consumed in the area overlying the aquifer. An SSA designation is one tool to protect drinking water supplies in areas where there are few or no alternative sources to the groundwater resource. There are 77 designated SSAs in the U.S. and its territories (US-EPA, 2011j).

Unlike a point source which is a “discernible, confined and discrete conveyance”, nonpoint source pollution (NSP) comes from many diffuse sources. Rainfall or snowmelt moving over the ground, also known as runoff, picks up and carries away natural and human-made pollutants, creating NSP. The pollutants may eventually be transported by runoff into lakes, rivers, wetlands, coastal waters and ground waters. Agricultural NPS pollution is the leading source of impacts to surveyed rivers and lakes and the third largest source of impairment to estuaries, as well as a major source of impairment to groundwater and wetlands (USDA-NRCS, 2011b). Agricultural NPS pollution includes animal wastes, fertilizers, and pesticides. Surface water may be contaminated by agricultural sediments transported by erosion that may also include pesticides, fertilizers, and sometimes fuel and pathogens. Agricultural practices that introduce contaminants into the groundwater include fertilizer and pesticide application, spilled oil and gasoline from farm equipment, nitrates, and pathogens from animal manure.

In regions of the U.S. that experience low amounts of rainfall during the growing season or during drought, soybean yields benefit from proper irrigation. Soybeans require approximately 20-25 inches of water during the growing season to produce a relatively high yield of 40-50 bushels per acre (U of Arkansas, 2006). In 2006 and 2008, approximately 9% of the planted acres of soybeans in the U.S. were irrigated (USDA-ERS, 2011c; USDA-NASS, 2010, 2011e). As shown in Figure 7, a majority (approximately 73%) of irrigated soybean farms occur in the Missouri and Lower Mississippi Water Resource Regions, with soybean farms in the states of Nebraska, Arkansas, Mississippi, Missouri, and Kansas accounting for 85% of all irrigated acres (USDA-NASS, 2010). In 2006, approximately 8.4 inches of water per irrigated acre was used, producing an average of over 51 bushels per irrigated acre (USDA-ERS, 2011c). This yield was approximately 19.8% higher than the national average (42.9 bushels per acre) for that year (USDA-NASS, 2011e).

Approximately 94% of the soybean acreage in the U.S. is planted with GE herbicide-tolerant soybean varieties (USDA-ERS, 2011b) (see Table 2, Subsection 2.1.1, Acreage and Area of Soybean Production). Farms planting GE herbicide-tolerant soybean varieties are more likely to use conservation tillage and no-till practices over conventional agricultural practices (Dill et al., 2008; Givens et al., 2009). This shift has resulted in reduced surface water run-off and soil erosion (Locke et al., 2008). As discussed in Subsection 2.2.1, Soil Quality, reduced tillage agricultural practices result in improved soil quality having high organic material that binds nutrients within the soil. An increased amount of plant residue on the soil surface reduces the effects of pesticide usage on water resources by forming a physical barrier to erosion and runoff, allowing more time for absorption into the soil, and slowing down soil moisture evaporation (Locke et al., 2008).

DAS-68416-4 soybean has conferred tolerance to 2,4-D and glufosinate. Both field crop and aquatic application for weed control are registered uses of 2,4-D (US-EPA, 2005a). The registered use of glufosinate is primarily terrestrial (Bayer CropScience, 2011; US-EPA, 2008b), but may be applied to certain confined waters for irrigated crops, such as rice (US-EPA, 2002a). Use of pesticides for field crop production may introduce these chemicals to water through spray drift, cleaning of pesticide equipment, soil erosion, or filtration through soil to groundwater. 2,4-D has a low binding affinity in mineral soils and is rapidly degraded in soils and aerobic aquatic environments; however, it is relatively persistent in anaerobic aquatic conditions (US-EPA, 2005a). This pesticide rapidly dissipates in soil, effectively minimizing leaching to groundwater, but heavy irrigation after application in sandy soils can increase leaching potential (Senseman, 2007), and it has been detected in groundwater, albeit below any level of concern (US-EPA, 2005a).

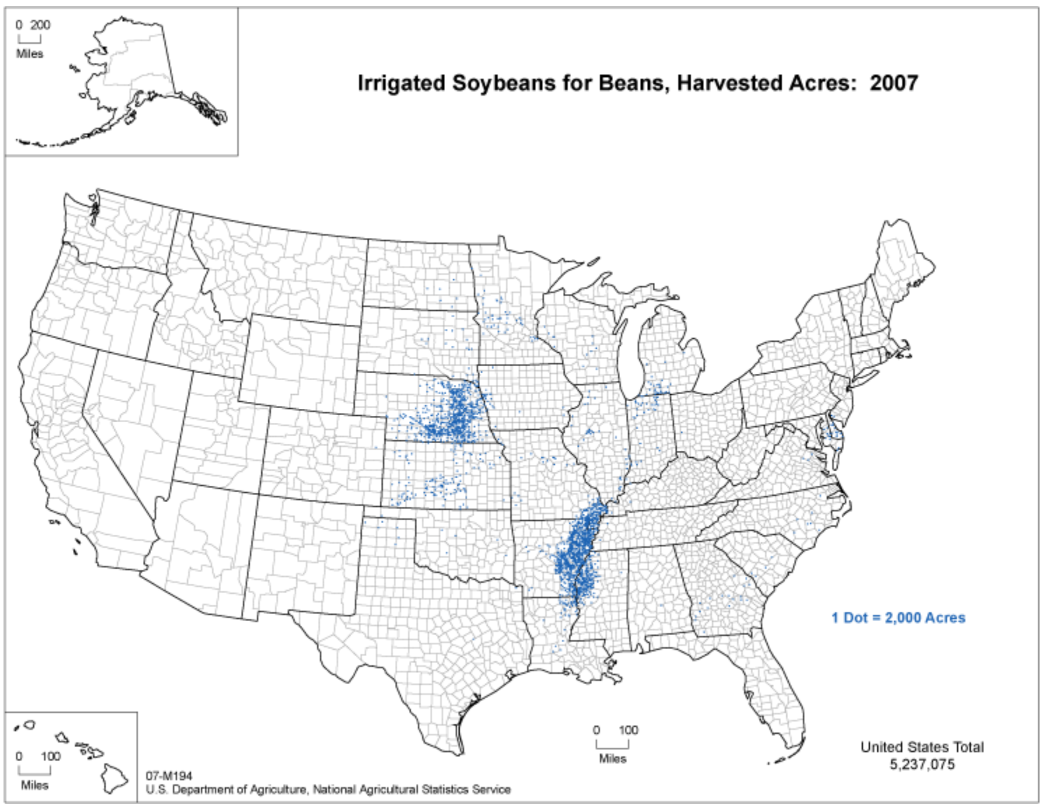


Figure 7. Irrigated soybeans for beans, harvested acres, 2007.

Source: (USDA-NASS, 2009c).
Notes: Dot distribution map where each dot represents 2,000 acres of irrigated soybeans for beans harvested in 2007. The largest concentrations of acres are in the Nebraska, eastern Arkansas, and northwestern Mississippi.

Glufosinate is weakly absorbed and highly mobile in soil, rapidly degrading in soil and water and having a short soil residual half-life of seven days (Senseman, 2007). Glufosinate has a high leaching potential in soil, but because it degrades so rapidly, it is rarely found deeper than 15 centimeters (approximately 6 inches) from the soil surface (Senseman, 2007), and thus has little potential impact to groundwater. Implementation of best management practices to slow soil erosion and filter pollutants from surface runoff, such as vegetated strips, control of spray drift, and adherence to label restrictions governing safe application and equipment cleanup, minimize the potential for pesticide impacts to surface and groundwater.

2.2.3 Air Quality

The Clean Air Act (CAA) requires the maintenance of National Ambient Air Quality Standards (NAAQS). The NAAQS, developed by the EPA to protect public health, establish limits for six criteria pollutants: ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), sulfur dioxide (SO_2), lead (Pb), and inhalable particulates (coarse particulate matter [PM] greater than 2.5 micrometers and less than 10 micrometers in diameter [PM_{10}] and fine particles less than 2.5 micrometers in diameter [$PM_{2.5}$]). The CAA requires states to achieve and maintain the NAAQS within their jurisdiction. Each state may adopt requirements stricter than those of the national standard and each is also required by EPA to prepare a State Implementation Plan (SIP) containing strategies to achieve and maintain the national standard of air quality within the state. Areas that violate air quality standards are designated as non-attainment areas for the criteria pollutant(s), whereas areas that comply with air quality standards are designated as attainment areas. Emissions contributing to greenhouse gases (GHG) associated with global warming are discussed in Subsection 2.2.4, Climate Change.

Primary sources of emissions associated with crop production include vehicle exhaust from motorized equipment such as tractors and irrigation equipment, suspended soil particulates from tillage and wind induced erosion, smoke from burning of fields, and drift from sprayed herbicides and pesticides, and nitrous oxide emissions from the use of nitrogen fertilizer (Aneja et al., 2009; Hoeft et al., 2000b; US-EPA, 2011d; USDA-NRCS, 2006b). These agricultural activities individually have the potential to cause negative impacts to air quality.

As mentioned in Subsection 2.1.2, Agronomic Practices, the majority of soybean grown in the U.S. is rotated with corn on a two-year rotation. Soybean fields typically are tilled and the new crop rotation planted in the following year. Use of herbicide-tolerant soybeans has facilitated conservation tillage and/or no-till soybean production, as it diminishes the need to till for weed control. Longer intervals between rotating crops and minimized earth disturbance from decreased tillage reduce the use of emission-producing equipment. This is illustrated in Table 9 utilizing the NRCS Energy Estimator: Tillage Tool (USDA-NRCS, 2011a). The tool estimates potential fuel savings of 3,010 gallons or 60% savings per year based upon producing 1,000 acres of no-till soybean compared to conventional till soybean in the Urbana, Illinois postal code[3]. NRCS is careful to note that this estimate is only approximate, as many variables could affect an individual operation's actual savings. Reduced tillage also generates fewer particulates (dust) and potentially contributes to lower rates of wind erosion releasing soil particulates into the air, benefitting air quality (Towery and Werblow, 2010).

[3] Postal codes are used in the NRCS Energy Estimator to estimate diesel fuel use and costs in the production of key crops for an area.

Table 9. Total farm diesel fuel consumption estimate (in gallons per year).

Estimate for 1,000-Acre Soybean Crop (Urbana, Illinois)	Tillage Method			
	Conventional Tillage	Mulch-till	Ridge-till	No-till
Total fuel use	4,980	4,110	3,330	1,970
Potential fuel savings over conventional tillage	--	870	1,650	3,010
Total savings	--	17%	33%	60%

Source: USDA-NRCS (2011a)

Prescribed burning is a land treatment, used under controlled conditions, to accomplish resource management objectives. Open combustion produces particles of widely ranging size, depending to some extent on the rate of energy release of the fire (US-EPA, 2011c). The extent to which agricultural and other prescribed burning may occur is regulated by individual SIPs to achieve compliance with the NAAQS. Prescribed burning of fields would likely occur only as a pre-planting option for soybean production based on individual farm characteristics.

Volatilization of fertilizers, herbicides and pesticides from soil and plant surfaces also introduces these chemicals to the air. The USDA Agricultural Research Service (ARS) is conducting a long-term study to identify factors that affect pesticide levels in the Chesapeake Bay region airshed (USDA-ARS, 2011a). This study has determined volatilization is highly dependent upon exposure of disturbed unconsolidated soils and variability in measured compound levels is correlated with temperature and wind conditions. Another ARS study of volatilization of certain herbicides after application to fields has found moisture in dew and soils in higher temperature regimes significantly increases volatilization rates (USDA-ARS, 2011a). The acid and salt forms of 2,4-D have low volatility (Senseman, 2007); however, ester formulations volatilize readily. Low volatility esters may become volatile at 90°F and above (US-EPA, 2005a). Glufosinate does not volatilize significantly due to low vapor pressure (US-EPA, 2008b).

Pesticide and herbicide spraying may impact air quality from drift and diffusion. Drift is defined by EPA as “the movement of pesticide through air at the time of application or soon thereafter, to any site other than that intended for application” (US-EPA, 2000b). Diffusion is gaseous transformation to the atmosphere (FOCUS, 2008). Factors affecting drift and diffusion include application equipment and method, weather conditions, topography, and the type of crop being sprayed (US-EPA, 2000b). EPA’s Office of Pesticide Programs (OPP), which regulates the use of pesticides and herbicides in the U.S., encourages pesticide applicators to use all feasible means available to them to minimize off-target drift. The Agency has introduced several initiatives to help address and prevent the problems associated with drift. Currently, EPA is evaluating new regulations for pesticide drift labeling and the identification of best management practices to control such drift (US-EPA, 2009c), as well as identifying scientific issues surrounding field volatility of conventional pesticides (US-EPA, 2010e). Additionally, EPA OPP and its Office of Research and Development are developing a new voluntary program, the Drift Reduction Technology (DRT) Program, which encourages the development, marketing and use of application technologies verified to significantly reduce spray drift (US-EPA, 2009c).

Other conservation practices, as required by USDA to qualify for crop insurance and beneficial Federal loans and programs (USDA-ERS, 2009a), effectively reduce crop production impacts to air quality through the employment of windbreaks, shelterbelts, reduced tillage, and cover crops that promote soil protection on highly erodible lands.

2.2.4 Climate Change

Climate change represents a significant and lasting statistical change in climate conditions that may be measured across both time and space. The EPA has identified carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) as the key GHG affecting warming temperatures. While each of these gases occurs naturally in the atmosphere, human activity has significantly increased the concentration of these gases since the beginning of the industrial revolution. The level of human produced gases accelerated even more so after the end of the Second World War, when industrial and consumer consumption flourished. With the advent of the industrial age, there has been a 36% increase in the concentration of CO_2, 148 % in CH_4, and 18 % in N_2O (US-EPA, 2011f).

U.S. agriculture may influence climate change through various facets of the production process (Horowitz and Gottlieb, 2010). The major sources of GHG emissions associated with crop production are soil N_2O emissions, soil CO_2 and CH_4 fluxes, and CO_2 emissions associated with agricultural inputs and farm equipment operation (Adler et al., 2007; Del Grosso et al., 2002; Robertson et al., 2000; West and Marland, 2002). Over the twenty-year period of 1990 to 2009, total emissions from the agricultural sector grew by 8.7%, with 7% of the total U.S. GHG emissions in 2009 generated from this sector (US-EPA, 2011f).

CH_4 and N_2O are the primary GHGs emitted by agricultural activities. Emissions from intestinal (enteric) fermentation and manure management represent about 20% and 7% of total CH_4 emissions from anthropogenic activities, respectively. Agricultural soil management activities including fertilizer application and cropping practices were the largest source of N_2O emissions, accounting for 69% of all U.S. N_2O emissions (US-EPA, 2011f). Agricultural practices that produce CO_2 emissions include liming and the application of urea fertilization to agricultural soils. The use of lime and urea fertilizers resulted in an increase of 11% of CO_2 in 2009 relative to 1990 emissions (US-EPA, 2011f). The agricultural sector is also responsible for CO_2 emissions from fossil fuel combustion by farm equipment such as tractors, as discussed in Subsection 2.2.3, Air Quality.

Since CO_2 and CH_4 are two of the key gases most responsible for the "Greenhouse Effect," scientists and policy makers are interested in carbon (C) gases and how they may be removed from the atmosphere and stored. The process of C moving from the atmosphere to the earth and back is referred to as the carbon cycle. Simplified components of the carbon cycle are:

- Conversion of atmospheric C to carbohydrates through the process of photosynthesis;
- The consumption of carbohydrates and respiration of CO_2;
- The oxidation of organic carbon creating CO_2; and

- The return of CO_2 to the atmosphere.

Carbon can be stored in four main pools other than the atmosphere: (1) the earth's crust, (locked up in fossil fuels and sedimentary rock deposits); (2) the oceans where CO_2 is dissolved and marine life creates calcium carbonate ($CaCO_3$) shells; (3) in soil organic matter; and (4) within all living and dead organisms that have not been converted to soil organic matter. These pools can store or sink C for long periods, as in the case of C stored in sedimentary rock and in the oceans. Conversely, C may be held for as short a period as the life span of an individual organism. Humans can affect the carbon cycle through activities such as the burning of fossil fuels, deforestation, or releasing soil organic carbon (SOC) through land disturbing activities. The process of storing C in the ecosystem is termed carbon sequestration. Carbon sequestration includes storing C in trees, plants and grasses (biomass) in both the above ground and the below ground plant tissues, and in the soil. Soil C can be found in the bodies of microorganisms (fungi, bacteria, etc.), in non-living organic matter, and attached to inorganic minerals in the soil.

Between 1990 and 2008, crop conservation tillage practices increased from approximately 30% of soybean acreage to 63% of soybean acreage in the U.S. (CTIC, 2011). Tillage is one agricultural practice that contributes to the release of GHG because of the loss of soil CO_2 to the atmosphere; conversely, reductions in GHG emissions from lower exposure and oxidation of soil organic matter are often attributed to conservation tillage practices (Adler et al., 2007; CAST, 2009; Towery and Werblow, 2010; US-EPA, 2009b). Expected reductions in GHG emissions associated with the production of GE soybeans result from a reduction in fuel use due to less frequent herbicide applications and soil cultivation (Brookes and Barfoot, 2006).

The impacts of GE crop varieties on climate change are dependent on many variables including cropping systems, production practices, geographic distribution of activities, and individual grower decisions. Agriculture influences emissions that may contribute to climate change, and climate change, in turn, potentially affects agriculture. In a review of several studies on corn, rice, sorghum, soybean, wheat, common forages, cotton, some fruits, and irrigated grains, Field et al. (2007) found that most studies projected likely climate-related yield increases of 5 - 20%; however, this positive impact would not be observed evenly across all regions as certain areas of the U.S. are expected to be negatively impacted by substantially reduced water resources (Field et al., 2007). In addition, the current range of weeds and pests of agriculture is expected to change in response to climate change (USGCRP, 2009).

2.3 Biological Resources

2.3.1 <u>Animal Communities</u>

Animal communities in this discussion include wildlife species and their habitats. Wildlife refers to both native and introduced species of mammals, birds, amphibians, reptiles, invertebrates, and fish/shellfish. Agriculture dominates human uses of land (Robertson and Swinton, 2005). In 2010, 920 million acres (47%) of the contiguous 48 states were devoted to farming, including: crop production, pasture, rangeland, Conservation Reserve Program, Wetlands Reserve Program, or other government program uses (USDA-NASS, 2011c). How these lands are maintained influences the function and integrity of ecosystems and the wildlife populations that they support.

A wide array of wildlife species occur within the 31 major soybean-producing U.S. states. During the spring and summer months, soybean fields provide browse for rabbits, deer, rodents, other mammals; birds such as upland gamebirds, while also providing a forage base for insects (Palmer et al., No Date). During the winter months, leftover and unharvested soybeans provide a food-source for wildlife; however, soybeans are poorly suited for meeting nutrient needs of wildlife, such as waterfowl, that require a high-energy diet (Krapu et al., 2004).

As discussed in Subsection 2.1.2, Agronomic Practices, a shift from conventional agricultural practices to conservation tillage and no-till practices has occurred on farms planting GE herbicide-tolerant soybean varieties (Dill et al., 2008; Givens et al., 2009). This increased use of conservation tillage practices has benefitted wildlife through improved water quality, availability of waste grain, retention of cover in fields, and increased populations of invertebrates (Brady, 2007; Sharpe, 2010). Conservation tillage practices that leave greater amounts of crop residue serve to increase the diversity and density of birds and mammals (USDA-NRCS, 1999a). Increased residue also provides habitat for insects and other arthropods, consequently increasing this food source for insect predators. Insects are important during the spring and summer brood rearing season for many upland game birds and other birds, as they provide a protein-rich diet to fast growing young, as well as a nutrient-rich diet for migratory birds (USDA-NRCS, 2003).

Insects and other invertebrates can be beneficial to soybean production, providing services such as nutrient cycling and preying on plant pests. Conversely, there are many insects and invertebrates that are detrimental to soybean crops, including: bean leaf beetle (*Cerotoma trifurcata*); beet armyworm (*Spodoptera exigua*); blister beetle (*Epicauta* spp.); corn earworm (*Helicoverpa zea*); grasshopper (*Acrididae* spp.); green cloverworm (*Hypena scabra*); seed corn beetle (*Stenolophus lecontei*); seedcorn maggot (*Delia platura*); soybean aphid (*Aphis glycines*); soybean looper (*Pseudoplusia includens*); soybean stem borer (*Dectes texanus*); spider mites (*Tetranychus urticae*); stink bug (green [*Acrosternum hiliare*]; brown [*Euschistus* spp.]); and velvetbean caterpillar (*Anticarsia gemmatalis*) (Palmer et al., No Date; Whitworth et al., 2011). While insects are considered less problematic than weeds in U.S. soybean production, insect injury can impact yield, plant maturity, and seed quality. Consequently, insect pests are managed during the growth and development of soybean to enhance soybean yield (Aref and Pike, 1998; Higley and Boethel, 1994).

The environmental effects associated with 2,4-D are described in EPA's 2,4-D RED report and fact sheet (US-EPA, 2005a, 2005c). As part of the RED, an assessment was conducted by EPA of the potential risks to terrestrial and aquatic organisms from the use of 2,4-D and its associated chemical forms. For terrestrial exposure, the risk quotient (RQ) for direct exposure exceeded the level of concern (LOC) for most organisms with potential impacts on non-target plants and mammals considered acute or chronic[4]; however, EPA's modeling utilized conservative assumptions based on maximum authorized application rates that tend to

[4] The EPA presumes a risk of concern to a specific category (e.g., aquatic organisms, endangered species) when the RQ exceeds the LOC.

overestimate the risks (US-EPA, 2005c). 2,4-D is classified as slightly toxic to small mammals on an acute oral basis, and when assessed using average application rates to most major crops, including soybean and corn, RQs were below the acute LOC, but were still greater than the restricted use LOC (US-EPA, 2005c). EPA concluded, however, that the benefits from the use of 2,4-D, such as the control of invasive and noxious weeds and its low toxicity to humans, outweigh potential impacts to small mammals. Testing indicates that 2,4-D's ecological toxicity is moderate to practically non-toxic to birds from acute oral exposure and does not exceed the agency's LOC (US-EPA, 2005c). Study results also indicate that 2,4-D is practically non-toxic to the honey bee, and for 2,4-D and its salts and esters are predicted to have minimal potential risks to pollinators and other beneficial insects (US-EPA, 2005c).

For aquatic animal communities, 2,4-D acid and amine salts were found to be practically non-toxic to freshwater and marine fish with no exceedance of acute or chronic LOCs (US-EPA, 2005a). 2,4-D esters were found to be highly toxic to fish; although, no RQs exceeded acute LOCs to water bodies from runoff or drift from the use on terrestrial sites. 2,4-D has a low binding affinity in mineral soils and is rapidly degraded in soils and aerobic aquatic environments; however it is relatively persistent in anaerobic aquatic conditions (US-EPA, 2005c). While 2,4-D is potentially mobile, it degrades rapidly in soil. Dissipation studies indicate that more than 95% of 2,4-D moves less than 6 inches in soil from the point of application, but somewhat more (12-18 inches) in sandy soils with heavy amounts of applied water (Senseman, 2007). The 2005 2,4-D RED decreased master label application rates of 2,4-D for corn and soybean to reduce potential exposure to non-target organisms (US-EPA, 2005a, 2005c).

The use of 2,4-D could also affect both terrestrial and aquatic animals from the alteration of habitat and the potential reduction of forage and cover as a result of spray drift and runoff. As such, in 2005, EPA specified that several mitigation steps were necessary for reregistration eligibility, including modification of the 2,4-D label for spray drift control measures and reductions of application rates and/or the number of applications to reduce the risk of exposure to non-target species (US-EPA, 2005a, 2005c).

Glufosinate-ammonium is currently in the reregistration process, with an estimated completion of the preliminary risk assessments in late 2012, and a reregistration review decision expected in April to June of 2013 (US-EPA, 2008b). As of the March 2008 *Glufosinate Summary Document Registration Review*, there were insufficient data available on terrestrial plant toxicity for an ecological assessment to be completed (US-EPA, 2008b). Based on the data collected as of the 2008 review summary, however, the areas of concern are impacts to non-target plants, chronic toxicity to mammals, and the indirect impacts to terrestrial animals from potential alterations in aquatic plant communities (US-EPA, 2008b). The EPA requires additional plant toxicity and field dissipation studies to determine potential impacts of typical end-use products. Existing environmental assessments of the toxicity of glufosinate to animal species indicated a relatively low direct risk, but high risk to plants composing the animals' habitat (US-EPA, 2008b). On an acute exposure basis, glufosinate is considered practically nontoxic to birds, mammals, and insects; slightly non-toxic to freshwater fish; slightly toxic to estuarine/marine fish; moderately toxic to freshwater and estuarine/marine invertebrates; and toxic to terrestrial and aquatic plants. For birds, glufosinate is practically non-toxic on an acute and subacute dietary basis; therefore, the risk potential is presumed to be low (US-EPA, 2008b).

2.3.2 Plant Communities

Soybeans are grown in 31 states (USDA-NASS, 2011a) throughout the Midwest, Delta, Mid-Atlantic, and Southeast regions of the U.S., encompassing a wide range of physiographic regions, ecosystems, and climatic zones. The types of vegetation, including the variety of weeds, within and adjacent to soybean fields can vary greatly, depending on the geographic area in which the field occurs. Non-crop vegetation in soybean fields is limited by the extensive cultivation and weed control programs practiced by soybean producers. Plant communities bordering soybean fields can range from forests and woodlands to grasslands, aquatic habitats, or residential areas. Adjacent crops frequently include other soybean varieties, corn, cotton, or other crops.

Weeds are classified as annuals or perennials. An annual is a plant that completes its lifecycle in one year or less and reproduces only by seed. Perennials are plants that live for more than 2 years. Weeds are also classified as broadleaf (dicots) or grass (monocots). Weeds can reproduce by seeds, rhizomes (underground creeping stems), or other underground parts. Annual grass and broadleaf weeds are considered the most common weed problems in soybeans (DAS, 2010; Krausz et al., 2001). However, with increased rates of conservation tillage, there has been a decrease in large-seeded broadleaf weeds and increases in perennial, biennial, and winter annual weed species being observed (Durgan and Gunsolus, 2003; Green and Martin, 1996). Winter perennials are particularly competitive and difficult to control, as these weeds re-grow every year from rhizomes or root systems (DAS, 2010). At least 55 weed species have been identified as commonly occurring in soybean production (DAS, 2010; Monsanto, 2010a). The most troublesome species are shown in Table 10. Recent surveys of U.S. agronomic crop producers suggest that pigweed species (*Amaranthus* spp.), morning glory species (*Ipomoea* spp.), Johnsongrass (*Sorghum halepense*), ragweed species (*Ambrosia* spp.), foxtail species (*Setaria* spp.), and velvetleaf (*Abutilon theophrasti*) are among the most problematic weeds (Heatherly et al., 2009).

An important concept in weed control is the seed bank, which is the reservoir of seeds that are in the soil and have the potential to germinate. Agricultural soils contain reservoirs of weed seeds ranging from 4,100 to 137,700 seeds per square meter of soil (May and Wilson, 2006). Climate, soil characteristics, cultivation, crop selection, and weed management practices affect the seed bank composition and size (May and Wilson, 2006).

Herbicide resistance is described by the Weed Science Society of America as the “inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type” (WSSA, 2011b). The first reports of weed resistance to herbicides were in the 1950s (WSSA, 2011a), which included 2,4-D-resistant spreading dayflower (*Commelina diffusa*) in a sugarcane field in Hawaii in 1957 (Sellers et al., 2011). Individual plants within a

Table 10. Common troublesome weeds in soybeans in 2006-2008.

Weed Species	Total Soybean Acres Treated[a]		
	2006	2007	2008
Annual Broadleaf Weeds			
Lambsquarters, Common	21,859,614	24,459,895	28,242,972
Velvetleaf	23,820,731	23,373,573	26,786,349
Pigweed, Redroot	21,093,224	21,788,121	26,715,150
Cocklebur, Common	23,657,980	22,389,376	23,962,063
Waterhemp, Common	18,399,609	15,970,794	21,364,980
Ragweed, Giant	13,369,296	14,684,000	16,565,209
Sunflower, Wild	5,558,526	5,759,216	5,709,292
Kochia	4,859,759	3,671,795	5,317,528
Smartweed Pennsylvania	2,366,851	1,835,825	3,529,114
Waterhemp, Tall	2,301,380	2,926,358	3,826,647
Horseweed	2,188,359	3,159,712	3,470,274
Mustard, Wild	2,019,346	1,975,291	2,688,590
Sicklepod	2,024,031	1,650,086	2,535,829
Sida, Prickly	1,639,261	1,567,275	2,432,701
Sunflower, Volunteer	1,089,460	1,007,691	1,913,860
Chickweed	1,652,712	1,259,096	1,823,638
Nightshade, Black	1,766,649	1,277,416	1,385,751
Buckwheat, Wild	1,167,746	855,879	1,331,675
Pigweed, Smooth	188,160	801,569	1,322,732
Annual Grass Weeds			
Foxtail Spp.	24,409,043	18,489,746	18,446,420
Foxtail, Giant	11,817,612	17,513,493	17,804,622
Foxtail, Yellow	10,870,761	11,217,512	13,947,018
Foxtail, Green	5,629,880	7,109,316	7,610,855
Crabgrass	5,170,684	5,928,919	7,424,879
Barnyardgrass	4,189,156	3,967,425	3,805,391
Corn, Volunteer	2,292,705	2,088,371	3,704,330
Oat, Wild	1,792,389	1,478,890	2,886,300
Cupgrass, Woolly	1,765,244	2,470,437	2,108,135
Shattercane	2,408,592	2,715,388	1,879,416
Panicum, Fall	2,251,014	2,241,088	1,852,417
Perennial / BiennialWeeds			
Johnsongrass	10,152,393	11,057,825	10,368,155
Thistle, Canada	4,123,437	3,584,676	4,840,383
Quackgrass	2,628,187	2,570,688	2,786,633
Dandelion	1,578,579	1,528,332	2,154,008
Thistle	1,479,038	647,315	1,513,566

Source: (DAS, 2010)(Data from DMR-Kynetec).
Notes:
a. Total soybean acres in 2006, 2007, and 2008 were 75.5, 64.7, and 75.7 million acres, respectively (USDA NASS, 2008). However, the total soybean herbicide-treated acreage is much more, due to multiple sprays on each acre.

species can exhibit different responses to the same herbicide rate. Initially, herbicide rates are set to work effectively on the majority of the weed population under normal growing conditions. Genetic variability, including herbicide resistance, is exhibited naturally in normal weed populations, although at very low frequencies. When only one herbicide is used year after year as the primary means of weed control, the number of weeds resistant to that herbicide compared to those susceptible to the herbicide may change as the surviving resistant weeds reproduce (see Figure 8). With no change in weed control strategies, in time, the weed population may be composed of more and more resistant weeds (US-NARA, 2010).

The adoption of glyphosate-tolerant crops, including soybean, resulted in growers changing historical weed management strategies and relying on a single herbicide, glyphosate, to control weeds in the field (Owen et al., 2011; Weirich et al., 2011). Reliance on a single management technique for weed control resulted in the selection for weeds resistant to that technique (Owen et al., 2011; Weirich et al., 2011). The development of glyphosate-resistant weeds has necessitated a diversification of weed management strategies by growers. Faced with glyphosate-resistant weeds, growers have responded to the problem by applying herbicides with

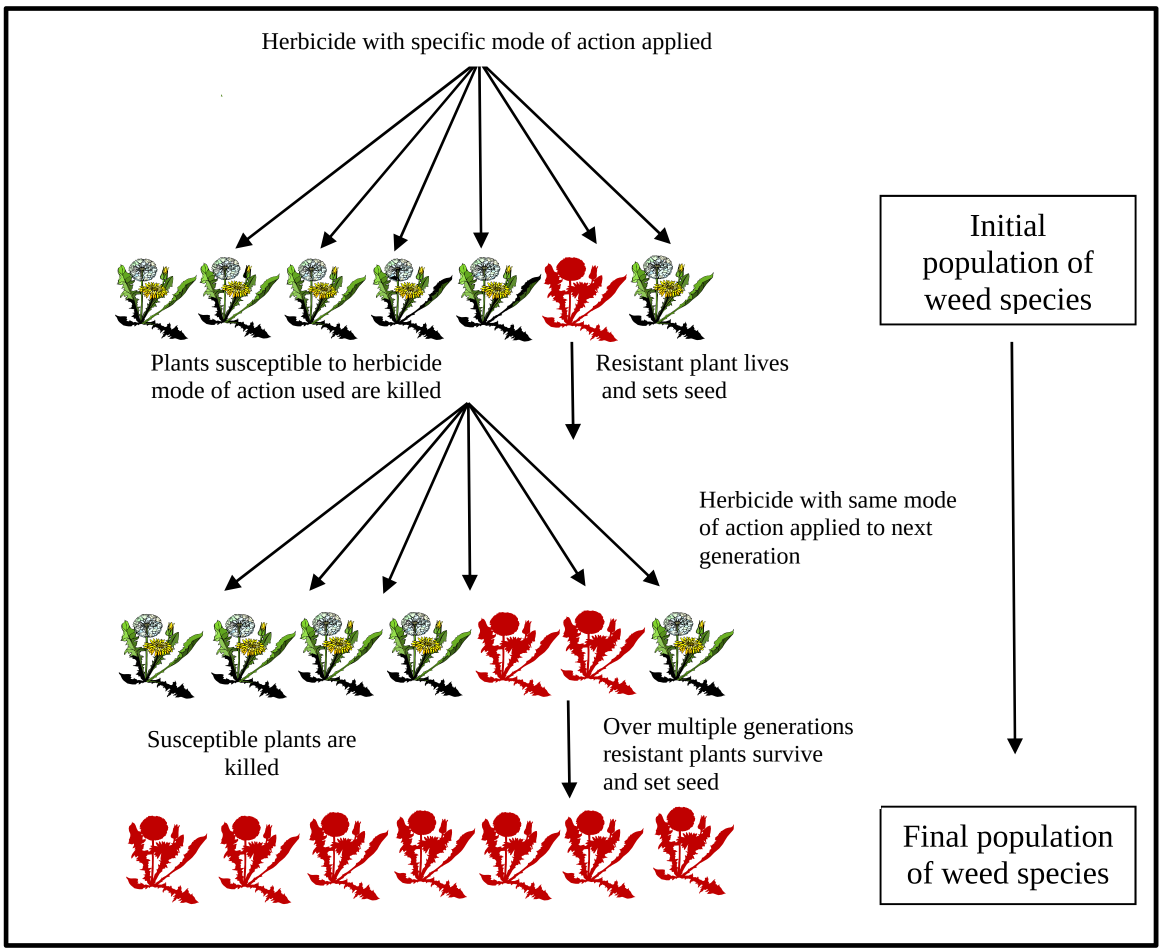


Figure 8. The evolution of herbicide resistance.

Source: Adapted from (Tharayil-Santhakumar, 2003).

a different modes of action, using tank mixes, increasing the frequency of glyphosate applications, and returning to tillage and other cultivation techniques to physically control these species when a specific herbicide proves to be ineffective (CAST, 2012; DAS, 2011f).

As previously discussed in Subsection 2.1.2, Agronomic Practices, the widespread adoption of glyphosate-tolerant GE crops has resulted in the increased use of glyphosate after 1995 and a decrease in the diversity of other herbicides applied in crop production to control weeds (Weirich et al., 2011). Glyphosate-resistant crops do not influence weeds any more than non-transgenic crops. It is the weed control methods selected by growers that create the ecological selection pressure that ultimately changes the weed communities (Owen, 2008). The recurrent and exclusive use of glyphosate in the production of many GE crops has resulted in the selection for weed populations (e.g., *Amaranthus tuberculatus*) that are tolerant to glyphosate. Currently, 21 weed species have evolved glyphosate-resistant biotypes, 13 of which are commonly found in association with the cultivation of glyphosate-tolerant crops (see Appendix D, Table D-1) (Heap, 2011). Furthermore, weeds that had previously been agronomically unimportant (e.g., *Commelina communis* L. or Asiatic dayflower) but had natural resistance to glyphosate have become major regional problems (Owen, 2008).

Glyphosate, however, is not the only herbicide to which weeds have developed resistance. To date, 28 known weed biotypes have been identified and confirmed that are resistant to synthetic auxin herbicides, including 2,4-D (see Appendix D, Table D-1) (Heap, 2011). Of these, eight have been confirmed in the U.S. Sixteen of these weeds are known to be resistant to 2,4-D, specifically (Egan et al., 2011), 12 biotypes are not indicated as having 2,4-D resistance specifically, and only one biotype has arisen where typical row cropping is practiced (Egan et al., 2011; Wright et al., 2011). Two known weed biotypes have been identified and confirmed to be resistant to glufosinate, a glutamine synthase inhibitor herbicide, namely goosegrass (*Eleusine indica*) and Italian ryegrass (*Lolium multiflorum*). Glufosinate resistant goosegrass has not been identiried in the U.S. In 2010, Italian ryegrass with resistance to both glyphosate and glufosinate was confirmed in Oregon (Heap, 2011).

The evolution of herbicide-resistant weeds has required that growers diversify weed management practices and use combinations of herbicides, tillage practices, and herbicide-tolerant traits. Integrated weed management programs that use herbicides from different groups, vary cropping systems, rotate crops, and that use mechanical, as well as chemical weed control methods, will delay or prevent the selection of herbicide-resistant weed populations (Gunsolus, 2002; Sellers et al., 2011), as is discussed in greater detail in Subsection 2.1.2, Agronomic Practices.

Runoff, spray drift, and volatilization of herbicides have the potential to impact non-target plant communities growing in proximity to fields in which herbicides are used. The extent of damage to a nontarget plant exposed to herbicide is determined by the overall vigor of the affected plant, the amount and type of herbicide to which the plant is exposed, and the growing conditions after contact (Ruhl et al., 2008).

The total rainfall the first few days after herbicide application can influence the amounts of leaching and runoff. However, it has been estimated that even after heavy rains, herbicide losses to runoff generally do not exceed five to ten percent of the total applied (Tu et al., 2001;

USDA-FS, 2009). Planted vegetation, such as grass buffer strips, or crop residues can effectively reduce runoff (IPPC, 2010). Volatilization typically occurs during application, but herbicide deposited on plants or soil can also volatilize. Most of the herbicdes considered highly volatile are no longer used (Tu et al., 2001)

Spray drift is a concern for non-target susceptible plants growing adjacent to fields when herbicides are used in the production of DAS-68416-4 soybean. This potential impact relates to exposure of non-target susceptible plants to the off-target herbicide drift (see, e.g., CBD, 2010). Damage from spray drift typically occurs at field edges or at shelterbelts (i.e., windbreaks), but highly volatile herbicides may drift further into a field. The risk of off-target herbicide drift is recognized by the EPA, which has incorporated both equipment and management restrictions to address drift in the EPA-approved herbicide labels. These EPA label restrictions include requirements that the grower manage droplet size, spray boom height above the crop canopy, restricted applications under certain wind speeds and environmental conditions, and using drift control agents (CBD, 2010).

Volunteer soybean are not a widespread problem, and when they occur, it is most often in parts of the Delta and the southeastern U.S. In production systems where soybean is rotated, such as corn or cotton, it has shown up as a volunteer weed, yet was not generally seen as a serious problem by farmers (Owen and Zelaya, 2005). Volunteer soybean is not considered difficult to manage, as soybean seeds rarely remain viable the following season and any interference they may pose to subsequent crops are minimal (Owen and Zelaya, 2005). Furthermore, herbicides usually used for weed control in corn are also effective at controlling volunteer soybean.

Conversely, volunteer glyphosate-tolerant corn in soybean is a greater concern (Owen and Zelaya, 2005). Glyphosate has been used to control all weeds, including corn in soybean, yet, the increase in cultivation of glyphosate-tolerant corn has created problems for growers in the Midwest managing volunteer corn with glyphosate. Growers must now often include graminicides (herbicides to control weedy grasses) as part of their weed management strategy (Owen and Zelaya, 2005). 2,4-D has been shown to be active on several glyphosate- and ALS inhibitor herbicide-resistant weeds (see Appendix D, Table D-2) (DAS, 2010; Heap, 2011).

2.3.3 Gene Flow and Weediness

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

The rate and success of gene flow is dependent on numerous external factors in addition to the donor/recipient plant. General external factors related to pollen-mediated gene flow include the presence/abundance/distance of sexually-compatible plant species; overlap of flowering phenology between populations; the method of pollination; the biology and amount of pollen produced; and weather conditions, including temperature, wind, and humidity (Zapiola et al., 2008). Seed-mediated gene flow also depends on many factors, including the absence/presence/magnitude of seed dormancy; contribution and participation in various dispersal pathways; and environmental conditions and events.

Soybean is not native to the U.S. and has no feral or weedy relatives. Soybean is considered a highly self-pollinated species, propagated by seed (OECD, 2000). Pollination typically takes place on the day the flower opens. The soybean flower stigma is receptive to pollen approximately 24 hours before anthesis (i.e., the period in which a flower is fully open and functional) and remains receptive for 48 hours after anthesis. Anthesis normally occurs in late morning, depending on the environmental conditions. The pollen usually remains viable for two to four hours, and no viable pollen can be detected by late afternoon. Natural or artificial cross-pollination can only take place during the short time when the pollen is viable. Additionally, soybean's reproductive characteristics (e.g., flower orientation that reduces its exposure to wind, internal anthers, and clumping and stickiness of the pollen) decreases the dispersion ability of pollen (Yoshimura, 2011).

As a highly self-pollinated species, cross-pollination of soybean plants to adjacent plants of other soybean varieties occurs at a very low frequency (0 to 6.3%) (Caviness, 1966; Ray et al., 2003; USDA-APHIS, 2011b; Yoshimura et al., 2006). A study of soybeans grown in Arkansas found that cross-pollination of soybeans in adjacent rows averaged between 0.1% and 1.6%, but may be as high as 2.5% (Ahrent and Caviness, 1994). Abud et al. (2007) illustrated that as distance is increased from the soybean pollination source, the chance of cross-pollination is decreased. This study found that at a distance of 1 meter (3.28 feet), outcrossing averaged about 0.5%, at 2 meters (approximately 6.5 feet) outcrossing averaged about 0.1%, at 4 meters (approximately 13 feet) it declined to approximately 0.05%, and at 10 meters (approximately 33 feet) the potential for outcrossing was less than 0.01%.

Generally, gene flow by seed is dependent on natural dispersal mechanisms, such as water, wind or animals, or by human actions and is favored by characteristics such as small and lightweight seed size, prolific production, seed longevity and dormancy, and long distance seed transport (Mallory-Smith and Zapiola, 2008). Soybean seeds do not possess the characteristics for efficient seed-mediated gene flow. Soybean seeds are heavy and, therefore, are not readily or naturally dispersed by wind or water (Mallory-Smith and Zapiola, 2008). Similarly, soybean seeds and seedpods do not have physical characteristics that encourage animal transport (OECD, 2000). In addition, soybeans lack dormancy, a characteristic that allows dispersal in time by maintaining seeds and their genes within the soil for several years (Mallory-Smith and Zapiola, 2008; OECD, 2000). As already mentioned, there are no wild populations of soybean within the U.S.

Any crop seeds that remain on the field after harvest and remain viable to germinate the following year in rotation crops are termed volunteers (Carpenter et al., 2002). Volunteer soybeans are limited by the geography in which soybean is planted. Soybean requires specific environmental conditions to grow as a volunteer (OECD, 2000). Mature soybean seeds are sensitive to cold and rarely survive in freezing winter conditions (Raper and Kramer, 1987); however, if temperature and moisture conditions are suitable, seeds may remain viable, germinate and become volunteers (Mallory-Smith and Zapiola, 2008). Volunteer soybeans can occur in regions with warmer climates where conditions for germination can occur year round, such as the Mississippi Delta and the Southeast U.S. But as discussed in Subsection 2.1.2, Agronomic Practices, volunteer soybean does not easily compete with other crops and are easily controlled with common agronomic practices. In addition, as discussed above, since soybean is principally self-pollinating, the potential for transgene movement from volunteers as a result of pollen movement is negligible (Owen and Zelaya, 2005).

Horizontal gene transfer and expression of DNA from a plant species to bacteria is unlikely to occur (Keese, 2008). Many bacteria (or parts thereof) that are closely associated with plants have been sequenced, including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2000; Kaneko et al., 2002; Wood et al., 2001). There is no evidence that these organisms contain genes derived from plants. Further, in cases where review of sequence data implied that horizontal gene transfer occurred, these events are inferred to occur on an evolutionary time scale on the order of millions of years (Brown, 2003; Koonin et al., 2001). The FDA has also evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (US-FDA, 1998c).

2.3.4 Microorganisms

Soil microorganisms play a key role in soil structure formation, decomposition of organic matter, toxin removal, nutrient cycling, and most biochemical soil processes (Garbeva et al., 2004). They also suppress soil-borne plant diseases and promote plant growth (Doran et al., 1996). The main factors affecting microbial population size and diversity include soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), plant type (providers of specific carbon and energy sources into the soil), and agricultural management practices (crop rotation, tillage, herbicide and fertilizer application, and irrigation) (Garbeva et al., 2004). Some types of soil micro-organisms share metabolic pathways with plants, and might be affected by herbicides. Tillage disrupts multicellular relationships among micro-organisms, and crop rotation changes soil conditions in ways that favor different microbial communities.

Plant roots, including those of soybean, release a variety of compounds into the soil creating a unique environment for microorganisms in the rhizosphere (root zone). Microbial diversity in the rhizosphere may be extensive and differs from the microbial community in the bulk soil (Garbeva et al., 2004). The following briefly focuses on the soybean, GE crop, and 2,4-D and glufosinate herbicide use factors with the potential to affect microbial population size and diversity.

2.3.4.1 Soybeans

An important group of soil microorganisms associated with legumes, including soybean, are the mutualists. These include mycorrhizal fungi, nitrogen-fixing bacteria, and some free-living microbes that have co-evolved with plants that supply nutrients to and obtain food from their plant hosts (USDA-NRCS, 2004). Legumes have developed symbiotic relationships with specific nitrogen-fixing bacteria in the family *Rhizobiaceae* that induce the formation of root nodules where bacteria may carry out the reduction of atmospheric nitrogen into ammonia (NH_3) that is usable by the plant (Gage, 2004). *Bradyrhizobium japonicum* is the rhizobium bacteria specifically associated with soybeans (Franzen, 1999). Since neither soybean nor *B. japonicum* is native to North America, if a field has not been planted with soybean within three to five years, either the seed or seed zone must be inoculated with *B. japonicum* prior to soybean planting (Berglund and Helms, 2003; Pedersen, 2007).

In addition to beneficial microorganisms, there are also several microbial pathogens that cause disease in soybean and vary somewhat depending on the region. These include fungal pathogens such as Rhizoctonia Stem Rot (*Rhizoctonia solani*), Brown Stem Rot (*Phialophora gregata*), Sudden Death Syndrome (*Fusarium solani* race A), and Charcoal Root Rot (*Macrophomina phaseolina*); bacterial pathogens Bacterial Blight (*Pseudomonas syringae*) and Bacterial Pustule (*Xanthomonas campestri*); the Soybean Cyst Nematode (*Heterodera glycines*); and viral pathogens Soybean Mosaic Virus and the Tobacco Ringspot Virus (Ruhl, 2007; SSDW, No Date). Management to control disease outbreaks varies by region and pathogen, but include common practices such as crop rotation, weed control, planting resistant cultivars, and proper planting and tillage practices.

2.3.4.2 GE crops

Identify and gauging the effects of GE crops on soil microbes in the rhizosphere can be challenging, as agricultural soils are complex and dynamic and numerous other factors can potentially influence the soil-borne ecosystem. Changes in agricultural practices and inputs and natural variations in season, weather, plant development stage, geographic location, soil type, and plant species or cultivar can all impact the microbial community (Kowalchuk et al., 2003; US-EPA, 2009e). It is assumed that direct impacts may include changes to the structure (species richness and diversity) and function of the microbial community in the rhizosphere due to the biological activity of the inserted gene(s). Indirect impacts may result from changes in the composition of root exudates, plant litter, or agricultural practices (Kowalchuk et al., 2003; US-EPA, 2009e). Several reviews of the investigations into the impact of GE plants on microbial soil communities found that most of the studies examining distinctive microbial traits concluded that there was either minor or no detectable non-target effects (Hart, 2006; Kowalchuk et al., 2003; US-EPA, 2009e).

2.3.4.3 Herbicides

The herbicide 2,4-D has been approved for use on a wide variety of crops for over 60 years and is currently approved for pre-plant burndown application in soybean (US-EPA, 2005c). Likewise, glufosinate has been approved for use since 1993, and is approved for pre-plant and post-emergence control of broadleaf weeds in soybean (OSTP, 2001; US-EPA, 2008b).

2,4-D is readily metabolized in soil by bacteria (Tu et al., 2001). Once 2,4-D reaches soil, it is rapidly converted to the acid form and dissipates (Senseman, 2007). Under warm, moist conditions 2,4-D undergoes microbial degradation at an increased rate as temperature, moisture, and organic matter increases; it has an average residual soil half-life of 10 days (Senseman, 2007).

Identifying and quantifying the environmental impact of 2,4-D use on microbial communities is difficult due to variations in 2,4-D formulation, concentrations, and the environment (Chinalia et al., 2007). Results from studies into the impact of 2,4-D on microbial communities vary. Two studies found that at rates of 6.7 and 16.7 pounds of acid equivalent per acre (lb ae/A)[5] per 2,4-D application (considerably above EPA registered use levels), the risk to soil microorganisms was low (FAO, 1997). Yet, a third study found that populations of aerobic bacteria, fungi, and actinomycetes in soil were reduced by approximately 13.2%, 9.8% and 15.0% respectively by a per application of 0.88 lb ae/A of 2,4-D salt (an acid form of the herbicide), and nearly twice as much by similar rates of 2,4-D ester. Reductions of less than 30% are not considered a cause for concern (FAO, 1997). Other studies have found that the application of 2,4-D acid did not quantitatively change the structure of the soil microbial communities (Breazeale and Camper, 1970; Xia et al., 1995). Conversely, in a 15-year study, Rai (1992) found that 2,4-D substantially affected the microbial community, with greater reductions of bacterial, fungal, and actinomycetal microbes resulting from the ester formulation than with the amine formulation.

Glufosinate is rapidly degraded in soil, acted upon by microbes that degrade it to CO_2 and natural phosphorus compounds (US-EPA, 2008b). As with 2,4-D, studies of the effects of glufosinate on the microbial community have also yielded varying results. Several found no differences in the microbial community from the application of glufosinate compared to either those treated with different herbicides or those left untreated (Lupwayi et al., 2004; Schmalenberger and Tebbe, 2002; Wibawa et al., 2010); however, Gyamfi et al. (2002) found the application of glufosinate caused minor, transient shifts in the bacterial community structure, potentially caused by the increase of herbicide-degrading microbes. Other research found that the use of glufosinate inhibits the activity of cultivar pathogens such as Bacterial Blight (Pline, 1999) and Grapevine Downy Mildew (*Plasmopara viticola*) (Kortekamp, 2010).

2.3.5 Biodiversity

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

The degree of biodiversity in an agroecosystem depends on four primary characteristics: (1) diversity of vegetation within and around the agroecosystem; (2) permanence of various crops within the system; (3) intensity of management; and (4) extent of isolation of the agroecosystem from natural vegetation (Altieri, 1999). Agricultural land subject to intensive farming practices, such as that used in crop production, generally has low levels of biodiversity compared with

[5] An herbicide product or formulation is composed of three parts: the parent acid, salt, and proprietary components. A formulation's acid equivalent is a measure of the parent acid (Johnson et al., 2006). In the case of 2,4-D, the ester, salt, and amine formulations are derivatives of the acid parent compound, 2,4-dichlorophenoxyacetic acid (Hager and Sprague, 2000). It is the parent acid which is the herbicidally active portion of the formulation, the salt, ester, or amine formulation has been developed to enhance plant absorption or otherwise facilitate herbicide delivery in the field (Hager and Sprague, 2000).

adjacent natural areas. Tillage, seed bed preparation, planting of a monoculture crop, pesticide use, fertilizer use, and harvesting limit the diversity of plants and animals (Lovett et al., 2003).

Biodiversity can be maintained or reintroduced into agroecosystems through the use of woodlots, fencerows, hedgerows, and wetlands. Agronomic practices that may be employed to support biodiversity include intercropping (the planting of two or more crops simultaneously to occupy the same field), agroforestry, crop rotations, cover crops, no-tillage, composting, green manuring (growing a crop specifically for the purpose of incorporating it into the soil in order to provide nutrients and organic matter), addition of organic matter (compost, green manure, animal manure, etc.), and hedgerows and windbreaks (Altieri, 1999). Integrated pest management strategies include several practices that increase biodiversity such as retaining small, diverse natural plant refuges and minimal management of field borders.

The potential impacts to biodiversity associated with the agricultural production of crops include a loss of diversity, which can occur at the crop, farm, and/or landscape level (Ammann, 2005; Carpenter, 2011; Visser, 1998). In this EA, crop diversity refers to the genetic uniformity within crops, farm-scale diversity refers to the level of complexity of organisms within the boundaries of a farm, and landscape level diversity refers to potential changes in land use and the impacts of area-wide weed suppression beyond the farm boundaries (Carpenter, 2011).

2.3.5.1 Crop Diversity

Genetic diversity in crops is beneficial as it may improve yields, pest and disease resistance, and quality in agricultural systems, and that greater varietal and species diversity enable growers to maintain productivity over a wide range of conditions (Krishna et al., 2009). There is concern that the adoption of GE technology potentially reduces grower-demand for crop genetic diversity because breeding programs could concentrate on a smaller number of high value cultivars, which could reduce the availability of, and demand for, non-GE varieties (Carpenter, 2011; Krishna et al., 2009). In contrast, several studies involving GE soybeans and cotton have found this not to be the case, indicating the introduction of GE crops has not decreased crop species diversity (Ammann, 2005; Carpenter, 2011; Krishna et al., 2009).

Concern for the loss of genetic variability has led to the establishment of a worldwide network of genebanks (van de Wouw et al., 2010). The USDA Soybean Germplasm Collection, which is part of the National Plant Germplasm System, acquires, maintains, and evaluates soybean germplasm and distributes seed samples to scientists in 35 states (U of Illinois, 2003). Nationwide, there are over 21,850 soybean varieties (USDA-ARS, 2011b) that provide a vast reservoir of genetic diversity for crop development.

2.3.5.2 Farm-scale Diversity

As noted previously, agricultural practices have the potential to impact diversity at the farm level by affecting a farm’s biota, including birds, wildlife, invertebrates, soil microorganisms, and weed populations. For example, an increase in adoption of conservation tillage practices is associated with the use of GE herbicide-tolerant crops (Givens et al., 2009). Less tillage provides more wildlife habitat by allowing other plants to establish between crop rows. Conservation tillage also leaves a higher rate of plant residue and increases soil organic matter

(Hussain et al., 1999), which benefit soil biota by providing additional food sources (energy) (USDA-NRCS, 1996) and increase the diversity of soil microorganisms, as discussed in Subsection 2.3.4, Microorganisms. In addition, invertebrates that feed on plant detritus and their predators and, in turn, birds and other wildlife that prey on them, may benefit from increased conservation tillage practices (Carpenter, 2011; Towery and Werblow, 2010). Ground-nesting and seed-eating birds, in particular, have been found to benefit from greater food and cover associated with conservation tillage (SOWAP, 2007).

Herbicide use in agricultural fields may impact biodiversity by decreasing weed quantities or causing a shift in weed species present in the field, which would affect those insects, birds, and mammals that utilize these weeds. The quantity and type of herbicide use associated with conventional and GE crops is dependent on many variables, including cropping systems, type and abundance of weeds, production practices, and individual grower decisions.

2.3.5.3 Landscape-scale Diversity

The greatest direct impact of agriculture on biodiversity on the landscape scale results from the loss of natural habitats caused by the conversion of natural ecosystems into agricultural land (Ammann, 2005). Increases in crop yields, such as has been observed in the last 10 years in soybean production, have the potential to reduce impacts to biodiversity by allowing less land to be converted to agriculture than would otherwise be necessary (Carpenter, 2011); however, substantial gains in yields have generally not been obtained by herbicide-tolerant cultivars unless higher yielding cultivars are modified with an herbicide-tolerant trait (NRC, 2010).

Similar to that discussed in farm-scale diversity, the use of herbicides at the landscape-level also has the potential to impact biodiversity. Increased conservation tillage practices associated with herbicide-tolerant crops over large areas may increase certain populations of invertebrates and wildlife that benefit from conservation tillage, whereas those species dependent on the targeted weeds may be negatively impacted. Potential impacts to landscape-scale diversity can also be related to the effects of herbicides on non-target plant and animal species.

Several recent studies (Brower et al., 2012; Hartzler, 2010; Pleasants and Oberhauser, 2012) have examined the potential causes of observed decreases in overwintering monarch butterfly *Danaus plexippus* populations, namely the reduced infestations of common milkweed (*Asclepias syriaca*), a perennial weed, in Corn Belt agricultural fields. The loss of host milkweed plants in agricultural fields is assumed to be a result of the increased use of glyphosate associated with the high adoption rate of GE crops (Brower et al., 2012), although slight declines in milkweed abundance in non-agricultural areas not related to glyphosate use were also observed. However, it was concluded that the observed reduced monarch abundance is likely based on several contributing factors including: degradation of the forest in the overwintering areas; the loss of breeding habitat (i.e., milkweed host plants) in the U.S. resulting from the use of herbicide associated with the expansion of GM herbicide-resistant crop acreage and from continued land development; and severe weather (Brower et al., 2012; Hartzler, 2010; Pleasants and Oberhauser, 2012).

The ecological toxicity of 2,4-D is moderate to practically non-toxic to birds on an acute basis; slightly toxic to small mammals on an acute oral basis; practically non-toxic to honey bees; and toxic to terrestrial plants (US-EPA, 2005a). Toxicity tests of glufosinate-ammonium found this substance to be practically non-toxic to birds, mammals, and insects; slightly non-toxic to freshwater fish; moderately toxic to estuarine/marine fish; moderately toxic to freshwater and estuarine/marine invertebrates; and toxic to terrestrial and aquatic plants (US-EPA, 2008b). See Subsections 2.3.1, Animal Communities, and 2.3.2, Plant Communities, for more detailed information regarding the ecological toxicity of these herbicides. While herbicide use potentially affects biodiversity, the application of pesticides in accordance with EPA registered label uses and careful management of chemical spray drift minimizes the potential biodiversity impacts from their use.

2.4 Human Health

2.4.1 Public Health

Human health concerns surrounding GE soybean focus primarily on human consumption and occupational exposure. Soybeans yield both solid (meal) and liquid (oil) products. Soybean meal is high in protein and is used for products such as tofu, soymilk, meat replacements, and protein powder; it also provides a natural source of dietary fiber (USB, 2009). Nearly 98% of soybean meal produced in the U.S. is used as animal feed, while less than 2% is used to produce soy flour and proteins for food use (Soyatech, 2011). Soybean liquids are used to produce salad and cooking oils, baking and frying fat, and margarine. Soy oil is low in saturated fats, high in poly and monounsaturated fats, and contains essential omega-3 fatty acids. Soybean oil comprises nearly 70% of the oils consumed in U.S. households (Soy Stats, 2010c).

Non-GE soybean varieties, both those developed for conventional use and for use in organic production systems, are not routinely required to be evaluated by any regulatory agency in the U.S. for human food or animal feed safety prior to release in the market. Under the FFDCA, it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. As a GE product, however, food and feed derived from DAS-68416-4 soybean must be in compliance with all applicable legal and regulatory requirements.

GE organisms for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market. Although a voluntary process, thus far, all applicants who have wished to commercialize a GE variety that would be included in the food supply have completed a consultation with the FDA. In such a consultation, a developer who intends to commercialize a bioengineered food meets with the agency to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food and then submits to FDA a summary of its scientific and regulatory assessment of the food. This process includes: 1) an evaluation of the amino acid sequence introduced into the food crop to confirm whether the protein is related to known toxins and allergens; 2) an assessment of the protein's potential for digestion; and 3) an evaluation of the history of safe use in food (Hammond and Jez, 2011). FDA evaluates the submission and responds to the developer by letter with any concerns it may have or additional information it may require. Several international agencies also review food safety associated with GE-derived food items, including the European Food Safety Agency (EFSA) and the Australia and New Zealand Food Standards Agency (ANZFS).

Foods derived through biotechnology also undergo a comprehensive safety evaluation before entering the market, including reviews under the CODEX, the European Food Safety Agency, and the World Health Organization (FAO, 2009; Hammond and Jez, 2011). Food safety reviews frequently will compare the compositional characteristics of the GE crop with non-transgenic, conventional varieties of that crop (Aumaitre et al., 2002; FAO, 2009). Moreover, this comparison also evaluates the composition of the modified crop under actual agronomic conditions, including various agronomic input. Composition characteristics evaluated in these comparative tests include moisture, protein, fat, carbohydrates, ash, minerals, dietary fiber, essential and non-essential amino acids, fatty acids, vitamins, and antinutrients.

There are multiple ways in which organisms can be genetically modified through human intervention. Traditional methods include breeding or crossing an organism to elicit the expression of a desired trait, while more contemporary approaches include the use of biotechnology such as genetic engineering to produce new organisms (NRC, 2004). As noted by the National Research Council (NRC), unexpected and unintended compositional changes arise with all forms of genetic modification, including both conventional hybridizing and genetic engineering (NRC, 2004). The NRC also noted that at the time, no adverse health effects attributed to genetic engineering had been documented in the human population. Reviews on the nutritional quality of GE foods have generally concluded that there are no significant nutritional differences in conventional versus GE plants for food or animal feed (Faust, 2002; Flachowsky et al., 2005).

Pursuant to FFDCA, before a pesticide can be used on a food crop, EPA must establish the tolerance value which is the maximum amount of pesticide residue that can remain on the crop or in foods processed from that crop (US-EPA, 2010c). In addition, the FDA and the USDA monitor foods for pesticide residues and enforce these tolerances (see USDA-AMS, 2011). If pesticide residues are found to exceed the tolerance value, the food is considered adulterated and may be seized. The USDA has implemented the Pesticide Data Program (PDP) in order to collect data on pesticides residues on food (USDA-AMS, 2010). The EPA uses PDP data to prepare pesticide dietary exposure assessments pursuant to the 1996 FQPA. Pesticide tolerance levels for 2,4-D and glufosinate-ammonium have been established for a wide variety of commodities, including soybean (US-EPA, 2011g). For 2,4-D, the tolerance for soybean seed is 0.02 parts per million (ppm) (EPA, 2012a), while the established tolerance of glufosinate ammonium is 2.0 ppm (EPA, 2012b).

2.4.2 Occupational Health and Safety

Agriculture is one of the most hazardous industries for U.S. workers. As a result, Congress directed the National Institute of Occupational Safety and Health to develop a program to address high-risk issues related to occupational workers. In consideration of the risk of pesticide exposure to field workers, EPA's Worker Protection Standard (WPS) (40 CFR Part 170) was published in 1992 to require actions to reduce the risk of pesticide poisonings and injuries among agricultural workers and pesticide handlers. The WPS offers protections to more than two and a half million agricultural workers who work with pesticides at more than 560,000 workplaces on farms, forests, nurseries, and greenhouses. 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. Furthermore, the Occupational Safety and Health Administration require all employers to protect their employees from hazards associated with pesticides and herbicides.

Pesticides, which includes herbicides, are used on most soybean acreage in the U.S., and changes in acreage, crops, or farming practices can affect the amounts and types of pesticides used and thus the potentional risks to farm workers. The EPA pesticide registration process, however, involves the design of use restrictions that, if followed, have been determined to be protective of worker health. EPA labels for herbicides include use restrictions and safety measures to mitigate against exposure risks. Growers are required to use pesticides consistent with the application instructions provided on the EPA-approved pesticide labels. Worker safety precautions and use restrictions are clearly noted on pesticide registration labels. These restrictions provide instructions as to the appropriate levels of personal protection required for agricultural workers to use herbicides. These may include instructions on personal protective equipment, specific handling requirements, and field reentry procedures. Used in accordance with the EPA label, these herbicides have been determined to not present a health risk to workers (US-EPA, 2005c, 2008b).

Under FIFRA, all pesticides (which is inclusive of herbicides) sold or distributed in the U.S. must be registered by the EPA (US-EPA, 2005c). Registration decisions are based on scientific studies that assess the chemical's potential toxicity and environmental impact. To be registered, a pesticide must be able to be used without posing unreasonable risks to people or the environment. All pesticides registered prior to November 1, 1984, such as 2,4-D, must also be reregistered to ensure that they meet the current, more stringent standards. The reregistration decision for 2,4-D was issued in 2005 (US-EPA, 2005c).

In the 2005 RED, EPA classified 2,4-D (inclusive of the salt, ester, and acid forms) as having low to slight acute toxicity (Category III or IV[6]), with the exception of the acid and salt forms being severe eye irritants (US-EPA, 2005a). Moreover, the EPA has concluded that there is no evidence that 2,4-D is either a mutagenic or a carcinogen. Additional human health studies were required by the 2005 EPA reregistration eligibility decision for 2,4-D and included a developmental neurotoxicity study, a multi-generation reproduction study, and a subchronic inhalation toxicity study (US-EPA, 2005c). The Industry Task Force II on 2,4-D Research Data has completed these additional data requests and they are currently in review at the EPA (Conner, 2010).

In 2008, the Natural Resources Defense Council (NRDC) petitioned EPA to revoke all tolerances and cancel all registrations of 2,4-D (US-EPA, 2008d). The petitioner (NRDC) claimed that EPA could not make a finding that there is a reasonable certainty of no harm from dietary residues of 2,4-D; therefore, the Agency must revoke all tolerances established under Section 408 of FFDCA, as amended by FQPA. As a part of the petition, NRDC claims that the agency did not consider the full spectrum of potential human health effects associated with 2,4-D in connection with EPA's reassessment of the existing 2,4-D tolerances, and EPA's environmental risk assessment for the reregistration of 2,4-D in 2005. EPA recently responded to NRDC's petition and denied the petition's request to cancel all tolerances and registrations of

[6] Category I indicates the highest degree of acute toxicity and Category IV the lowest.

2,4-D (US-EPA, 2012a, 2012c). As previously discussed, EPA's reregistration review of 2,4-D is scheduled to begin in 2013 (US-EPA, 2011i).

Glufosinate-ammonium is classified as not likely to be a human carcinogen and has no mutagenicity concern (US-EPA, 2008b). During the registration decision, the EPA must find that a pesticide does not cause unreasonable adverse effects to human health or the environment if used in accordance with the approved label instructions (OSTP, 2001).

2.5 Animal Feed

Animal agriculture consumes 98% of the U.S. soybean meal produced (Soyatech, 2011) and 70% of soybeans worldwide (USB, 2011d). Poultry consume more than 45% of domestic soybean meal or 590 million bushels of the U.S. soybean crop, with soy oil increasingly replacing animal fats and oils in broiler diets (USB, 2011c). Soybean can be the dominant component of livestock diets, such as in poultry, where upwards of 66% of their protein intake is derived from soy (Waldroup and Smith, No Date). Other animals fed domestic soybean (by crop volumes consumed) include swine (26%), beef cattle (12%), dairy cattle (9%), other (e.g., poultry, farm-raised fish 3%), and household pets (2%) (Soy Stats, 2010d; USB, 2011a).

Although the soybean market is dominated by seed production, soybean has a long history in the U.S. as a nutritious grazing forage, hay, and silage crop for livestock (Blount et al., 2009). Soybean may be harvested for hay or grazed from the flowering stage to near maturity; the best soybean for forage is in the beginning pod stage (Johnson et al., 2007). For silage, it should be harvested at maturity before leaf loss, and mixed with a carbohydrate source, such as corn, for optimal fermentation characteristics (Blount et al., 2009). Varieties of soybean have been developed specifically for grazing and hay, but use of the standard grain varieties are recommended by some because of the whole plant feeding value (Weiderholt and Albrecht, 2003).

Similar to the regulatory oversight for direct human consumption of soybean under the FFDCA, it is the responsibility of feed manufacturers to ensure that the products they market are safe and properly labeled. Feed derived from GE soybean must comply with all applicable legal and regulatory requirements, which in turn protects human health. To help ensure compliance, GE organisms used for feed may undergo a voluntary consultation process with FDA before release onto the market, which provides the applicant with any needed direction regarding the need for additional data or analysis, and allows for interagency discussions regarding possible issues.

Although a voluntary process, thus far all applicants who wish to commercialize a GE variety that will be included in the food supply have completed a consultation with the FDA. A developer who intends to commercialize a bioengineered food consults with the agency to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food and then submits to FDA a summary of its scientific and regulatory assessment of the food (BNF No. 000124). FDA evaluates the submission and responds to the developer by letter (US-FDA, 2011a).

Growers must adhere to EPA label use restrictions for pesticides used to produce a soybean crop before using it as forage, hay, or silage. Under Section 408 of FFDCA, EPA regulates the levels of pesticide residues that can remain on food or food commodities from pesticide applications (US-EPA, 2010c). The tolerance level is the maximum residue level of a pesticide that can legally be present in food or feed, and if pesticide residues are found to exceed the tolerance value, the food is considered adulterated and may be seized. For 2,4-D, tolerances for soybean forage, hay and seed are 0.02, 2.0, and 0.02 parts per million (ppm), respectively (US-EPA, 2010a). GE glufosinate-tolerant soybean treated with glufosinate are not to be grazed or cut for hay (Bayer CropScience, 2011).

2.6 Socioeconomic

2.6.1 Domestic Economic Environment

The value of U.S. soybean production exceeded $38.9 billion in 2010 (USDA-NASS, 2011b), which was a quarter of the value of all field crops (Figure 3). The top ten producing states (Iowa, Illinois, Minnesota, Indiana, Nebraska, Ohio, Missouri, South Dakota, Kansas, and North Dakota) accounted for more than 80% of this production (Table 11). These states are located in the USDA-ERS's Heartland (Iowa, Illinois, Indiana, Minnesota, Missouri, Nebraska, Ohio, and South Dakota), Northern Crescent (Minnesota and Ohio), Northern Great Plains (Nebraska, Minnesota, North Dakota, and South Dakota), Prairie Gateway (Kansas and Nebraska), and Eastern Uplands (Missouri and Ohio) resource regions (Fernandez-Cornejo and McBride, 2002), which vary in terms of land productivity and cost of production (Figure 4). The most productive of these regions are the Heartland and Northern Crescent. While these regions have higher production cost, their higher productivity still results in greater profitability. In 2010, the U.S. total gross average value of soybean production per planted acre was $449.32 and the average price of a bushel of soybeans at harvest was $9.56 (USDA-ERS, 2011g).

Production cost data are provided by USDA-ERS and collected in surveys conducted every four to eight years for each commodity as part of the annual ARMS (USDA-ERS, 2011d). In 2010, typical operating costs are reported in dollars per planted acre and included purchased seed ($59.20), fertilizer and soil amendments ($17.87), other chemicals ($17.04), and irrigation water ($0.14) (USDA-ERS, 2011g). Total 2010 operating costs were $132.29 per planted soybean acre (USDA-ERS, 2011g). In comparison, forecasted 2011 typical U.S. soybean production operating costs per planted acre total 149.62, including $67.37 for purchased seed, $22.63 for fertilizer and soil amendments, and $16.85 for other chemicals; costs for irrigation water were not estimated (USDA-ERS, 2011e). The rise in crop production input prices is attributed to the increased use of more expensive seeds with complex genetic traits, increased use of fertilizer that has increased in price primarily in response to rising natural gas prices, and a 4% rise in pesticide costs coupled with an increase in overall crop acreage (USDA-ERS, 2011f).

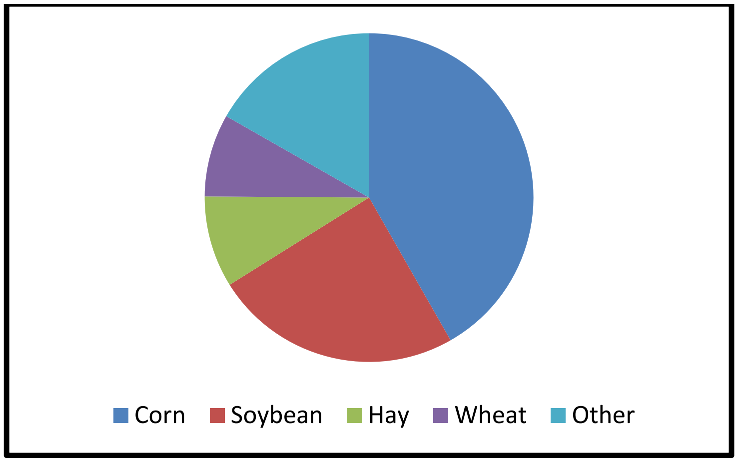


Figure 8. Distribution of crop value in 2010.

Source: USDA-NASS (2011b)

Table 11. Soybean crop value by state.

State	Crop Value ($ millions)			Percent of Total Soybean Crop Value		
	2008	2009	2010	2008	2009	2010
Alabama	126	172	100	0.4	0.5	0.3
Arkansas	1,191	1,185	1,246	4.0	3.7	3.2
Delaware	50	74	66	0.2	0.2	0.2
Florida	9	12	8	0.03	0.04	0.02
Georgia	122	155	76	0.4	0.5	0.2
Illinois	4,372	4,215	5,779	14.8	13.1	14.9
Indiana	2,492	2,612	3,050	8.5	8.1	7.8
Iowa	4,586	4,627	5,806	15.6	14.4	14.9
Kansas	1,129	1,506	1,658	3.8	4.7	4.3
Kentucky	476	675	572	1.6	2.1	1.5
Louisiana	298	354	456	1.0	1.1	1.2
Maryland	134	190	188	0.5	0.6	0.5
Michigan	687	759	1,012	2.3	2.4	2.6
Minnesota	2,675	2,674	3,717	9.1	8.3	9.6
Mississippi	728	713	846	2.5	2.2	2.2
Missouri	1,862	2,216	2,546	6.3	6.9	6.5
Nebraska	2,212	2,459	3,026	7.5	7.7	7.8
New Jersey	26	34	25	0.1	0.1	0.1
New York	107	99	147	0.4	0.3	0.4
North Carolina	514	571	496	1.7	1.8	1.3
North Dakota	1,022	1,075	1,564	3.5	3.3	4.0

Table 11. Soybean crop value by state (continued).

State	Crop Value ($ millions)			Percent of Total Soybean Crop Value		
	2008	2009	2010	2008	2009	2010
North Dakota	1,022	1,075	1,564	3.5	3.3	4.0
Ohio	1,661	2,171	2,600	5.6	6.8	6.7
Oklahoma	82	114	132	0.3	0.4	0.3
Pennsylvania	175	192	245	0.6	0.6	0.6
South Carolina	153	132	120	0.5	0.4	0.3
South Dakota	1,332	1,615	1,762	4.5	5.0	4.5
Tennessee	469	671	507	1.6	2.1	1.3
Texas	46	44	56	0.2	0.1	0.1
Virginia	166	198	166	0.6	0.6	0.4
West Virginia	7	7	7	0.02	0.02	0.02
Wisconsin	545	623	938	1.9	1.9	2.4
United States	29,458	32,145	38,915	100	100	100

Source: USDA-NASS (2011b)

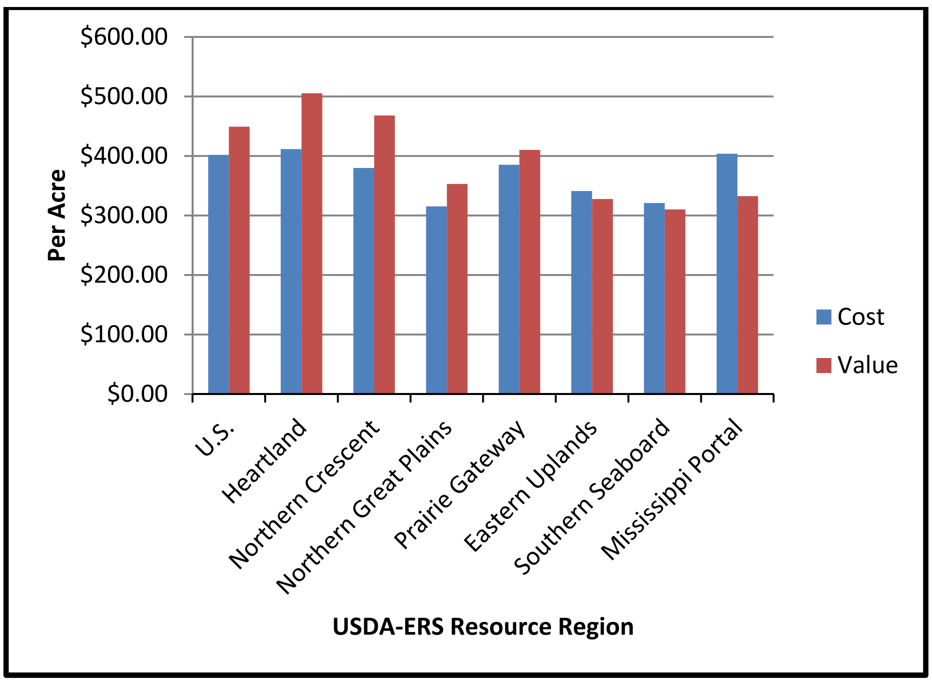


Figure 9. U.S. soybean cost and value of production estimates for 2010 (excluding government payments).

Source: USDA-ERS(2011g)

Almost all of the U.S. soybean supply (95.6% in 2009/10) comes from domestic production and almost all of this supply (96.8%) is either exported or crushed for meal and oil (Table 12). In any given year, the resulting meal and oil is modestly supplemented with carryover stocks and imports before being consumed domestically or exported. In the U.S., almost all of the soybean meal is used for animal feed (97.5% in 2002/03) (Soybean Meal Information Center, 2011). The vast majority of the oil (86% in 2010) is used for human consumption, with the balance going to industrial products (Figure 10). Soybean oil represents almost 70% of the oils consumed by U.S. households. It is notable that higher petroleum prices and an increased interest in biofuels are increasing the demand for soybean-based biodiesel. From 1999 to 2009, the consumption of soybean biodiesel has increased from 0.5 to 545 million gallons (Soy Stats, 2010a).

Table 12. U.S. soybean supply and disappearance[1] 2009/10.

	Soybeans	Soybean Meal	Soybean Oil
	(Million Metric Tons)		
Total	95.58	38.19	10.24
	Supply		
Beginning Stocks	3.76	0.21	1.3
Production	91.42	37.83	8.90
Imports	0,40	0.15	0.05
	Disappearance		
Crush	47.67	--[2]	--
Feed, Seed & Residual	2.95	--	--
Domestic	--	27.78	7.20
Exports	40.85	10.14	7.54
Ending Stocks	4.11	0.27	1.53

Source: USDA-ERS (2011i, 2011j, 2011k)
[1]Disappearance is the consumed supply
[2] No data

Soybean production has been transformed over the past fifteen years by the commercial release of Roundup Ready® soybean varieties in 1996 and LibertyLink® soybean varieties in 2009. Roundup Ready® soybeans are GE to be tolerant to glyphosate, while LibertyLink® soybeans are engineered to be tolerant to glufosinate. GE soybeans were planted on 94% of U.S. soybean acreage in 2011 (see Subsection 2.1.1, Acreage and Area, Table 2). In terms of weed control costs, Johnson et al. (2008) estimated a $1.562 billion reduction in production costs associated with grower's adoption of herbicide-tolerant soybeans in 2006. A more recent study found a $17.75 net cost saving per U.S. GE soybean acre in 2008 (Brookes and Barfoot, 2010).

As of the 2010 growing season, there were 12 different weed species with glyphosate-resistant populations ranging across 25 different U.S. states (see Appendix D, Table D-1) (Heap, 2011). Resistant weed populations have been found in all but 1 (South Dakota) of the 10 major soybean producing states. Surveys show that farmers expect glyphosate resistance will or has increased the cost of weed control from $10.87 to $16.30 per acre (Foresman and Glasgow, 2008; Hurley et al., 2009b), and that farmers prefer to address the problem by using additional herbicides with different modes of action (Foresman and Glasgow, 2008; Johnson and Gibson, 2006; Johnson et al., 2009; Scott and VanGessel, 2007).

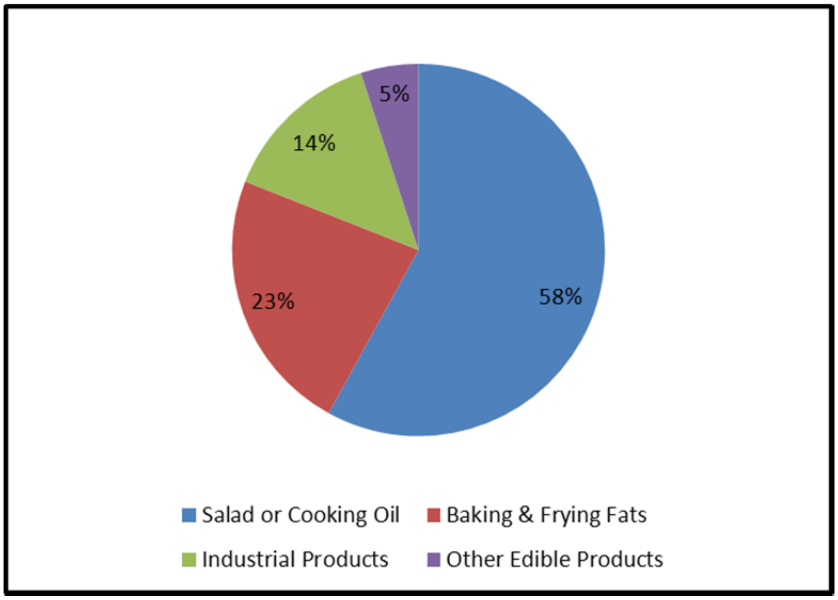


Figure 10. Distribution of U.S. soybean oil consumption in 2010.

Source: Soy Stats (2010b)

In response to the increasing incidence of glyphosate-resistant weeds, a recent trend has been a return to using older herbicides and an increased adoption of newer glufosinate-tolerant crop varieties (Paytosh, 2011). The price of glyphosate dropped in half in 2011 to a rate as low as \$2.50/acre for typical use rates in Pennsylvania, and Ignite 280® glufosinate for use on LibertyLink® systems dropped to \$53/gallon or approximately \$9/acre per application in this region; even though herbicide resistance management is important, pricing offers little incentive to change systems in some areas of the country (Lingenfelter, 2011). The estimated price per gallon of 2,4-D amine salt in Florida for 2011 crop season was \$13 (Feller and Sellers, 2011) and in the Mississippi Delta area, the 2010/2011 estimate for 2,4-D used for pre-plant burndown applications in Roundup Ready® soybean was \$3.48 per acre (MSU, 2010). Herbicide prices vary among dealers and regions of the U.S., vary by company offerings that differ in regard to rebates and guarantees of certain seed/herbicide packages, and may vary based on volume of purchases (Lingenfelter, 2011).

There is consistent evidence that farmers obtain substantial financial and non-financial benefits as a result of adoption of GE crops. These benefits include an opportunity to increase income from off-farm labor; increased flexibility and simplicity in the application of pesticides; an ability to adopt more environmentally friendly farming practices; increased consistency of weed control; increased human safety; equipment savings; and labor savings (Duke and Powles, 2009; Fernandez-Cornejo et al., 2005; Fernandez-Cornejo and McBride, 2000; Fernandez-Cornejo and McBride, 2002; Hurley et al., 2009a; Marra et al., 2004).

2.6.2 <u>Trade Economic Environment</u>

Soybean exports in the form of bulk beans, meal, and oil are a major share of the total agricultural exports for the U.S., representing 20.1% of the total value of U.S. exports. The value of U.S. agricultural exports was \$108.67 billion in 2010 (USDA-ERS, 2010d). Bulk soybeans accounted for $16.9 billion of this total, ranking first among all agricultural commodities, while soybean meal, at a value of $3.78 billion, and soybean oil, at a value of $1.35 billion, ranked 6th and 16th, respectively (USDA-ERS, 2010c). The U.S. was responsible for 44.0% of the world's bulk soybean exports, 18.2% of the world's soybean meal exports, and 16.8% of the world's soybean oil exports (Tables 13, 14).

Table 13. U.S. and rest of world (ROW) soybean supply and disappearance[1] 2009/10.

	Soybeans		Soybean Meal		Soybean Oil	
	U.S.	ROW	U.S.	ROW	U.S.	ROW
	(Million Metric Tons)					
Total	95.58	294.71	38.19	184.03	10.24	40.13
	-----Supply-----					
Beginning Stocks	3.76	38.82	0.21	4.20	1.30	1.62
Production	91.42	168.85	37.83	127.45	8.90	29.87
Imports	0.40	87.04	0.15	52.38	0.05	8.64
	-----Disappearance-----					
Crush	47.67	161.84	--[2]	--	--	--
Feed, Seed & Residual	2.95	26.09	--	--	--	--
Domestic	--	--	27.78	132.84	7.20	31.06
Exports	40.85	51.89	10.14	45.56	1.52	7.54
Ending Stocks	4.11	54.89	0.27	5.63	1.52	1.53

Source: USDA-ERS (2011h)
[1] Disappearance is the consumed supply
[2] No data

ROW = Rest of World
U.S. = United States

Soybean meal represented 68% of the protein meal produced worldwide , though soybean ranked behind palm in terms of worldwide vegetable oil production (USDA-FAS, 2011a). Similarly, soybean held the largest share of protein meal consumed worldwide mainly as animal feed (USDA-FAS, 2011a), with soybean oil again coming in second behind palm oil in terms of worldwide vegetable oil consumption (USDA-FAS, 2011a).

The U.S. was responsible for 35.1% of the world's soybean production, 22.9% of world's soybean meal production, and 23.0% of the world's soybean oil production (Table 13) (USDA-ERS, 2011h). The U.S., China, Argentina, and Brazil are the major producers of soybean (88.1%), soybean meal (78.2%), and soybean oil (78.6%) (Table 14).

The U.S., along with Brazil, Argentina, Paraguay, and Canada, account for 97% of the bulk soybean exported, while Argentina, Brazil, the U.S., India, and Paraguay account for 94.1% of the soybean meal exported (Table 15). Argentina, the U.S., and Brazil are the dominant countries in terms of soybean oil exports accounting for 80.2% (Table 15). Table 16 presents the top 10 U.S. export markets for soybean by volume for 2010 and 2011, during which China, Mexico, and the European Union 27 member countries (EU-27) were the top 3 importers (USDA-ERS, 2011l). As of March 2011, U.S. exports of soybean valued $13.79 billion, soybean meal approximately $2.1 billion, and soybean oil approximately $1.2 billion (USDA-ERS/USDA-FAS, 2011). China, the EU-27, Mexico, and Japan are the major importers of world bulk soybean, accounting for 80.1% of total imports, whereas the EU-27, Vietnam, Thailand, Indonesia, and Japan are the largest importers of soybean meal with a world share of 57.6% (USDA-FAS, 2011a). For soybean oil, China and India are the major importers with a world share of 35.8% (USDA-FAS, 2011a). U.S. soybean exports are projected to increase to approximately 1.5 billion bushels (33.2 million metric tons) in 2020 (USDA-OCE, 2011).

Table 14. World soybean production in 2009/10.

Location	Soybean	Soybean Meal	Soybean Oil
	(million metric tons)		
Argentina	55	26.62	6.48
Brazil	69	26.12	6.47
Canada	4	--[1]	--
China	15	38.64	8.73
EU-27[2]	--	9.88	2.28
India	10	5.99	1.34
Mexico	--	2.83	0.64
Paraguay	7	--	--
United States	91	37.83	8.90
Other	11	17.37	4.06

Source: USDA-FAS (2011a).
[1]-- = No Data
[2] European Union 27 member countries

Table 15. World soybean exports in 2009/10.

Location	Soybean Bulk	Soybean Meal	Soybean Oil
	(million metric tons)		
Argentina	13.09	24.91	4.45
Bolivia	--[1]	--	0.26
Brazil	28.58	12.99	1.45
Canada	2.25	--	--
EU-27[2]	--	--	0.38
India	--	3.15	--
Paraguay	5.35	1.12	0.24
Russia	--	--	0.17
United States	40.85	10.14	1.52
Other	2.53	3.29	0.79

Source: USDA-FAS (2011a).
[1] -- = No Data
[2] European Union 27 member countries

Table 16. Top 10 U.S. soybean export markets in 2010/11.

Location	January-October 2010	January-October 2011	October 2010	October 2011
	(million metric tons)			
China	15.65	13.86	5.58	3.88
Mexico	3.02	2.76	0.56	0.51
EU-27[1]	1.30	1.41	0.99	0.12
Japan	2.03	1.39	0.24	0.11
Taiwan	2.03	1.32	0.44	0.067
Indonesia	1.18	1.11	0.16	0.081
Egypt	0.86	0.57	0.12	0.14
Turkey	0.58	0.42	0.17	0.0029
South Korea	0.26	0.31	0.036	0.012
Syria	0.14	0.23	0.039	0.018
World Total	29.92	25.26	8.00	5.26

Source: USDA-ERS (2011l).
[1] European Union 27 member countries

3 ALTERNATIVES

This document analyzes the potential environmental consequences of a determination of nonregulated status of DAS-68416-4 soybean. To respond favorably to a petition for nonregulated status, APHIS must determine that DAS-68416-4 soybean is unlikely to pose a plant pest risk. Based on its PPRA (USDA-APHIS, 2012), APHIS has concluded that DAS-68416-4 soybean is unlikely to pose a plant pest risk. Therefore, APHIS must determine that DAS-68416-4 soybean is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act.

Two alternatives are evaluated in this EA: (1) no action and (2) determination of nonregulated status of DAS-68416-4 soybean. APHIS has assessed the potential for environmental impacts for each alternative in the Environmental Consequences section.

3.1 No Action Alternative: Continuation as a Regulated Article

Under the No Action Alternative, APHIS would deny the petition. DAS-68416-4 soybean and progeny derived from DAS-68416-4 soybean would continue to be regulated articles under the regulations at 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of DAS-68416-4 soybean and measures to ensure physical and reproductive confinement would continue to be implemented. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of DAS-68416-4 soybean.

This alternative is not the Preferred Alternative because APHIS has concluded through a PPRA that DAS-68416-4 soybean is unlikely to pose a plant pest risk (USDA-APHIS, 2012). Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status.

3.2 Preferred Alternative: Determination that DAS-68416-4 Soybean is No Longer a Regulated Article

Under this alternative, DAS-68416-4 soybean and progeny derived from them would no longer be regulated articles under the regulations at 7 CFR part 340. DAS-68416-4 soybean is unlikely to pose a plant pest risk (USDA-APHIS, 2012). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of DAS-68416-4 soybean and progeny derived from this event. This alternative best meets the purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the Plant Protection Act. Because the agency has concluded that DAS-68416-4 soybean is unlikely to pose a plant pest risk, a determination of nonregulated status of DAS-68416-4 soybean is a response that is consistent with the plant pest provisions of the Plant Protection Act, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework.

Under this alternative, growers may have future access to DAS-68416-4 soybean and progeny derived from this event if the developer decides to commercialize DAS-68416-4 soybean. DAS has indicated its intention to develop a stacked hybrid through conventional breeding techniques (DAS, 2010, 2011e, 2011f). In this process, the 2,4-D and glufosinate resistance traits in DAS-

68416-4 soybean would be combined with glyphosate resistance from another soybean variety that is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. APHIS does not have jurisdiction under the PPA and Part 340 to review such stacked hybrids developed using nonregulated articles and conventional hybridization techniques where there is no evidence of a plant pest risk. Accordingly, this EA focuses on the cultivation of the DAS-68416-4 soybean. Issues associated with potential future stacking, particularly cultivation of a stacked hybrid incorporating glyphosate resistance from a variety previously determined to be nonregulated, are presented and discussed in the cumulative effects analyses (see Section 5.0), where appropriate.

3.3 Alternatives Considered But Rejected from Further Consideration

APHIS assembled a list of alternatives that might be considered for DAS-68416-4 soybean. The agency evaluated these alternatives, in light of the agency's authority under the plant pest provisions of the Plant Protection Act, and the regulations at 7 CFR part 340, with respect to environmental safety, efficacy, and practicality to identify which alternatives would be further considered for DAS-68416-4 soybean. Based on this evaluation, APHIS rejected several alternatives. These alternatives are discussed briefly below along with the specific reasons for rejecting each.

3.3.1 Prohibit Any DAS-68416-4 Soybean from Being Released

In response to public comments that stated a preference that no GE organisms enter the marketplace, APHIS considered prohibiting the release of DAS-68416-4 soybean, including denying any permits associated with the field testing. APHIS determined that this alternative is not appropriate given that APHIS has concluded that DAS-68416-4 soybean is unlikely to pose a plant pest risk (USDA-APHIS, 2012).

In enacting the Plant Protection Act, Congress found that:

> [D]ecisions affecting imports, exports, and interstate movement of products regulated under [the Plant Protection Act] shall be based on sound science…§ 402(4).

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 (EO) 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 EO 13563 and, consistent with that EO, the following principle, among others, to the extent permitted by law, when regulating emerging technologies:

> “[D]ecisions 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 (USDA-APHIS, 2012) and the scientific data evaluated therein, APHIS concluded that DAS-68416-4 soybeanis unlikely to pose a plant pest risk. Accordingly, there is no basis in science for prohibiting the release of DAS-68416-4 soybean.

3.3.2 Approve the Petition in Part

The regulations at 7 CFR 340.6(d)(3)(i) state 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. Because APHIS has concluded that DAS-68416-4 soybean is unlikely to pose a plant pest risk, there is no regulatory basis under the plant pest provisions of the Plant Protection Act for considering approval of the petition only in part.

3.3.3 Isolation Distance between DAS-68416-4 and Non-GE Soybean Production and Geographical Restrictions

In response to public concerns of gene movement between GE and non-GE plants, APHIS considered requiring an isolation distance separating DAS-68416-4 soybean from non-GE soybean production. However, because APHIS has concluded that DAS-68416-4 soybean is unlikely to pose a plant pest risk (USDA-APHIS, 2012), an alternative based on requiring isolation distances would be inconsistent with the statutory authority under the plant pest provisions of the Plant Protection Act and regulations in 7 CFR part 340.

APHIS also considered geographically restricting the production of DAS-68416-4 based on the location of production of non-GE soybean in organic production systems or production systems for GE-sensitive markets in response to public concerns regarding possible gene movement between GE and non-GE plants. However, as presented in APHIS' PPRA for DAS-68416-4 soybean, there are no geographic differences associated with any identifiable plant pest risks for DAS-68416-4 soybean (USDA-APHIS, 2012). This alternative was rejected and not analyzed in detail because APHIS has concluded that DAS-68416-4 soybean does not pose a plant pest risk, and will not exhibit a greater plant pest risk in any geographically restricted area. Therefore, such an alternative would not be consistent with APHIS' statutory authority under the plant pest provisions of the Plant Protection Act and regulations in Part 340 and the biotechnology regulatory policies embodied in the Coordinated Framework.

Based on the foregoing, the imposition of isolation distances or geographic restrictions would not meet APHIS' purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the Plant Protection Act. Individuals, however, might choose on their own to geographically isolate their non-GE soybean production systems from DAS-68416-4 soybean or to use isolation distances and other management practices to minimize gene movement between soybean fields. Information to assist growers in making informed management decisions for DAS-68416-4 is available from AOSCA (AOSCA, 2010).

3.3.4 Requirement of Testing for DAS-68416-4

During the comment periods for other petitions for nonregulated status, some commenters requested USDA to require and provide testing for GE products in non-GE production systems. APHIS notes there are no nationally-established regulations involving testing, criteria, or limits of GE material in non-GE systems. Such a requirement would be extremely difficult to implement and maintain. Additionally, because DAS-68416-4 soybean does not pose a plant pest risk (USDA-APHIS, 2012), the imposition of any type of testing requirements is inconsistent with the plant pest provisions of the Plant Protection Act, the regulations at 7 CFR part 340 and biotechnology regulatory policies embodied in the Coordinated Framework. Imposing such a requirement for DAS-68416-4 soybean, therefore, would not meet APHIS' purpose and need to respond appropriately to the petition in accordance with its regulatory authorities.

3.4 Comparison of Alternatives

Table 17 presents a summary of the potential impacts associated with selection of either of the alternatives evaluated in this EA. The impact assessment is presented in Section 4 of this EA.

Table 17. Summary of issues of potential impacts and consequences of alternatives.

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Meets Purpose and Need and Objectives	No	Yes
Unlikely to pose a plant pest risk	Satisfied through use of regulated field trials	Satisfied – risk assessment (USDA-APHIS, 2012)
Management Practices		
Acreage and Areas of Soybean Production	Unchanged	Unchanged
Agronomic Practices	Unchanged	Unchanged
Soybean Seed Production	Unchanged	Unchanged
Organic Soybean Production	Unchanged	Unchanged
Environment		
Water Resources	Unchanged	Unchanged
Soil Quality	Unchanged	Unchanged
Air Quality	Unchanged	Unchanged
Climate Change	Potential for some increase if tillage increases	Unchanged
Animal Communities	Unchanged	Unchanged
Plant Communities	Unchanged	Unchanged
Gene Flow and Weediness	Unchanged gene flow. Glyphosate resistant weed development may increase since another mode of action for controlling weeds would not be available for use.	Unchanged
Soil Microorganisms	Unchanged	Unchanged
Biological Diversity	Unchanged	Unchanged

Table 17. Summary of issues of potential impacts and consequences of alternatives (continued).

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Human and Animal Health		
Risk to Human Health	Unchanged	Unchanged
Risk to Animal Feed	Unchanged	Unchanged
Socioeconomic		
Domestic Economic Environment	Unchanged	Unchanged
Trade Economic Environment	Unchanged	Unchanged
Other Regulatory Approvals		
U.S.	Unchanged for existing nonregulated GE organisms	FDA consultation completed, EPA pesticide registration being reviewed
Other Countries	Unchanged	Filed in Canada, Japan, Korea, Taiwan, European Union, Australia, New Zealand, South Africa, Brazil, Argentina and Mexico
Compliance with Other Laws		
CWA, CAA, EOs[1]	Fully compliant	Fully compliant

[1] CWA = Clean Water Act; CAA = Clean Air Act; EOs = Executive Orders

4 ENVIRONMENTAL CONSEQUENCES

Potential environmental impacts from the No Action Alternative and the Preferred Alternative for DAS-68416-4 soybean are described in detail throughout this section. An impact would be the result of any change, positive or negative, from the existing (baseline) conditions of the affected environment (described for each resource area in Section 2.0). Impacts may be categorized as direct, indirect or cumulative. A direct impact is an effect that results solely from a proposed action without intermediate steps or processes. Examples include soil disturbance, air emissions, and water use. An indirect impact may be an effect that is related to but removed from a proposed action by an intermediate step or process. Examples include surface water quality changes resulting from soil erosion due to increased tillage, and worker safety impacts resulting from an increase in herbicide use.

A cumulative impacts analysis is also included for each environmental issue. A cumulative impact may be a consequence on the human environment which results from the incremental impact of the proposed action when added to other past, present, and reasonably foreseeable future actions. Examples include breeding DAS-68416-4 soybean with other events previously approved for nonregulated status. If there are no direct or indirect impacts identified for a resource area, then there can be no cumulative impacts. Cumulative impacts are discussed in Section 5.

Where it is not possible to quantify impacts, APHIS provides a qualitative assessment of potential impacts. Certain aspects of the DAS-68416-4 soybean and its cultivation may be no different between the alternatives; those are described below.

4.1 Scope of Analysis

For the discussion of environmental consequences, this section addresses the following principal areas of potential environmental concern:

- Agricultural Production of Soybean (Subsection 4.2);
- Physical Environment (Subsection 4.3);
- Biological Resources (Subsection 4.4);
- Human Health (Subsection 4.5);
- Animal Feed (Subsection 4.6); and
- Socioeconomic Impacts (Subsection 4.7).

Under the Preferred Alternative, DAS-68416-4 soybean could be planted anywhere in the U.S.; however, APHIS has limited the environmental analysis to those areas that currently support soybean production. According to USDA National Agricultural Statistics Service (NASS) (2011a) annual crop statistics, soybean-producing states in the U.S. include 31 states that largely encompass the southeast and midwest regions (see Subsection 2.1.1, Acreage and Area of Soybean Production, Table 1).

The environmental consequences of the different alternatives described above are analyzed under the assumption that farmers who produce conventional soybean, DAS-68416-4 soybean or soybean using organic methods are using reasonable, commonly accepted best management practices for their chosen system and varieties during agricultural soybean production; however, APHIS recognizes that not all farmers follow these best management practices for soybean. Thus, the analyses of potential environmental impacts will also include the assumption that some farmers do not follow these best management practices.

DAS has developed new herbicide tolerance traits and herbicide technology aimed at providing alternatives to current soybean production sytems to help address glyphosate-resistant and other hard-to-control weeds. A part of this system, DAS-68416-4 soybean is a GE soybean line that has increased tolerance to treatment with phenoxy auxin herbicides and pyridyloxy auxin herbicides (DAS, 2010). The most well-known and widely-used phenoxy auxin herbicide is 2,4-D which has been used for many decades as a pre-plant or post-emergent herbicide to control broadleaf (dicot) weeds and for pre-plant weed control in soybean fields. In addition to 2,4-D, DAS-68416-4 soybean has demonstrated tolerance to the herbicides 2,4-DB, MCPA, triclopyr, and fluroxypry. According to DAS, of these additional herbicides, DAS-68416-4 soybean only has commercially acceptable tolerance to MCPA (DAS, 2012). DAS-68416-4 soybean, like currently produced GE soybeans containing the *pat* protein, also has tolerance to post-emergence applications of the herbicide glufosinate.

Additionally, DAS has developed a new formulation of 2,4-D created using a choline salt (DAS, 2010) to be used with DAS-68416-4 soybean. The new formulation of 2,4-D is chemically identified as 2,4-dichlorophenoxyacetic acid (2-hydroxyethyl) trimethylammonium salt or 2,4-D choline (DAS, 2010). 2,4-D choline is a quaternary ammonium salt that is reported to be chemically more stable, making it less volatile, than the currently used amine or ester formulations of 2,4-D. DAS has submitted applications to the EPA for a label for this new 2,4-D formulation for use with DAS-68416-4 soybean. APHIS considered the possible introduction of DAS-68416-4 soybean and this new 2,4-D formulation as a potential future action and takes this into consideration in the environmental assessment where appropriate. The conferred tolerance of DAS-68416-4 soybean to glufosinate and use of glufosinate on the Event are considered in the assessmentof potential environmental impacts since glufosinate currently is labeled for use on glufosinate-tolerant GE soybeans. APHIS will use the DAS draft 2,4-D label (Appendix A) and previous EPA analyses on 2,4-D (see US-EPA, 2005c) as the basis for its evaluation of the potential impacts associated with the use of and exposure to 2,4-D choline salt. DAS has indicated there is no change in the use pattern of glufosinate on the glufosinate-tolerant DAS-68416-4 soybean variety; hence, APHIS will use the current glufosinate label (Bayer CropScience, 2011) and EPA analyses on glufosinate (US-EPA, 2008b) as the basis for its potential impacts analysis. DAS has indicated that only DAS-approved herbicides would be allowed to be used on DAS-68416-4 soybean and 2,4-D would be the only auxin contained in their herbicides (DAS, 2012). Although, DAS-68416-4 soybean has varying levels of demonstrated tolerance to the herbicides 2,4-DB, MCPA, triclopyr, and fluroxypry, because these herbicides are not approved for use on soybean and DAS intends to only allow use of their herbicide formulation containing 2,4-D (DAS, 2012), impacts of these other herbicides are not considered in this EA.

DAS has indicated that a premix of the 2,4-D choline salt and glyphosate would be marketed for use with DAS-68416-4 (DAS, 2012) The draft premix label and application rates for use on DAS-68416-4 have not yet been submitted to EPA. However, the premix application directions will be the same as those for DAS-40278-9 corn, since the DAS 2,4-D choline salt and glyphosate premix will be made to use on either DAS-68416-4 and DAS-40278-9 corn (DAS, 2012). APHIS assumes, for the purposes of this analysis, that if and when the new premix becomes available, that the mixture of herbicides will be used by growers consistent with the EPA label application rate. Similar to other EPA-registered herbicides, when used in accordance with the EPA label restrictions, this premix is anticipated to present a reasonable certainty of no harm to humans and no unreasonable adverse effects on environment. The potential impacts associated with the additional application of glyphosate as part of the DAS premix is evaluated as part of cumulative impacts in Section 5.0.

DAS plans to stack varieties of soybean where the DAS-68416-4 soybean is combined using traditional hybridization techniques with other herbicide-tolerant soybean varieties (DAS, 2010). The range of potential stacked varieties could include stacked hybrids incorporating glyphosate tolerance, insect resistance, or other traits. APHIS does not have jurisdiction under the PPA and Part 340 to review such hybrids expressing stacked traits from nonregulated articles developed using conventional hybridization techniques where there is no evidence of a plant pest risk. APHIS considers the future development of these stacked hybrids a speculative event, and, accordingly, evaluates these stacked varieties only in the cumulative impacts analyses where appropriate. DAS has indicated its intention to develop a stacked hybrid through conventional breeding techniques combining the 2,4-D tolerance from DAS-68416-4 soybean with glyphosate tolerance from another nonregulated soybean variety (DAS, 2010, 2011a). Issues associated with potential future stacking in which glyphosate tolerance is incorporated with the DAS-68416-4 soybean are presented and discussed in the cumulative impacts analyses where appropriate.

4.2 Agricultural Production of Soybean

Best management practices are commonly accepted, practical ways to grow soybean, regardless of whether the soybean farmer is using organic practices or conventional practices with non-GE or GE varieties. These management practices consider crop-specific planting dates, seeding rates, and harvest times, among others. Over the years, soybean production has resulted in well-established management practices that are available through local Cooperative Extension Service offices and their respective websites. The National Information System for the Regional Integrated Pest Management (IPM) Centers publishes crop profiles for major crops on a state-by-state basis. These crop profiles provide production guidance for local growers, including recommended practices for specific pest control. Crop profiles for many of the soybean production states can be reviewed at www.ipmcenters.org/cropprofiles/index.cfm.

4.2.1 Acreage and Area of Soybean Production

4.2.1.1 No Action Alternative: Acreage and Area of Soybean Production

Under the No Action Alternative, DAS-68416-4 soybean would remain regulated and would not be commercially available for production. Soybeans will continue to be a major crop in the U.S. for the foreseeable future (USDA-OCE, 2011). Existing trends related to the cultivation and proportion of crop acreage planted with soybean in the U.S. are expected to continue. The majority of soybeans grown in the U.S. utilize GE technology (94% of all planted soybean acreage) with some form of herbicide tolerance (Fernandez-Cornejo and Caswell, 2006; USDA-

ERS, 2011b); this trend is likely to remain unchanged under the No Action Alternative. Therefore, the number of states and areas of the U.S. involved in soybean cultivation is not expected to change.

4.2.1.2 Preferred Alternative: Acreage and Area of Soybean Production

DAS conducted field trials with DAS-68416-4 soybeans and a non-transgenic soybean control variety (Maverick) at 6 locations in the U.S. and Canada in 2008 and at 21 locations in the U.S in 2009 (DAS, 2010; USDA-APHIS, 2012). The results of the field trials demonstrated that there was no substantial agronomic or phenotypic differences between DAS-68416-4 soybean and its comparator control variety, including no statistically significant difference in stand count, emergence, seedling vigor, days to flower, lodging, disease incidence, insect damage and yield from the control lines (DAS, 2010; USDA-APHIS, 2012). Although DAS-68416-4 soybean might be expected to replace other varieties of herbicide-tolerant soybean currently grown, because DAS-68416-4 confers no special agronomic benefit compared to other soybean varieties, no change in soybean production area or in the total amount of soybean acrage in the U.S is expected to result from a determination of nonregulated status of DAS-68416-4 soybean. Potential impacts to the acreage and area of soybean production under the Preferred Alternative would be be the same as for the No Action Alternative.

4.2.2 Agronomic Practices: Crop Rotation, Tillage, and Agronomic Inputs

As discussed in Subsection 2.1.2, soybean cultivation requires significant management considerations regarding tillage, rotation strategy, agricultural inputs, and pesticide inputs. Decisions concerning soybean agronomic practice are dependent on grower want and need, and ultimately reflective of external factors including geography, weed and disease pressure, economics of management of yield, and production system (rotation) flexibility (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008).

4.2.2.1 No Action Alternative: Agronomic Practices

Under the No Action Alternative, DAS-68416-4 soybean would continue to be subject to the regulatory requirements of 7 CFR part 340 and plant pest provisions of the Plant Protection Act. Growers will continue to have access to existing nonregulated GE soybean varieties, as well as conventional corn varieties. Current soybean management practices would be expected to continue under the No Action Alternative. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008).

Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008). Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-D, and glufosinate, for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011h).

4.2.2.2 Preferred Alternative: Agronomic Practices

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides. DAS' studies demonstrate DAS-68416-4 soybean is essentially indistinguishable from other soybean varieties in terms of agronomic characteristics and cultivation practices (DAS, 2010; USDA-APHIS, 2012). As DAS-68416-4 soybean is essentially equivalent to other GE herbicide-tolerant and non-GE soybeans, no changes in agronomic practices (such as crop rotation), cultivation, geographic range, seasonality or insect susceptibility, are expected to occur.

DAS' Enlist™ Weed Control System includes the new Colex-D™ Technology which includes the newly developed 2,4-D choline salt formulation. According to DAS, the new 2,4-D choline is characterized by low volatility, as observed in laboratory and field trials conducted by DAS (DAS, 2011c). In addition, the new formulation is reported to have minimized potential for physical drift in comparison to the currently used 2,4-D ester and 2,4-D dimethylamine (DMA) formulations, as well as decreased odor and improved handling (DAS, 2011a, 2011c).

The proposed use pattern of the new formulation of 2,4-D on DAS-68416-4 soybean would allow application of 2,4-D at pre-plant/burndown or pre-emergence (1.0 lb ae/A) without plant back restrictions and/or one or two over-the-top post-emergence applications (0.5 - 1.0 lb ae/A) at least 10 days apart up to the R2 stage (full flower) of development (see Figure 11) (DAS, 2010). Thus, the proposed maximum total seasonal application rate of 2,4-D on DAS-68416-4 soybean would increase from 1.0 lb ae/A (current) to 3.0 lb ae/A per year (the same current EPA-approved maximum annual use rate of 2,4-D for popcorn and field corn). Post-emergence application of 2,4-D, as specified on the draft label, could not occur within a preharvest interval of 30 days (DAS, 2011a, 2011b). The new use pattern and draft label are subject to regulatory approval by EPA. Table 18 presents a comparison of the current and proposed application rates of 2,4-D on soybean (DAS, 2010).

Glufosinate is registered by EPA for use on LibertyLink® (i.e., glufosinate-tolerant) soybeans at an intial application rate no higher than 0.66 lb ai/A (36 fl oz/A) and a single second application up to 0.53 lb ai/A (29 fl oz/A). Glufosinate applications on LibertyLink® soybean should be made from emergence up to but not including the bloom growth stage and within 70 days of harvesting. A seasonal glufosinate maximum rate of 1.2 lb ai/A (65 fl oz/A of Ignite/Liberty herbicide) is the approved use pattern on LibertyLink® soybeans (Bayer CropScience, 2011). The glufosinate application rate for use on DAS-68416-4 soybean will be consistent with the current use pattern of glufosinate on other glufosinate-tolerant soybean (DAS, 2010).

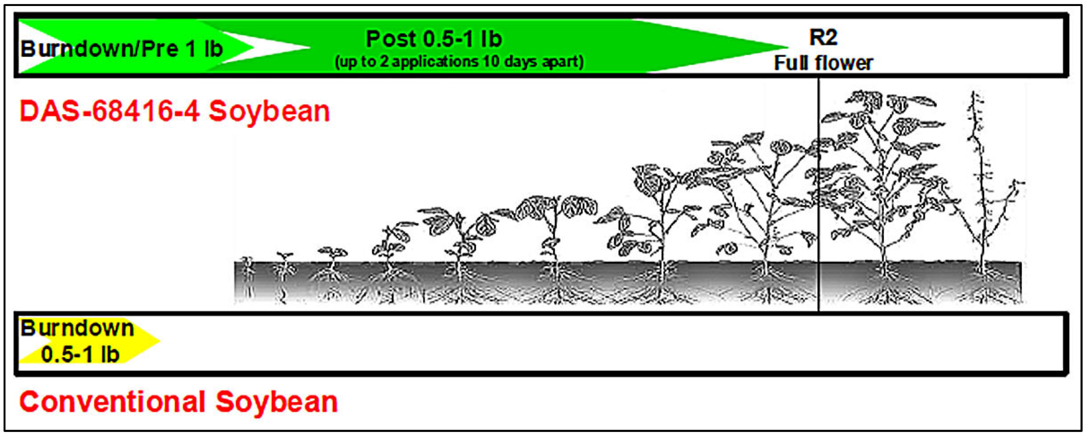


Figure 11. Proposed 2,4-D application rates on DAS-68416-4 soybean compared to current application rates permitted for conventional soybean.

Source: (DAS, 2010).

An increase in diversity of weed control tactics is necessary to mitigate selection pressures for more glyphosate-resistant broadleaf weeds (Powles, 2008). The practice of using herbicides with alternative modes of action is expected to potentially diminish the populations of glyphosate-resistant and hard to control weeds (Dill et al., 2008; Duke and Powles, 2008, 2009; Owen, 2008). Also, applications of herbicides with mixed modes of action also are expected to prolong the development of new herbicide-resistant weed populations (Duke and Powles, 2009; Owen, 2008). The 2,4-D and glufosinate tolerance in DAS-68416-4 soybean could provide growers with additional options to manage glyphosate-resistant weeds and could reduce applications of other herbicides needed to manage glyphosate-resistant weeds.

DAS has indicated that their stewardship program will include the technological advancements in application and off-target movement of 2,4-D choline salt, as discussed above (DAS, 2010). Additionally, DAS intends to incorporate grower education and training on these management strategies and protocols as part of its product stewardship program (DAS, 2011d). The program will include the following components:

- Colex-D Technology;
- Comprehensive product use guidelines;
- Authorized use through grower agreements;
- Education and training;
- Labeled for ground application only on crops with the Enlist Weed Control System; and
- Compliance monitoring and reporting

Adherence to the stewardship program is expected to minimize the development of weeds with expressing resistance to multiple herbicides (DAS, 2011d; Wright et al., 2011)Although farmers may have to change their management strategies to adopt varieties with the DAS-68416-4 soybean traits, these changes will not necessitate a major departure from well-established and broadly used agricultural protocols currently in use under the No Action Alternative.

Table 18. Comparison of current and proposed application rates of 2,4-D on soybean.

Crop Stage	Current Use Pattern - Conventional Soybean		Proposed New Use Pattern – DAS-68416-4 Soybean	
	Maximum Application Rate (lb/acre)[1]	Directions and Timing	Maximum Application Rate (lb/acre)[1]	Directions and Timing
Pre-plant (burndown) or Pre-emergence	0.5 -1.0	**Pre-plant:** Apply before soybean emerges to control emerged broadleaf weed seedlings or existing cover crops	1.0	**Pre-plant:** Apply any time prior to and up through soybean planting but before soybean emerges to control emerged broadleaf weed seedlings or existing cover crops. **Pre-emergence:** Apply anytime after planting but before soybean emerges to control broadleaf weed seedlings or existing cover crops.
Post-emergence	--[2]	--	0.5-1.0	Apply when weeds are small and soybean growth stage is no later than R2 (full flowering stage). Make one to two applications with a minimum of 12 days between applications.
Total Annual Maximum Application	1.0	--	3.0	--

Source: DAS (2011b).
[1]All values expressed as acid equivalents
[2]Not applicable

The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment. While 2,4-D is already used for burndown application prior to planting soybean, a determination of nonregulated status of DAS-68416-4 soybean, with the associated new application rates and timing of 2,4-D on soybean, has the potential to result in an increase in the amount of 2,4-D that may be used and the time of year that it may be applied on soybeans. The 2,4-D applied may likely replace other hebicides currently used on soybean, and, as a result, the overall amount of herbicides used on soybeans may not change. 2,4-D is approved by the EPA for use on other major agricultural crops at rates greater than those proposed for DAS-68416-4 soybean. The proposed maximum 2,4-D application rate for soybean is the same as that currently approved for use on field corn and popcorn (US-EPA, 2005c) which is typically grown in the same areas as soybeans and often in the same fields in rotation with soybean. Similar to the current use of 2,4-D under the No Action Alternative, the new application rate and timing of 2,4-D on soybean would be used in accordance with the per application and per year rates approved by EPA.

Also, under this alternative, depending on the extent of adoption of DAS-68416-4 soybean, glufosinate use on soybean could increase in comparison to use under the No Action Alternative. However, as with 2,4-D, glufosinate use may likely replace other herbicides currently being used on soybean; thus, the overall amount of herbicides being applied to soybeans may not change. Similar to the current use of glufosinate under the No Action Alternative, the use of glufosinate on soybean would be in accordance with the per application and per year rates approved by EPA.

4.2.3 Soybean Seed Production

As discussed in Subsection 2.1.3, soybean seed production is conducted under standard procedures specified by AOSCA to prevent gene flow between varieties (AOSCA, No Date). Several best management practices to preserve varietal identity include:

- Maintaining isolation intervals to prevent pollen movement from other soybean sources;
- Planting border rows to capture any pollen present or employing natural pollen barriers; and
- Field monitoring for off types, other crops, etc.

Soybean is considered to be highly self-pollinated; therefore, cross-pollination to adjacent soybean plants occurs at a very low frequency (Abud et al., 2007; Caviness, 1966; OECD, 2000; Ray et al., 2003). Other research has also demonstrated that pollen dispersal is restricted to small areas and wind mediated pollination is negligible (Yoshimura, 2011).

4.2.3.1 No Action Alternative: Soybean Seed Production

Under the No Action Alternative, current soybean seed production practices are not expected to change. It is expected that soybean seed producers would continue to implement measures to preserve the identity of their seed varieties.

4.2.3.2 Preferred Alternative: Soybean Seed Production

Field trials conducted by DAS have not demonstrated any agronomic or phenotypic differences between DAS-68416-4 soybean and conventional soybean varieties (DAS, 2010). Based on the data provided, as well as previous experience with other GE herbicide-tolerant soybean varieties that have been widely adopted by growers since their commercial availability in 1996, APHIS has concluded that the availability of DAS-68416-4 soybean under the Preferred Alternative would not alter the agronomic practices, cultivation locations, seed production practices or quality characteristics of conventional and non-GE soybean seed production (USDA-APHIS, 2012). A determination of nonregulated status of DAS-68416-4 soybean would not require change to seed production practices. The potential impacts to soybean seed production associated with the Preferred Alternative would not be any different than practices under the No Action Alternative.

4.2.4 Organic Soybean Production

Organic production plans prepared pursuant to the NOP include practical methods to prevent the unintended presence of GE materials. Typically, organic growers use multiple methods to prevent unwanted material from entering their fields, many of them following the same system utilized for the cultivation of certified seed under the AOSCA procedures. These include planting organic seed only, planting at times earlier or later than neighbors, and using field isolation practices (NCAT, 2003).

APHIS recognizes that producers of non-GE soybean, particularly producers who sell their products to markets sensitive to GE traits (e.g., organic or some export markets), can be reasonably assumed to use practices on their farms that protect their crop from unwanted substances. APHIS's baseline for analysis of the alternatives will therefore assume that growers of organic soybean are already using, or have the ability to use, these common practices.

4.2.4.1 No Action Alternative: Organic Soybean Production

Under the No Action Alternative, DAS-68416-4 soybean would remain subject to the regulatory requirements of 7 CFR part 340 and the plant pest provisions of the Plant Protection Act. GE, non-GE and organic soybean seed availability would not change as a result of the continued regulation of DAS-68416-4 soybean. Organic soybean farmers would continue to utilize the same methods as applied in certified seed production systems designed to maintain soybean seed identity and meet National Organic Standards as established by the NOP. Acreage devoted to organic soybean production is small relative to that of GE varieties and has remained relatively steady, only fluctuating between 122,000 to 126,000 acres between 2005 and 2008 (USDA-ERS, 2010d, 2011b). As described in Subsection 2.1.4, Organic Soybean Production, organic soybean production is a very small portion of the soybean market which would not be expected to change under the No Action Alternative. Also, agronomic practices employed to produce organic soybean would remain unaffected by selection of the No Action Alternative.

It is important to note that the current NOP regulations do not specify an acceptable threshold level for the adventitious presence of GE materials in an organic-labeled product. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan (Ronald and Fouche, 2006; USDA-AMS, 2008). However, certain markets or contracts may have defined thresholds (Non-GMO Project, 2010).

4.2.4.2 Preferred Alternative: Organic Soybean Production

GE herbicide-tolerant soybean lines are already extensively used by farmers, while organic soybean production represents a small percentage (less than 1.0%) of the total U.S. soybean acreage (USDA-ERS, 2010b). Similar to the No Action Alternative, organic soybean acreage is likely to remain small, regardless of whether new varieties of GE or non-GE corn varieties, including DAS-68416-4 soybean, become available for commercial soybean production.

When compared to other GE varieties of soybean, DAS-68416-4 soybean should not present any new and different issues and impacts for organic and other specialty soybean producers and consumers. Organic producers employ a variety of measures to manage identity and preserve the integrity of organic production systems (NCAT, 2003). Agronomic tests conducted by DAS found DAS-68416-4 soybean substantially equivalent to the non-GE control variety; hence, pollination characteristics would be similar to other soybean varieties currently available to growers (DAS, 2010). Given the largely self-fertilized nature and the limited pollen movement of soybean (Abud et al., 2007; Caviness, 1966; OECD, 2000; Ray et al., 2003; Yoshimura, 2011), it is not likely that organic farmers will be substantially affected by a determination of nonregulated satatus of DAS-68416-soybean when organic soybeans are produced in accordance with agronomic practices designed to meet National Organic Standards.

The trend in the cultivation of GE soybean, non-GE, and organic soybean varieties, and the corresponding production systems to maintain varietal integrity are likely to remain the same as the No Action Alternative. Accordingly, impacts of a determination of nonregulated status of DAS-68416-4 soybean on organic soybean production would be similar to the No Action Alternative.

4.3 Physical Environment

4.3.1 Soil Quality

4.3.1.1 No Action Alternative: Soil Quality

Under the No Action Alternative, current soybean management practices would be expected to continue. Agronomic practices that benefit soil quality, such as contouring, use of cover crops to limit the time soil is exposed to wind and rain and introduce certain soil nutrients, crop rotation, and windbreaks would not change as a result of the continued regulated status of DAS-68416-4 soybean. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Some of these adjustments may have the potential to impact soil quality. Residue management that employs intensive tillage and leaves low amounts of crop residue on the surface results in greater losses of SOM (USDA-NRCS, 1996).

Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008). Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-D, and glufosinate for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011l).

The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

4.3.1.2 Preferred Alternative: Soil Quality

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS' studies demonstrate DAS-68416-4 soybean is essentially indistinguishable from other soybean varieties in terms of agronomic characteristics and cultivation practices (DAS, 2010; USDA-APHIS, 2012). DAS-68416-4 soybean is essentially equivalent to other GE herbicide-tolerant and non-GE soybeans, no changes in agronomic practices (such as crop rotation), cultivation, geographic range, seasonality or insect susceptibility, are expected to occur. Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues. This in turn could reduce the potential loss of SOM and soil erosion that may result when more aggressive tillage practices are used to combat herbicide restistant weeds. Becasue DAS-68416-4 soybean is agronomically and nutritionally similar to conventional soybean, cultivation of DAS-68416-4 soybean is not expected to impact soil differently than other currently available herbicide-tolerant soybean varieties.

DAS-68416-4 soybean and the associated production of the AAD-12 and PAT proteins are not expected to cause an impact to the physicochemical characteristics of the soil. The *aad-12* gene which has been introduced into DAS-68416-4 soybean is derived from *Delftia acidovorans*, a gram negative soil bacterium (DAS, 2010). The gene is present in nature and can be found in soil, fresh water, activated sludge, and clinical specimens (DAS, 2010; Tamaoka et al., 1987; Von Gravenitz, 1985; Wen et al., 1999). The *pat* gene expressing the PAT protein in DAS-68416-4 soybean was derived from *Streptomyces viridochromogenes*, a gram-positive soil bacterium. Similar to the No Action Alternative, the *pat* gene produced in DAS-68416-4 soybean is equivalent to that produced in other transgenic crops that are no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act (e.g., USDA 1996, USDA 2001, USDA 2004, USDA 2005).

Adoption of DAS-68416-4 soybean by soybean producers and the potential additional new use of 2,4-D on DAS-68416-4 soybean (pending EPA approval) has the potential to result in an increase in the annual amount of 2,4-D that may be used and the time of year that it may be applied on soybeans. 2,4-D is approved by the EPA for use on other major agricultural crops at rates greater than those proposed for DAS-68416-4 soybean. The proposed maximum 2,4-D application rate for soybean is the same as that currently approved for use on field and popcorn which is typically grown in the same areas as soybeans and often in the same fields in rotation with soybean. Similar to the current use of 2,4-D under the No Action Alternative, the new application rate and timing of 2,4-D on soybean would be used in accordance with the per application and per year rates approved by EPA. When used in accordance with the EPA label, 2,4-D accumulation in soil has not been shown to be significant. Soil half-life (the time it takes for half the amount of substance to degrade to other substances) estimates for 2,4-D range from

1.1 to 30.5 days, with an average half-life of 10 days (Senseman, 2007; US-EPA, 2005c). The degradation products of 2,4-D are 1,2,4-benzenetriol, 2,4-dichlorophenol (2,4-DCP), 2,4-dichloroanisole (2,4-DCA), 4-chlorophenol, chlorohydroquinone (CHQ), volatile organics, bound residues, and carbon dioxide (US-EPA, 2005c). The EPA has determined that residues other than 2,4-D are not of risk concern due to low occurrence under environmental conditions, comparatively low toxicity, or a combination thereof (US-EPA, 2005c).

As DAS-68416-4 soybean also would have tolerance for glufosinate, the use of this pesticide on soybean could also increase. Similar to the current use of glufosinate under the No Action Alternative, the use of glufosinate on soybean would be in accordance with the per application and per year rates approved by EPA. Glufosinate is weakly adsorbed to and is highly mobile in soil, undergoes rapid microbial degradation in soil, and has a short soil residual half-life of seven days (Senseman, 2007).

Based on this information, overall impacts to soil under the Preferred Alternative are expected to be similar to the No Action Alternative.

4.3.2 Water Resources

4.3.2.1 No Action Alternative: Water Resources

Under the No Action Alternative, current soybean management practices, including irrigation, pesticide use and fertilizer application would be expected to continue. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Some of these adjustments may have the potential to impact surface water quality through increased sedimentation and agricultural chemical loading derived from exposed soils (Owen, 2011; Towery and Werblow, 2010).

Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008). Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-D, and glufosinate for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011l). The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registred pesticide continues to meet the highest standards of safety to protect human health and the environment.

4.3.2.2 Preferred Alternative: Water Resources

No differences in morphological characteristics and agronomic requirements were found between DAS-68416-4 soybean and the hybrid control (DAS, 2010; USDA-APHIS, 2012). Therfore, cultivation of DAS-68416-4 soybean would not necessitate changes in current agronomic practices for soybean production. Also, as previously discussed, the use of DAS-68416-4 soybean would not increase the total acres and range of U.S. soybean production areas. For these reasons, a determination of nonregulated status of DAS-68416-4 soybean is unlikely to change the current irrigation practices in commercial soybean production. Because DAS-68416-4 soybean is expected to simply replace GE soybean varieties already in use, the effects of the Preferred Action Alternative on water use would be the same as the No Action Alternative.

Adoption of DAS-68416-4 soybean by soybean producers and the potential additional new use of 2,4-D on DAS-68416-4 soybean have the potential to result in an increase in the annual amount of 2,4-D that may be used and the time of year that it may be applied on soybeans. 2,4-D is approved by the EPA for use on other major agricultural crops at rates greater than those proposed for DAS-68416-4 soybean. The proposed maximum 2,4-D application rate for soybean is the same as that currently approved for use on field and popcorn which is typically grown in the same areas as soybeans and often in the same fields in rotation with soybean. Similar to the current use of 2,4-D under the No Action Alternative, the new application rate and timing of 2,4-D on soybean would be used in accordance with the per application and per year rates approved by EPA. When mixed with water, the new DAS 2,4-D choline salt formulation readily dissociates into 2,4-D and choline cation (75 FR 169, 760). Choline is a water-soluble, naturally-occuring nutrient with low toxicity. Upon evaluation of all available information and a detemination of reasonable certainty of no harm to any human population, EPA has approved an inert tolerance exemption for residues of choline hydroxide under 40 CFR 180.920 when used as an inert ingredient in pesticide formulations (75 FR 760).

2,4-D is currently approved by EPA for aquatic applications to control aquatic weeds in food use areas (i.e., rice and fish farms) as well as industrial areas (i.e., drainage systems) (US-EPA, 2005c). When used for aquatic treatments (direct application to water for aquatic vegetation control), 2,4-D has a half-life of between 3.2 days and 27.8 days (US-EPA, 2005c): the half-life of 2,4-D in aerobic aquatic environments is approximately 45 days and the half-life of 2,4-D esters in normal agricultural soil and natural waters is less than 3 days (US-EPA, 2005c, 2009e). The EPA has stated that the 2,4-D acid and amine salts are practically non-toxic to freshwater or marine fish (US-EPA, 2005c).

Similar to the No Action Alternative, the potential for applications of 2,4-D to impact groundwater is considered low. 2,4-D rapidly degrades in soil, effectively minimizing leaching to groundwater; however, heavy irrigation after application in sandy soils can increase leaching potential (Senseman, 2007). EPA label restrictions also are intended to minimize groundwater contamination by 2,4-D and its metabolites (US-EPA, 2005c).

As DAS-68416-4 soybean also would have tolerance for glufosinate, the use of this herbicide on soybean could also increase; however, no changes to the currently authorized use of glufosinate on other glufosinate-tolerant soybean are proposed. Similar to the current use of glufosinate under the No Action Alternative, the use of glufosinate on soybean would be in accordance with the per application and per year rates approved by EPA. Glufosinate is highly water soluble and stable in water. The chemical is considered to be essentially nonvolatile from soil and surface water. Adsorption to suspended solids and sediment has been observed to be low to high (HSDB, 2010; US-EPA, 2000a). Biodegradation occurs in anaerobic water bodies with a half life greater than 64 days (US-EPA, 2000a). Surface water may be impacted by glufosinate residues transported by runoff, but EPA label restrictions require actions be taken to minimize impacts, such as not applying the herbicide when rainfall is forecasted to occur within 48 hours (US-EPA, 2007b). Glufosinate has not been found to be a source of impairment for any water body designated as impaired under section 303(d) of the Clean Water Act (US-EPA, 2008b). Glufosinate may leach to groundwater under certain conditions (such as soils with high permeability and shallow groundwater), but generally, because it degrades, it is rarely found deeper than 15 centimeters (approximately 6 inches) from the soil surface (Senseman, 2007), minimizing its potential to enter groundwater. Glufosinate ammonium does not bioaccumulate in fish and has low potential for bioconcentration in aquatic organisms (US-EPA, 2008b).

Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues. This in turn this could reduce the need for more aggressive tillage practices that may lead to increased potential for sedimentation and chemical loading of nearby waters through soil erosion.

Based on the above information, the impacts to water resources from a determination of nonregulated status of DAS-68416-4 soybean are expected to be similar to the No Action Alternative.

4.3.3 Air Quality

4.3.3.1 No Action: Air Quality

Under the No Action Alternative, air quality would continue to be affected by current agronomic practices associated with soybean production, such as tillage, cultivation, pesticide and fertilizer applications, and the use of agricultural equipment. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Some of these adjustments may have potential to impact air quality by increased emissions from tillage equipment and windborne dust from exposed soils.

Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008). Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-

D, and glufosinate for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011l). The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for a each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

4.3.3.2 Preferred Alternative: Air Quality

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS' studies demonstrate DAS-68416-4 soybean is essentially indistinguishable from other soybean varieties in terms of agronomic characteristics and cultivation practices (DAS, 2010; USDA-APHIS, 2012). As DAS-68416-4 soybean is essentially equivalent to other GE herbicide-tolerant and non-GE soybeans, no changes in agronomic practices (such as crop rotation), cultivation, geographic range, seasonality or insect susceptibility, are expected to occur. Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues. This in turn this could reduce the need for more aggressive tillage practices that may impact air quality by increased emissions from tillage equipment and windborne dust from exposed soils.

Adoption of DAS-68416-4 soybean by soybean producers and the potential additional new use of 2,4-D on DAS-68416-4 soybean (pending EPA approval) has the potential to result in an increase in the annual amount of 2,4-D that may be used and the time of year that it may be applied on soybeans. 2,4-D is approved by the EPA for use on other major agricultural crops at rates greater than those proposed for DAS-68416-4 soybean. The proposed maximum 2,4-D application rate for soybean is the same as that currently approved for use on field corn and popcorn which is typically grown in the same areas as soybeans and often in the same fields in rotation with soybean. Similar to the current use of 2,4-D under the No Action Alternative, the new application rate and timing of 2,4-D on soybean would be used in accordance with the per application and per year rates approved by. As DAS-68416-4 soybean also would have tolerance for glufosinate, the use of this pesticide on soybean could also increase. Similar to the current use of glufosinate under the No Action Alternative, the use of glufosinate on soybean would be in accordance with the per application and per year rates approved by EPA. The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continuesto meet the highest standards of safety to protect human health and the environment.

Spray drift or volatilization of herbicides and subsequent off-site movement is an air quality concern with direct potential impacts to non-target plants (CBD, 2010; US-EPA, 2005d; Vogel et al., 2008). Herbicides that are sufficiently volatile (i.e., easily change from a solid or liquid to a gas or vapor) may be transported from the target site (i.e., point of application in a crop field) and affect nearby sensitive plants. Amine formulations of 2,4-D are considered nonvolatile, while ester formulations of 2,4-D are more volatile, although, low-volatile (long-chain) 2,4-D esters formulations are now also available. DAS' newly developed 2,4-D choline salt formulation to be used with DAS-68416-4 soybean is characterized, according to DAS, by low volatility compared against other 2,4-D formulations, as observed in laboratory (humidome and volatility chamber) and field tests conducted by DAS (DAS, 2011a, 2011c). Therefore, use of this 2,4-D choline formulation with DAS-68416-4 soybean is not expected to to impact air quality by vapor drift. Glufosinate has low volatility and a short soil residual life (average half-life of 7 days) (Senseman, 2007); thus, it is not considered an atmospheric contaminant with any potential impacts to air quality.

Pesticides are typically applied to crops by ground spray equipment or aircraft. Small, light weight droplets are produced by equipment nozzles, with many droplets small enough that they remain suspended in air and are moved by air currents until they touch a surface or drop to the ground. Weather conditions, topography, the crop or area being sprayed, application equipment and methods, and applicator decisions influence the amount of drift. Spray drift is a concern with all herbicides applied in a liquid form (CBD, 2010; Vogel et al., 2008). The use of 2,4-D, particularly the ester formulation applied in liquid form, has raised a concern for potential off-site impacts to terrestrial plants adjacent to treated fields (CBD, 2010; US-EPA, 2005d). The EPA has addressed these concerns regarding spray drift through the EPA label restriction requirement of spray drift controls when 2,4-D is applied (US-EPA, 2005d). The EPA provides several spray drift risk management procedures, including stipulations on droplet size for liquid sprays, wind speed, ambient temperature, proximity to sensitive plants, and buffer zones of unsprayed or untreated crop (US-EPA, 2005d). The EPA notes that if applied in accordance with these EPA label restrictions, spray drift impacts can be avoided (US-EPA, 2005d). Additionally, the new 2,4-D choline salt formulation is reported to have minimized potential for physical drift in comparison to the currently used 2,4-D ester and 2,4-D dimethylamine (DMA) formulations, as well as decreased odor and improved handling (DAS, 2011a, 2011c). According to the application direction on the new DAS draft label for new 2,4-D formulation (GF 2654 TS), the product may not be applied aerially (DAS, 2011b). The potential impacts to non-target plants from spray drift are discussed below in Subsection 4.4.2, Plant Communities, and Section 6.0, Threatened and Endangered Species.

Based on the above information, the impacts to air quality from a determination of nonregulated status of DAS-68416-4 soybean are expected to be similar to the No Action Alternative.

4.3.4 <u>Climate Change</u>

4.3.4.1 No Action Alternative: Climate Change

Under the No Action Alternative, agronomic practices associated with soybean production which contribute to GHG emissions, including tillage, cultivation, irrigation, pesticide application, fertilizer applications, and use of agriculture equipment are expected to continue. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicide use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Some of these adjustments may have the potential to impact air quality improvements that have been gained with the adoption of conservation tillage practices. As a result, under the No Action Alternative, there may be a slight increase in GHG emissions.

4.3.4.2 Preferred Alternative: Climate Change

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS' studies demonstrate DAS-68416-4 soybean is essentially indistinguishable from other soybean varieties in terms of agronomic characteristics and cultivation practices (DAS, 2010; USDA-APHIS, 2012). As DAS-68416-4 soybean is essentially equivalent to other GE herbicide-tolerant and non-GE soybeans, no changes in agronomic practices (such as crop rotation), cultivation, geographic range, seasonality or insect susceptibility, are expected to occur. Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues. This in turn this could reduce the need for more aggressive tillage practices that may impact conservation tillage practices. The continued use of conservation tillage associated with GE crops may reduce GHG emissions as a result of increased carbon sequestration in soils, decreased fuel consumption, and the reduction of nitrogen soil amendments (Towery and Werblow, 2010).

Based on the above information, the availability of DAS-68416-4 soybean is not expected to change the cultivation or agronomic practices or agricultural land acreage associated with growing soybean. It may provide some benefit to reducing GHG contributions to climate change in the form of sustaining the adoption of conservation tillage practices, but overall the impacts to climate change is expected to be similar to the No Action Alternative.

4.4 Biological Resources

4.4.1 <u>Animal Communities</u>

4.4.1.1 No Action Alternative: Animal Communities

Under the No Action Alternative, terrestrial (insect, bird, and mammal) and aquatic (fish, benthic invertebrate, and herptile) species would continue to be affected by current agronomic practices associated with conventional methods of soybean production, such as tillage, cultivation, pesticide and fertilizer applications, and the use of agricultural equipment. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Some of these adjustments may have potential to impact the adoption of conservation tillage practices. If tillage rates were to increase as a means of weed suppression, it could possibly diminish the benefits to wildlife provided by conservation tillage practices.

Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008). Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-D, and glufosinate for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011l). The environmental risks of pesticide use on wildlife and wildlife habitat are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

4.4.1.2 Preferred Alternative: Animal Communities

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS' studies demonstrate DAS-68416-4 soybean is essentially indistinguishable from other soybean varieties in terms of agronomic characteristics and cultivation practices (DAS, 2010; USDA-APHIS, 2012). As DAS-68416-4 soybean is essentially equivalent to other GE herbicide-tolerant and non-GE soybeans, no changes in agronomic practices (such as crop rotation), cultivation, geographic range, seasonality or insect susceptibility, are expected to occur. Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues. This in turn this could reduce the need for more aggressive tillage practices that may impact conservation tillage practices. The continued use of conservation tillage would continue to provide benefits to various animal communities (Wilson et al., 2011).

AAD-12 and PAT Proteins

DAS has evaluated the potential allergenicity and toxicity of the AAD-12 protein following the weight-of-evidence approach (DAS, 2010). The AAD-12 protein does not share any meaningful amino acid similarities with known allergens. The AAD-12 protein is degraded rapidly and completely in simulated gastric fluids and the protein is not present in a glycosylated state (DAS, 2010). The protein does not share any amino acid sequence similarities with known toxins (DAS, 2010). The results presented by DAS suggest that the AAD-1 protein is unlikely to be a toxin in animal diets. Based on a review of this information and the assumption that these studies serve as surrogates for direct testing, APHIS has found no evidence that the presence of the *aad-12* gene or the expression of the AAD-12 protein would have any impact on animals, including animals beneficial to agriculture (USDA-APHIS, 2012). FDA completed its early food safety evaluation of the AAD-12 protein in DAS-68416-4 soybean on May 19, 2010. The FDA had no further questions concerning DAS' conclusion that "the AAD-12 protein would not raise food safety concerns when it is in a new food plant variety that is present at low levels in the food (US-FDA, 2010)."

The PAT protein present in DAS-68416-4 soybean is equivalent to that produced in other GE crops that are no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act (USDA, 1996, 2001, 2004, 2005). The food and feed safety of the protein was reviewed as part of these previous assessments and was shown to present no significant food or feed safety risk. A biotechnology consultation on the PAT protein was conducted in 1998 and does not require additional evaluation by the FDA (US-FDA, 1998a, 1998b). Additionally, the FDA evaluated the safety and nutritional characteristics of food and feed derived from DAS-68416-4 soybean and responded on November 14, 2011, having no questions concerning DAS's findings that "food and feed derived from DAS-68416-4 soybean are not materially different in composition, safety, and other relevant parameters from soybean-derived food and feed currently on the market, and that the genetically engineered DAS-68416-4 soybean does not raise issues that would require premarket review or approval by FDA (US-FDA, 2011a)."

Based on the above information, there are no expected hazards associated with its consumption of DAS-68416-4 soybean and therefore it is unlikely to pose a hazard to wildlife species. Further discussion on the potential impacts from the consumption of DAS-68416-4 soybean is presented in Subsection 4.6, Animal Feed.

2,4-D and Glufosinate

A determination of nonregulated status of DAS-68416-4 soybean could result in a new post-emergence use of 2,4-D on soybean or additional use of glufosinate on this crop, and may potentially replace other herbicides currently used for weed control in soybean acreage. Pesticide use changes, such as the new post-emergence use of 2,4-D on DAS-68416-4 soybean are regulated by the EPA through the FIFRA labeling process. The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment. Label application rates and use restrictions for both herbicides are assessed by the EPA for a reasonable certainty of no harm to humans and no unreasonable adverse effects on the environment. APHIS assumes that both herbicides will be used in accordance with these EPA label restrictions.

Currently, 2,4-D amine and ester formulations are registered by EPA for use on soybeans only for pre-plant burndown. Under the Preferred Alternative, if the new 2,4-D choline salt formulation is approved by EPA, 2,4-D could be used for pre-plant and pre- and post-emergence weed management in commercially grown DAS-68416-4 soybean, potentially increasing the amount of 2,4-D applied to fields with DAS-68416-4 soybean. Currently, 2,4-D can be applied for burndown prior to soybean planting up to 1.0 lbs ae/A per crop or year (US-EPA, 2005c). The proposed maximum application rate for 2,4-D for the DAS-68416-4 soybean is 3.0 lb ae/A per year; the same maximum application rate currently authorized by EPA for popcorn and field corn (US-EPA, 2005c). Since soybean is often grown in rotation with corn, the use of 2,4-D at the proposed maximum application rate would not be new on those fields where these crops are grown in rotation. The proposed maximum application rate of 2,4-D to DAS-68416-4 soybean is not greater than the level evaluated for ecological risks by EPA and found by the Agency not to be adverse. The new label submitted for 2,4-D choline salt limits the use of the herbicide to terrestrial use only, thereby, limiting the potential exposures to aquatic animal species and habitats to drift, deposition, and runoff of the herbicide, all of which would be well below exposures to terrestrial organisms. Thus, compared to the No Action Alternataive, no new impacts to animal communities from the new 2,4-D use would be expected.

DAS indicates the glufosinate application rate associated with DAS-68416-4 soybean will be the same as the current EPA label rates for glufosinate-tolerant soybean (seasonal maximum rate of 1.2 lb ai/A); however, the total amount of glufosinate used for soybean production throughout the U.S. could potentially increase as a result of adoption of DAS-68416-4 soybean under the Preferred Alternative. Currently, the maximum amount of glufosinate currently approved for application to other crops, such as orchard nuts and fruits, grapes, grasses grown for seed, and golf course turf, is 1.5 lb ai/A per application, a higher application rate than that approved for use on soybean (US-EPA, 2008b). Since current herbicide label application rates and the associated use restrictions are evaluated and approved by the EPA, it is expected that the use of glufosinate with DAS-68416-4 soybean in accordance with the EPA label restrictions will not result in new impacts to animal communities in comparison to the No Action Alternative.

To the extent that DAS-68416-4 soybean displaces other soybean varieties, the expression of herbicide tolerance could have an overall positive impact on animal communities. In those fields where DAS-68416-4 soybean is cultivated, growers would be expected to take advantage of the weed control offered by 2,4-D and glufosinate and incorporate these herbicides into a diverse weed management strategy. DAS-68416-4 soybean, and the associated use of 2,4-D and glufosinate, provides several potential advantages over conventional weed management programs, including increased flexibility to manage problem weeds, reduced use of soil-applied herbicides, potential reduced total use of herbicides, and continued use of conservation tillage. The associated adoption of conservation tillage and reduced use of soil-applied herbicides have the potential to positively impact animal communities in fields planted with DAS-68416-4 soybean (Eggert et al., 2004). Conservation tillage benefits wildlife and habitat value through increased water quality, availability of waste grain, retention of cover in fields, and increased populations of invertebrates (Brady, 2007; Sharpe, 2010).

Based on the above information, APHIS has determined that the impacts to the animal community from a determination of nonregulated status of DAS-68416-4 soybean are similar to those under the No Action Alternative.

4.4.2 Plant Communities

Plants communities within agroecosystems are generally less diverse than the plant communities that border crop fields. This lack of diversity is attributable to ecological selection that is imposed by crop production practices such as tillage and herbicide use (Owen, 2008). The plants communities that inhabit crop production fields are represented by plants (including weeds) that are able to adapt and thrive in an environment that is directed specifically to the production of crops, such as soybean. In crop production systems, the plant community is controlled using a number of tactics to maximize the production of food, fiber, and fuel (Green and Owen, 2011); however, herbicides are the most common and accepted tactic to manage plants communities within agroecosystems (Gianessi and Reigner, 2007). The landscape surrounding a soybean field may be bordered by other soybean (or any other crop) fields or may also be surrounded by woodland, rangelands, and/or pasture/grassland areas. These plant communities represent natural or managed plant buffers for the control of soil and wind erosion and also serve as habitats for a variety of transient and non-transient wildlife species. The potential impacts to off-site plant communities is discussed in Subsection 4.4.5, Biodiversity.

Weed control programs are important aspects of soybean cultivation. In this context, weeds are those plants which, when growing in the soybean field, compete with the soybean for space, water, nutrients, and sunlight, and may thus include native species (US-EPA, 2007c). The types of weeds in and around a soybean field will vary depending on the geographic region where the soybean is grown. Common weeds in soybean include grasses, broad-leaf weeds, and sedges (*Cyperus* spp.). Some of these have been discussed in Subsection 2.3.2, Plant Communities.

4.4.2.1 No Action Alternative: Plants Communities

Under the No Action Alternative, DAS-68416-4 soybean would remain under APHIS regulation. Soybean production would likely continue as it does today, with the majority of acres being planted with GE herbicide-tolerant soybean. Plant species that typically inhabit soybean production systems will be managed through the use of mechanical, cultural, and chemical control methods. Multiple herbicides, including glyphosate, glufosinate, and 2,4-D (for preplant burndown), will likely continue to be used for weed control in soybean fields.

Growers will continue to respond to the development of glyphosate- and other herbicide-resistant weeds by diversifying weed management strategies. This includes utilizing herbicides with a different modes of action, using tank mixes, increasing the frequency of glyphosate applications, and returning to tillage and other cultivation techniques to physically control these species when a specific herbicide proves to be ineffective (CAST, 2012; DAS, 2011f).

Runoff, spray drift, and volatilization of herbicides have the potential to impact non-target plant communities growing in proximity to fields in which herbicides are used. The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. In this process, where appropriate, steps to reduce pesticide drift and volatilization are included on a pesticide's label approved by EPA. EPA addressed spray drift concerns in the 2005 reregistration of 2,4-D by adding label language on required spray droplet size, wind speeds, ambient temperature, avoidance of certain sensitive plants, and specific equipment requirements regarding boom length and height above the canopy (US-EPA, 2005d). The EPA label for glufosinate identifies similar practices to manage spray drift (Bayer CropScience, 2011). By following equipment and management restrictions addressing drift detailed in EPA-approved herbicide labels, growers can control the potential impacts to non-target plants.

Volunteer soybeans are typically not a major problem in agroecosystems and regionally where volunteer soybean populations can develop, the volunteer plants are manageable and do not represent a serious weed threat (York et al., 2005). Glyphosate-tolerant volunteer soybean could continue to be controlled effectively by the application of currently available EPA registered herbicides (e.g., nicosulfuron, dicamba, clopyralid, etc.) and mechanical means (NDSU, 2011).

4.4.2.2 Preferred Alternative: Plants Communities

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. Field trials and laboratory analyses conducted by DAS showed no differences between DAS-68416-4 soybean and nontransgenic soybean in growth, reproduction, or interactions with pests and diseases (DAS, 2010). Similar to the No Action Alternative, weeds within fields of DAS-68416-4 soybean could be managed using mechanical, cultural, and chemical control. Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues.

A determination of nonregulated status of DAS-68416-4 soybean is not expected to affect plant communities due to toxicity or allergenicity of the inserted AAD-12 and PAT proteins. Both proteins are not derived from organisms that are known for pathogenic or toxic effects on plants; these traits themselves are effectively benign in the environment (DAS, 2010; USDA-APHIS, 2012).

A determination of nonregulated status of DAS-68416-4 soybean would provide soybean growers with the option to use 2,4-D post-emergence in addition to using glufosinate, which would provide different modes of action to control glyphosate-resistant weeds. Pesticide use changes, such as the new post-emergence use of 2,4-D on DAS-68416-4 soybean are regulated by the EPA. The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. In this process, steps to reduce pesticide drift and volatilization are included on a pesticide's label approved by EPA. While the total volume of 2,4-D applied to soybean could potentially increase from the introduction of DAS-68416-4 soybean, the herbicide would be used in locations where 2,4-D is already in use and at the same rates already being applied to corn in those locations. Additionally, according to DAS, the new 2,4-D choline is reported as having low volatility and minimized potential for physical drift in comparison to the currently used 2,4-D formulations, as well as decreased odor and improved handling (DAS, 2011a, 2011c). The use pattern for glufosinate on DAS-68416-4 soybean would be consistent with the current registered use on glufosinate-tolerant soybean. Thus, although growers may change their weed management strategies due to DAS-68416-4 soybean, these changes will not necessitate a major departure from well-established and broadly used agricultural protocols (USDA-APHIS, 2012). Under the Preferred Alternative, potential impacts to plants communities from runoff, spray drift, and volatilization of herbicides are not expected to be substantially different from those associated with the No Action Alternative.

Diversifying herbicide weed management strategies is an effective alternative to tillage for mitigating the evolution of weed resistance to glyphosate (Wilson et al., 2011b). Weeds with 2,4-D and glufosinate resistance have been identified by Heap (2011) in U.S. agriculture and are most likely to develop when overall weed management is not adequately diversified (Egan et al., 2011; Wright et al., 2011). Under the Preferred Alternative, adoption of DAS-68416-4 soybean would result in an increase of use of 2,4-D and glufosinate; however, the stewardship program initiated by DAS for the new formulation of 2,4-D to be used in conjunction with the DAS-68416-4 soybean emphasizes best management practices, including maintaining a diverse weed control strategy. This is accomplished by the use of multiple herbicides with different modes of action and overlapping weed spectrum (with or without tillage operations or other cultural practices) to preclude the evolution of weed resistance to 2,4-D and glufosinate (DAS, 2011d). DAS would require growers that purchase DAS-68416-4 soybean to sign an agreement providing the terms and conditions for the authorized use of the technology (DAS, 2011d). The grower agreement will include a provision requiring them to follow the product use guide and all EPA pesticide label requirements that mitigate the potential for the development of 2,4-D and glufosinate-resistant weeds. As such, weed resistance to 2,4-D and glufosinate would be less likely to evolve as a result of a determination of nonregulated status of DAS-68416-4 soybean.

When 2,4-D choline salt and glufosinate are used on DAS-68416-4 soybean consistently with their respective EPA labels and DAS' stewardship program, impacts to non-target plants would not be expected to be substantially different from those associated with current herbicide use under the No Action Alternative. These changes would be consistent with the practices currently employed by growers to control weeds found within soybean fields, as well as those practices undertaken to protect plants located outside of the soybean field. Based on the above information, APHIS has determined that the impacts to the plant community from a determination of nonregulated status of DAS-68416-4 soybean are similar to those under the No Action Alternative.

4.4.3 <u>Gene Flow</u>

4.4.3.1 No Action Alternative: Gene Flow and Weediness

Under the No Action Alternative, DAS-68416-4 soybean would remain under APHIS regulation. The availability of GE, non-GE and organic soybeanswould not change as a result of the continued regulation of DAS-68416-4 soybean. Soybean cultivation practices are expected to remain the same. Gene flow from current commercially available GE cultivars to non-GE soybean cultivars is expected to remain unchanged from the current conditions.

4.4.3.2 Preferred Alternative: Gene Flow and Weediness

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. APHIS evaluated information on the inserted genetic material, the potential for vertical and horizontal gene transfer, and weedy characteristics of DAS-68416-4 soybean in its PPRA and concluded it would not represent any plant pest risk (USDA-APHIS, 2012).

Field trials and laboratory data collected on DAS-68416-4 soybean indicate no plant pathogenic properties or weediness characteristics. Based on agronomic data and compositional analyses, DAS-68416-4 soybean was found to be substantially equivalent to conventional soybean and would no more likely to become a plant pest than conventional soybean. The reproductive characteristics of the DAS-68416-4 soybean are substantially equivalent to other GE and non-GE soybean varieties (DAS, 2010). Additionally, the AAD-12 and PAT proteins inserted in DAS-68416-4 soybean are unlikely to increase the weediness potential of any other plant or wild species (DAS, 2010). Given the reproductive nature of soybean, the potential for cross-pollination of DAS-68416-4 soybean with other soybean cultivars is highly unlikely.

Studies have indicated horizontal gene transfer and expression of DNA from a plant species to bacteria is unlikely to occur (Keese, 2008). Furthermore, there is no evidence that bacteria closely associated with plants and/or their constituent parts contain genes derived from plants (Kaneko et al., 2000; Kaneko et al., 2002; Wood et al., 2001), and when horizontal gene transfer has been found to occur, it has been on an evolutionary time scale of millions of years (Brown, 2003; Koonin et al., 2001). Finally, FDA has determined the chance of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (US-FDA, 1998c). Based on this information, APHIS has concluded that horizontal gene flow from DAS-68416-4 soybean to other unrelated organisms would be highly unlikely (USDA-APHIS, 2012).

In the event of a determination of nonregulated status of DAS-68416-4 soybean, the risks to wild plants and agricultural productivity from weedy DAS-68416-4 soybean populations are low, as volunteer soybean populations can be easily managed and there are no feral or weedy relatives (Carpenter et al., 2002). If present as volunteer soybean, DAS-68416-4 soybean would not be considered difficult to control, as soybean seeds rarely remain viable the following season and are easily managed with cultivation or hand weeding, or the application of herbicides with differing modes of action for control of herbicide-tolerant varieties (Owen and Zelaya, 2005). In addition, since no feral or weedy species of soybean exist in the U.S. (Ellstrand et al., 1999; OECD, 2000), DAS-68416-4 soybean poses no potential for transgene introgression (USDA-APHIS, 2012).

Based on the above information, APHIS has determined that the impacts to other vegetation in soybean and the surrounding landscapes from a determination of nonregulated status of DAS-68416-4 soybean are similar to those under the No Action Alternative.

4.4.4 Microorganisms

4.4.4.1 No Action Alternative: Microorganisms

Under the No Action Alternative, DAS-68416-4 soybean would remainunder APHIS regulation. The availability of GE, non-GE and organic soybeans would not change as a result of the continued regulation of DAS-68416-4 soybean. Agronomic practices used for soybean production, such as tillage and the application of agricultural chemicals (pesticides and fertilizers), would be expected to continue. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Some of these adjustments may have potential to impact soil quality. Residue management that employs intensive tillage and leaves low amounts of crop residue on the surface results in greater losses of SOM (USDA-NRCS, 1996). A reduction of residue and SOMcould, in turn, impact soil microbial communities.

Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008). Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-D, and glufosinate for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011l). The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA’s process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

4.4.4.2 Preferred Alternative: Microorganisms

The main factors affecting microbial population size and diversity include soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), plant type (providers of specific carbon and energy sources into the soil), and agricultural management practices (crop rotation, tillage, herbicide and fertilizer application, and irrigation) (Garbeva et al., 2004). A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management.

Similar to other GE crops, DAS-68416-4 soybean has the potential to directly and indirectly impact microbial communities. DAS-68416-4 soybean could have some impact on the structure of the soil microbial community in which it is planted, which could include mutualists, such as *B. japonicum*, and pathogens alike. In a review of studies of the below ground impacts of GE plants, Kowalchuk et al. (2003) found that GE crops investigated to date had minor to no detectable effects on important soil microorganisms, and the effects that had been observed were minimal when compared to “normal” sources of variation such as agricultural practices (e.g., tillage, planting, fertilization), season, weather, plant development, location and plant genotype. Field testing by DAS did not indicate changes to DAS 68416-4 soybean plant success when compared to control varieties, indicating no significant changes to the microbial community that would impact plant health had occurred (DAS, 2010). Similarly, field testing by DAS determined no greater incidence of diseases between either DAS-68416-4 soybean treated and not treated with 2,4-D or between DAS-68416-4 soybean and the non-GE control soybean (DAS, 2010).

Adoption of DAS-68416-4 soybean by soybean producers and the potential additional new use of 2,4-D on DAS-68416-4 soybean (pending EPA approval) has the potential to result in an increase in the annual amount of 2,4-D that may be used and the time of year that it may be applied on soybeans. 2,4-D is approved by the EPA for use on other major agricultural crops at rates greater than those proposed for DAS-68416-4 soybean. The proposed maximum 2,4-D application rate for soybean is the same as that currently approved for use on field corn and popcorn which is typically grown in the same areas as soybeans and often in the same fields in rotation with soybean. Similar to the current use of 2,4-D under the No Action Alternative, the new application rate and timing of 2,4-D on soybean would be used in accordance with the per application and per year rates approved by EPA. When used in accordance with the EPA label, 2,4-D accumulation in soil has not been shown to be significant.

As discussed in Subsection 2.3.4, Microorganisms, identifying and quantifying the impact of 2,4-D use on microorganisms is difficult due to the environmental variables, formulation, and concentrations that affect its fate and impact on the microbial community (Chinalia et al., 2007). Several studies have found varying impacts on the microbial community ranging from little to no detectable impact on the soil microbial community (Breazeale and Camper, 1970; FAO, 1997; Xia et al., 1995), to reductions in population that did not rise to a level of concern (FAO, 1997), while others have found significant impacts to the microbial community (Rai, 1992).

Two laboratory studies found that the risk to soil microorganisms from 2,4-D is low at rates of 6.7 and 16.7 lb ae/A per application (FAO, 1997), greater rates than the DAS application rate proposed in the new label. Yet, a third study determined that populations of aerobic bacteria, fungi, and actinomycetes found in soil were reduced by approximately 13.2%, 9.8% and 15.0%, respectively, by an application rate of 0.88 lb ae/A of the 2,4-D salt, and nearly twice as much by 2,4-D ester (FAO, 1997). The Council of Europe/ European and Mediterranean Plant Protection Organization (CoE/EPPO) do not consider any loss of soil microorganisms below 30% a cause for concern (FAO, 1997). The field testing conducted by DAS revealed no significant differences in agronomic performance between 2,4-D and glufosinate sprayed and non-sprayed DAS-68416-4 soybean (DAS, 2010) suggesting no meaningful changes to the soil microbial community that influence soybean growth and health.

As DAS-68416-4 soybean also would have tolerance for glufosinate, the use of this pesticide on soybean could also increase. Similar to the current use of glufosinate under the No Action Alternative, the use of glufosinate on soybean would be in accordance with the per application and per year rates approved by EPA. The potential impacts from the application of glufosinate to the microbial community are subject to the same variables as 2,4-D and are also difficult to quantify. As discussed in Subsection 2.3.4, Microorganisms, studies on the effect of glufosinate on the microbial community revealed varied results. Several studies found no differences in the microbial community from the application of glufosinate compared to either those treated with different herbicides or those left untreated (Lupwayi et al., 2004; Schmalenberger and Tebbe, 2002; Wibawa et al., 2010). Another study found minor, transient shifts in the bacterial community (Gyamfi et al., 2002), while others found reductions in the activity of pathogens (Kortekamp, 2010; Pline, 1999). Glufosinate use may potentially increase due to adoption of DAS-68416-4, but application rates would remain consistent with the current EPA approved application rates on other soybean cultivars. Therefore, no changes to the microbial community associated with glufosinate application to the DAS-68416-4 soybean are anticipated.

Residue management tactics that use intensive tillage and leave low amounts of residue crops on the surface result in greater losses of SOM (USDA-NRCS, 1996). Organic matter is a vital component in maintaining soil microbial populations. Maintaining adequate residue in the first three inches of the surface provides for a cooler and moister environment, increasing substrates and food for microorganisms (USDA-NRCS, 1996). Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues. This in turn this could reduce the potential loss of SOM and soil erosion that may result when more aggressive tillage practices are used to combat herbicide restistant weeds.

Based on the above information, overall impacts to microorganisms under the Preferred Alternative are expected to be similar to the No Action Alternative.

4.4.5 Biodiversity

Impacts to biodiversity can occur at the crop, farm, and/or landscape level (Ammann, 2005; Carpenter, 2011; Visser, 1998). For purposes of this EA, crop diversity refers to the genetic uniformity within crops, farm-scale diversity refers to the level of complexity of organisms within the boundaries of a farm, and landscape level diversity refers to potential changes in land use and the impacts of area-wide weed suppression beyond the farm boundaries (Carpenter, 2011).

4.4.5.1 No Action Alternative: Biodiversity

Under the No Action Alternative, DAS-68416-4 soybean would remain under APHIS regulation. The availability of GE, non-GE and organic soybeans would not change as a result of the continued regulation of DAS-68416-4 soybean. Agronomic practices used for soybean production, such as tillage and the application of agricultural chemicals (pesticides and fertilizers), would be expected to continue. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Agronomic practices that benefit biodiversity both on cropland (e.g., intercropping, agroforestry, crop rotations, cover crops, and no-tillage) and on adjacent non-cropland (e.g., woodlots, fencerows, hedgerows, and wetlands) would continue.

4.4.5.2 Preferred Alternative: Biodiversity

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. Field trials and laboratory analyses conducted by DAS showed no differences between DAS-68416-4 soybean and nontransgenic soybean in growth, reproduction, or interactions with pests and diseases (DAS, 2010). Similar to the No Action Alternative, weeds within fields of DAS-68416-4 soybean could be managed using mechanical, cultural, and chemical control. Based on individual grower needs, DAS-68416-4 soybean could provide growers with an alternative to intensive tillage practices that may be used to address herbicide weed resistance issues.

The environmental risks of pesticide use are assessed by EPA in the pesticide registration process and are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment. Adoption of DAS-68416-4 soybean by soybean producers and the potential additional new use of 2,4-D on DAS-68416-4 soybean (pending EPA approval) has the potential to result in an increase in the annual amount of 2,4-D that may be used and the time of year that it may be applied on soybeans. 2,4-D is approved by the EPA for use on other major agricultural crops at rates greater than those proposed for DAS-68416-4 soybean. The proposed maximum 2,4-D application rate for soybean is the same as that currently approved for use on field corn and popcorn which is typically grown in the same areas as soybeans and often in the same fields in rotation with soybean. Similar to the current use of 2,4-D under the No Action Alternative, the new application rate and timing of 2,4-D on soybean would be used in accordance with the per application and per year rates approved by EPA. The use pattern for glufosinate on DAS-68416-4 soybean would be consistent with the current registered use on glufosinate-tolerant soybean. Thus, although growers may change their weed management strategies due to DAS-68416-4 soybean, these changes will not necessitate a major departure from well-established and broadly used agricultural protocols (USDA-APHIS, 2012). Under the Preferred Alternative, potential impacts to biodiversity from runoff, spray drift, and volatilization of herbicides are not expected to be substantially different from those associated with the No Action Alternative.

A determination of nonregulated status of DAS-68416-4 soybean is anticipated to have similar effects on crop, farm or landscape level biodiversity as the No Action Alternative. As such, the impacts of biodiversity under the Preferred Alternative are expected to be similar to the No Action Alternative.

4.5 Human Health

4.5.1 No Action Alternative: Public Health

Under the No Action Alternative, DAS-68416-4 soybean would remain under APHIS regulation. Human exposure to existing GE and non-GE soybean varities would not change under this alternative. Grower and consumer exposure to DAS-68416-4 soybean would be limited to those individuals involved in the cultivation under regulated conditions.

4.5.2 Preferred Alternative: Public Health

The AAD-12 protein in DAS-68416-4 soybean is derived from the common gram-negative soil bacterium *Delftia acidovorans*. *D. acidovorans* is non glucose-fermenting, gram-negative, non spore-forming rod present in soil, fresh water, activated sludge, and clinical specimens. It has a history of safe use in the food processing industry, including having been used in transforming ferulic acid into vanillin and related flavor metabolites (DAS, 2010).

The *pat* gene is derived from *Streptomyces viridochromogenes,* a gram-positive soil bacterium. The PAT protein in DAS-68416-4 soybean is the same as that used as a selectable marker during development and to confer herbicide tolerance in other previously deregulated GE crops (USDA 1996, USDA 2001, USDA 2004, USDA 2005). Additionally, FDA has previously reviewed submissions regarding the safety of food and feed derived from crops containing the *pat* gene (BNFs 000055, 000073, 000081, 000085, and 000092).

DAS conducted safety evaluations based on Codex Alimentarius Commission procedures to assess any potential adverse effects to humans or animals resulting from environmental releases and consumption of DAS-68416-4 soybean (DAS, 2010; FAO, 2009; US-FDA, 2011c). These safety studies included evaluating protein structure and function, including homology searches of the amino acid sequences with comparison to all known allergens and toxins, an *in vitro* digestibility assay of the proteins, an acute oral toxicity feeding study in mice, and a feeding study in broiler chickens (DAS, 2010; Herman et al., 2011a; Herman et al., 2010; US-FDA, 2011c). DAS initiated a consultation with the FDA by submitting an early food safety evaluation of the AAD-12 protein expressed in DAS-68416-4 soybean (NPC 000009) on December 15, 2008 (Krieger, 2008) (Appendix C). The information presented by DAS indicated that the AAD-12 protein was determined to have no amino acid sequence similar to known allergens, lacked toxic potential to mammals, and was degraded rapidly and completely in gastric fluid. As such, the submission concluded that the presence of the AAD-12 protein in food or feed should be of no significant concern (Krieger, 2008). FDA completed its evaluation with no further questions on May 19, 2010 (US-FDA, 2010).

DAS submitted a safety and nutritional assessment of food and feed derived from DAS-68416-4 soybean to the FDA on December 22, 2009 (BNF No. 000124) in support of the consultation process with FDA for the commercial distribution of DAS-68416-4 soybean. FDA evaluated the submission and responded to the developer by letter on November 14, 2011. Based on the information DAS submitted, FDA has no further questions regarding food and feed derived from DAS-68416-4 soybean (US-FDA, 2011a). An FDA biotechnology consultation on soybean lines containing the PAT protein (BNF No. 000055) (US-FDA, 1998a) was submitted April 21, 1998 and completed on May 15, 1998 (US-FDA, 1998a), and does not require reevaluation. Copies of the completed FDA reviews are provided in Appendix C.

Additionally, the EPA previously concluded, after reviewing data on the acute toxicity and digestibility of the PAT protein, that there is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to the PAT protein and the genetic material necessary for its introduction (US EPA, 1997). EPA has consequently established an exemption from tolerance requirements pursuant to FFDCA section 408(j)(3) for PAT and the genetic material necessary for its production in all plants.

Recently, Food Standards Australia New Zealand (FSANZ) in its evaluation of the food safety of DAS-68416-4 soybean did not find any public health or safety concerns associated with the expression of AAD-12 protein . The PAT protein was evaluated in previous FSANZ assessments, which concluded that the protein it is essentially nontoxic to mammals and does not exhibit any allergenic potential to humans (FSANZ, 2011).

Pesticide residue tolerances have been published for 2,4-D (US-EPA, 2011a) and glufosinate (US-EPA, 2011g) for food consumption for a variety of crop products and animals. The EPA establishes tolerances to regulate the amount of pesticide residues that can remain on food or feed commodities as the result of pesticide applications (see, e.g., http://www.epa.gov/pesticides/bluebook/chapter11.html). The tolerance level is the maximum residue level of a pesticide that can legally be present in food or feed, and if pesticide residues are found to exceed the tolerance value, the food is considered adulterated and may be seized. The EPA has determined that residue tolerances for glufosinate and 2,4-D meet FQPA safety standards for the U.S. population and designated sensitive populations (i.e., infants and children), finding that there is a reasonable certainty of no harm to the general population and any subgroup from the use of glufosinate and 2,4-D at the approved levels and methods of application (US-EPA, 2011a, 2011b).

The FSANZ recent review also concluded that residues found on DAS-68416-4 soybean would be similar to those found on conventional crops treated with 2,4-D and the residue levels posed no safety concerns. Additionally, FSANZ indicated that glufosinate ammonium residues did not need to be considered in the Safety Assessment since the herbicide residues have been previously considered in a wide range of food crops, including soybean (FSANZ, 2011).

Based on this information, including field and laboratory data and scientific literature provided by DAS (DAS, 2010) and safety data available on other GE soybean, APHIS has concluded that a determination of nonregulated status of DAS-68416-4 soybean would have no adverse impacts on human health. Overall impacts are similar to the No Action Alternative.

4.5.3 No Action Alternative: Occupational Health and Safety

The availability of GE, non-GE and organic soybeans would not change as a result of the continued regulation of DAS-68416-4 soybean. Agronomic practices used for soybean production, such as the application of agricultural chemicals (pesticides and fertilizers), would be expected to continue. Growers likely will still experience the continued emergence of glyphosate-resistant weeds, requiring modifications of crop management practices to address these weeds. These changes may include diversifying the mode of action of herbicides applied to soybean and making adjustments to crop rotation and tillage practices (Owen et al., 2011). Herbicides use may increase to meet the need for additional integrated weed management tactics to mitigate herbicide-resistant weeds in different cropping systems (Culpepper et al., 2008; Heap, 2011; Owen and Zelaya, 2005; Owen, 2008). Growers will continue to choose certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Farnham, 2001; Heiniger, 2000; University of Arkansas, 2008).

Agricultural production of existing nonregulated herbicide-tolerant GE and non-GE soybean would continue to utilize EPA-registered pesticides, including glyphosate, 2,4-D, and glufosinate for weed management. 2,4-D would continue to be used as currently authorized by EPA for pre-plant application to soybean. Glufosinate use is likely to continue to follow the recent trend of increased use associated with the adoption of glufosinate-tolerant soybeans (DAS, 2011l). Worker safety is taken into consideration by EPA in the pesticide registration process and reregistration process. Pesticides are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

4.5.4 Preferred Alternative: Occupational Health and Safety

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. Similar to the No Action Alternative, it is expected that EPA-registered pesticides that currently are used for soybean production will continue to be used by growers, including the use of 2,4-D and glufosinate. EPA's core pesticide risk assessment and regulatory processes ensure that each registered pesticide continues to meet the highest standards of safety including all populations of non-target species and humans. These assessments provide EPA with information needed to develop label use restrictions for the pesticide. EPA's baseline criteria for registering a pesticide and providing a label for its use is whether the pesticide use in accordance with the label can be demonstrated to pose "a reasonable certainty of no harm to humans" and "no unreasonable adverse effects to the environment". Growers are required to use pesticides, such as 2,4-D and glufosinate, consistently with the application instructions provided on the EPA-approved pesticide label. These label restrictions carry the weight of law and are enforced by EPA and the states (Federal Insecticide, Fungicide, and Rodenticide Act 7 USC 136j (a)(2)(G) Unlawful Acts). Therefore, it is expected that 2,4-D and and glufosinate use on the DAS-68416-4 soybean product would be consistent with the EPA-approved label.

It is likely that adoption of DAS-68416-4 soybean could result in a increase in use of 2,4-D. As illustrated in Table 18, DAS-68416-4 soybean would allow the application of 2,4-D at pre-plant/burndown or pre-emergence and/or over-the-top post-emergence applications. The proposed EPA label changes for 2,4-D provide for maximum total annual applications are identical to the current label rate for field corn and popcorn (DAS, 2010). In situations where the maximum total annual application is reached, worker exposure to 2,4-D would be similar to that which currently occurs in those farms where 2,4-D currently is applied to corn at the maximum annual rate.

The EPA evaluated occupational risk from exposure to 2,4-D in the product reregistration (US-EPA, 2005d). In that analysis, the EPA concluded that the short-term and intermediate-term exposures to workers, including mixers, loaders, and applicators, were not a human health concern provided that the workers used appropriate personal protective equipment (US-EPA, 2005d). The EPA has reviewed additional information on the potential risks of non-Hodgkins lymphoma, a type of cancer, from 2,4-D exposures (US-EPA, 2007a). Based on the review of epidemiological data and animal studies, the EPA concluded that 2,4-D is not likely to be a human carcinogen (US-EPA, 2005c).

Agricultural workers that routinely handle 2,4-D and glufosinate (mixers, loaders, and applicators) may be exposed during and after use. In the 2,4-D RED, EPA evaluated 18 occupational exposure scenarios that included mixing/loading and applying 2,4-D for crop and non-crop uses. The exposure and risk assessment for occupational handlers and applicators used the maximum application rates and daily acreage to evaluate short-term exposure and the average application rates for intermediate exposure (US-EPA, 2005c). For 2,4-D, the current maximum application rate is 4.0 lb ae/A per year or season and the maximum average per application rate is 1.8 lb ae/A for granular formulations and 2.0 lb ae/A for liquid forms (US-EPA, 2005c), which are higher than the application rates of 3.0 lb ae/A per year or season and 0.5 to 1.0 lb ae/A per application proposed under the Preferred Alternative. For occupational exposure, the EPA is concerned about any Margin of Exposure[7] (MOE) less than 100. With the exception of mixing or loading wettable powder, which is not applicable for the new 2,4-D choline salt formulation, all short-term and intermediate-term MOEs for 2,4-D exceed 100 and are not a concern for human health, provided minimal PPE is worn and approved maximum application rates are observed (US-EPA, 2005a). The PPE standards specified in DAS's proposed label for the new 2,4-D choline salt formulation meet those outlined in the 2,4-D RED.

For glufosinate, the 2003 EPA risk assessment based the occupational risk assessment on the highest supported application rates for cotton, bushberry, and rice (0.79, 0.52 and 0.44 lb ai/A per application, respectively), which are greater or equal to the approved application rate of 0.44 lb ai/A per application for soybean (US-EPA, 2002a, 2008b). This study concluded the modeled exposure levels were adequate for the determination of potential adverse human health effects posed by glufosinate (US-EPA, 2008b). Potential human health effects from glufosinate use are currently under review in the EPA reregistration review process for this herbicide (United States Geological Survey, No Date-b; US-EPA, 2008b). The current EPA-approved label for glufosinate includes precautions and measures to protect human health. Applications of pesticides in accordance with the registered use and label instructions minimize the potential for human health impacts.

Based on this information, occupational risks from exposure to these herbicides were deemed to be well below the EPA's level of concern (US-EPA, 2007c). The proposed application rates and annual maximum total annual applications for 2, 4-D and glufosinate are equivalent to those rates currently approved for other crops (DAS, 2010).

EPA uses maximum application rates for human health and ecological risk assessment and has determined that both the chronic and acute population adjusted dose (PAD) of 2,4-D and glufosinate do not pose any adverse human health effects (US-EPA, 2005d, 2008a). Given the proposed maximum application rate of these herbicides to DAS-68416-4 soybean would be no more than the maximum level previously evaluated for human health effects by EPA and found by the Agency not to be adverse, exposure to DAS-68416-4 soybean under the Proposed Alternative is not expected to pose any change in existing human health. Based on the above information, overall impacts to occupational health and safety under the Preferred Alternative are expected to be similar to the No Action Alternative.

4.6 Animal Feed

The majority of the soybean cultivated in the U.S. is grown for animal feed and is usually fed as soybean meal. Animal agriculture consumes 98% of the U.S. soybean meal

[7] The ratio of the No Observed Adverse Effect Level to the estimated exposure dose or estimated exposure concentration.

produced (Soyatech, 2011). As with human health, the consumption of the inserted genes and proteins in DAS-68416-4 soybean is considered the primary concern relative to animal feed. This subsection also considers the potential impacts of herbicide residues on animal feed.

4.6.1 No Action Alternative: Animal Feed

Under the No Action Alternative, DAS-68416-4 soybean would remain under APHIS regulation. Soybean-based animal feed would still be available from currently cultivated soybean crops, including both GE and non-GE soybean varieties. No change in the availability of these crops as animal feed is expected under this alternative.

4.6.2 Preferred Alternative: Animal Feed

APHIS' assessment of the potential impacts of the consumption of the AAD-12 protein by animals considers the source of the gene and the expressed protein, as well as safety evaluations conducted by DAS. Animals are already exposed to the *Delftia acidovorans* soil bacteria that is the source of the *aad-12* gene and corresponding AAD-12 protein expressed in DAS-68416-4 soybean (DAS, 2010). Similarly, *Streptomyces viridochromogenes*, the source of the pat *gene*, is found naturally and in other previously deregulated GE crops, including soybean (USDA 1996, USDA 2001, USDA 2004, USDA 2005). Therefore, the incorporated *aad-12* and *pat* genes and the expression of the AAD-12 and PAT proteins in DAS-68416-4 soybean is not a novel exposure to animals.

The FDA has concluded its consultation regarding DAS' submittal of safety and nutritional data for DAS-68416-4 soybean (US-FDA, 2011c). DAS conducted safety evaluations based on Codex Alimentarius Commission procedures to assess any potential adverse effects to humans or animals resulting from environmental releases and consumption of DAS-68416-4 soybean (DAS, 2010; FAO, 2009; US-FDA, 2011c). These safety studies included evaluating protein structure and function, including homology searches of the amino acid sequences with comparison to all known allergens and toxins, an *in vitro* digestibility assay of the proteins, an acute oral toxicity feeding study in mice, and a feeding study in broiler (DAS, 2010; Herman et al., 2011a; Herman et al., 2011b; US-FDA, 2011c). In this study, chickens were fed soybean diets for 42 days (6 weeks) to assess dietary exposure to the AAD-12 and PAT proteins in DAS-68416-4 soybean. In this study, chickens were fed diets of toasted DAS-68416-4 soybean meal containing approximately 40, 36, and 32 percent of soybean meal in the starter, grower, and finisher feeds, respectively. The birds were exposed through dietary consumption to 16.6 ppm (ng/mg) of the AAD-12 protein and 1.7 ppm (ng/mg) of the PAT protein (Herman et al., 2011a). The results of this study found no difference between any of the feeding cohorts in growth, feed conversion, and carcass weight (Herman et al., 2011a). The DAS-68416-4 soybean AAD-12 protein was determined to have no amino acid sequence similar to known allergens, lacked toxic potential to mammals, and was degraded rapidly and completely in gastric fluid (DAS, 2010; US-FDA, 2011c). At this time, the FDA considers the consultation on DAS-68416-4 soybean to be complete (US-FDA, 2011a).

DAS also has evaluated the compositional and nutritional characteristics of DAS-68416-4 soybean grain and forage, comparing the composition of the GE soybean with conventional products (DAS, 2010; Herman et al., 2011b). In these studies, compositional comparisons were made among samples from unsprayed DAS-68416-4 soybean, DAS-68416-4 soybean sprayed with 2,4-D, DAS-68416-4 soybean sprayed with glufosinate, DAS-68416-4 soybean sprayed with both 2,4-D and glufosinate, and several conventional soybean varieties from six different field trial locations and analyzed for comparable nutritional components in accordance with Organisation for Economic Co-operation and Development (OECD) guidelines (OECD, 2001). Compositional elements compared included proximates (protein, fat, carbohydrates, fiber, ash, and moisture), minerals, amino acids, fatty acids, vitamins, isoflavones, and antinutrients (i.e., lectin, phytic acid, trypsin inhibitor, raffinose, and stachyose) (DAS, 2010; Herman et al., 2011b). There were no biologically meaningful differences for any of these compositional characteristics between the DAS-68416-4 soybean and the conventional soybean varieties (DAS, 2010; Herman et al., 2011b).

FDA evaluated the submission and responded to the developer by letter on November 14, 2011. Based on the information DAS submitted, including the composition and nutritional characteristics of DAS-68416-4 soybean, FDA has no further questions regarding feed derived from DAS-68416-4 soybean (US-FDA, 2011a). An FDA biotechnology consultation on soybean lines containing the PAT protein (BNF No. 000055) (US-FDA, 1998a) was submitted April 21, 1998 and completed on May 15, 1998 (US-FDA, 1998a), and does not require reevaluation. Copies of the completed FDA reviews are provided in Appendix C.
The proposed label for the new 2,4-D choline salt prohibits the subsequent use of sprayed soybean as animal hay or forage (DAS, 2011a), as do current labels for formulations of glufosinate such as Ignite® (Bayer CropScience, 2011).

The results of studies conducted by DAS confirm that the crops containing this protein can be safely used as animal feed. There are no differences in feed safety between the DAS-68416-4 soybean and other varieties currently available under the No Action Alternative. Based on the above information, analysis of field and laboratory data and scientific literature provided by DAS (2010), as well as safety data available on other GE soybean, APHIS has concluded that a determination of nonregulated status of DAS-68416-4 soybean would have no adverse impacts on animal health with regard to animal feed. Overall impacts are similar to the No Action Alternative.

4.7 Socioeconomic Impacts

4.8 Compliance with Clean Water Act and Clean Air Act

This EA evaluated the potential changes in soybean production associated with a determination of nonregulated status of DAS-68416-4 soybean (see Subsection 4.2, Agricultural Production of Soybean) and determined that the cultivation of DAS-68416-4 soybean would not lead to the increased production or acreage of soybean in U.S. agriculture. The herbicide tolerance conferred by the genetic modification to DAS-68416-4 soybean would not result in any changes in water usage for cultivation. As discussed in Subsection 4.3.2, Water Resources, and Subsection 4.3.3, Air Quality, there are no expected significant negative impacts to water resources or air quality from potential use of 2,4-D or glufosinate associated with DAS-68416-4 soybean production. Based on these analyses, APHIS concludes that a determination of nonregulated status for DAS-68416-4 soybean would comply with the CWA and the CAA.

4.8.1 <u>Domestic Economic Environment</u>

4.8.1.1 No Action Alternative: Domestic Economic Environment

In 2010, 76 million acres of soybeans were cultivated in the U.S., yielding 3.3 billion bushels at a value of 38.9 billion U.S. dollars (USDA-NASS, 2011d). The majority of soybeans produced in the U.S. is utilized domestically for animal feed, with less amounts and byproducts used for oil or fresh consumption (GINA, 2011; USDA-ERS, 2010a).

Under the No Action Alternative, DAS-68416-4 soybean would remain under APHIS regulation. Growers and other parties who are involved in production, handling, processing, or consumption of soybean would continue to have access to nonregulated GE and non-GE soybean varieties. Domestic growers would continue to utilize GE and non-GE soybean varieties based upon availability and market demand.

Increasing weed resistance to herbicides has elevated the importance of farmers diversifying weed management strategies. The shift in weed management practices in response to glyphosate-resistant weeds has been shown to increase crop production cost, reduce profitability, and reduce non-financial benefits like weed control simplicity, convenience, and labor savings (Duke and Powles, 2009; Fernandez-Cornejo et al., 2005; Fernandez-Cornejo and McBride, 2000; Fernandez-Cornejo and McBride, 2002; Hurley et al., 2009a; Lin et al., 2001; Marra et al., 2004; Sankula, 2006; Sankula and Blumenthal, 2004). The fuel, equipment, and labor costs associated with the need for increased tillage and/or increased number of applications of herbicides to control herbicide-resistant weeds can diminish the economic benefits of herbicide-tolerant GE crops (Duke and Powles, 2009; NRC, 2010). There is also an additional cost from the reduction in yield associated with the competition of the crop with the weeds (NRC, 2010; Weirich et al., 2011).

Under the No Action Alternative, the economic trends associated with the increase in costs for agronomic inputs to control herbicide-resistant weedy species are likely to continue.

4.8.1.2 Preferred Alternative: Domestic Economic Environment

In field tests accomplished by DAS, the performance and composition of DAS-68416-4 soybean was determined to not be substantially different from that of the non-GE comparator Maverick Soybean (DAS, 2010). With the exception of the benefits associated with the conferred tolerance to 2,4-D and glufosinate, the potential economic impacts from a determination of nonregulated status of DAS-68416-4 soybean would be no different than those currently observed for other soybean varieties under the No Action Alternative. GE seed is generally more expensive than conventional seed; producers that use DAS-68416-4 soybean would likely be charged a technology fee as part of the seed purchase price (NRC, 2010). Technology fees are charged by the product developer to cover research and development, production, marketing and distribution expenses. The amount of the fee is determined by producers' willingness to purchase the seed, the competiveness of the seed market and the pricing behavior of firms that hold large shares of the market (NRC, 2010). APHIS has no control over the establishment of technology fees, but assumes that the fee for DAS-68416-4 soybean would be consistent with the fee charges for other GE crops. Growers must make an independent assessment as to whether the benefits of DAS-68416-4 soybean would offset seed cost.

Under the Preferred Alternative, an additional herbicide-tolerant soybean variety would be available, providing farmers more choice for controlling weeds. Which weed control strategies are chosen by farmers would depend on seed prices, herbicide prices, and other individual differences across farming operations, including whether an operation has glyphosate-resistant weeds. Since 2,4-D has a different mode of action than glyphosate and glufosinate, farmers would have an additional choice for controlling glyphosate-resistant weeds. Farmers would also have more options for rotating herbicides, which is one of several strategies recommended for reducing the risk of herbicide-resistant weeds emerging and mitigating the effect of herbicide-resistant weeds that have emerged (Beckie, 2006; Boerboom, 1999; Frisvold et al., 2009; Sammons et al., 2007). Herbicide rotation and mixes are also among the alternatives farmers prefer to use for managing herbicide-resistant weeds (Foresman and Glasgow, 2008; Johnson and Gibson, 2006; Johnson et al., 2009; Scott and VanGessel, 2007) and can reduce production costs (Duke and Powles, 2009; NRC, 2010). The option to rotate a post-emergent soybean herbicide to 2,4-D and glufosinate rather than utilizing a weed control tactic, such as tillage, to address glyphosate-resistant weeds may reduce soybean production costs. In comparison to the No Action Alternative, the increased options for a diverse herbicide management program from the availability of DAS-68416-4 soybean could potentially provide increased benefits to the domestic economic environment.

4.8.2 <u>Trade Economic Environment</u>

4.8.2.1 No Action Alternative: Trade Economic Environment

The U.S. produces approximately 35 percent of the global soybean supply (Soy Stats, 2011b). In 2010, the U.S. exported 1.6 billion bushels of soybean, which accounted for 44 percent of the world's soybean exports (Soy Stats, 2011a). The global demand for soybeans is expected to increase by a full third over 2010 consumption in the next ten years. China is expected to account for 80 percent of the increased demand (FAPRI, 2009; Hartnell, 2010). China and India are predicted to import 46 percent of the total soybean market by 2018/2019 (FAPRI, 2009). The USDA has predicted that U.S. exports will remain flat during much of this period, as a result of increase in domestic consumption and competition from South America (FAPRI, 2009; USDA-ERS, 2009b).

Under the No Action Alternative, there is unlikely to be any change to the current soybean market. A majority (93 percent) of the non-organic soybean varieties currently cultivated in the U.S. are GE varieties and this is not expected to change significantly in the future (USDA-ERS, 2011a). U.S. soybeans will continue to play a role in global soybean production, and the U.S. will continue to be a supplier in the international market.

4.8.2.2 Preferred Alternative Trade Economic Environment

A determination of nonregulated status of DAS-68416-4 soybean is not expected to adversely impact the trade economic environmentand may have a positive impact through increased yields in soybean areas affected by glyphosate-resistant weeds. . The subsequent development and global adoption of the DAS-68416-4 soybean could provide another herbicide-tolerant management choice for growers. As the value and benefits of the product are realized, particularly where glyphosate-resistant weeds have emerged, DAS-68416-4 soybean may have potential for export as a seed product.

DAS has submitted applications to several international agencies, including the regulatory authorities in Canada, Japan, Korea, Taiwan, the EU, Australia/New Zealand, South Africa, Brazil, Argentina and Mexico (DAS, 2010). These authorities include U.S. trade partners for import clearance and production approval (see USDA-FAS, 2011b). FSANZ has concluded their review of the application to permit the sale and use of food derived from DAS-68416-4 soybean and determined that there were no potential public health and safety concerns (FSANZ, 2011). As of the time of the preparation of this EA, conclusions of the other international agencies had not been published. Approval in these export countries is intended to mitigate global sensitivities to GE productions and work in accordance with international regulations. The trade economic impacts associated with a determination of nonregulated status of DAS-68416-4 soybean are anticipated to be very similar to the No Action Alternative.

5 CUMULATIVE IMPACTS

A cumulative impact may be an effect on the environment which results from the incremental impact of the proposed action when added to other past, present, and reasonably foreseeable future actions. For example, the potential effects associated with a determination of nonregulated status for a GE crop in combination with the future production of crop seeds with multiple deregulated traits (i.e., "stacked" traits), including drought tolerance, herbicide tolerance, and pest resistance, would be considered a cumulative impact.

5.1 Assumptions Used for Cumulative Impacts Analysis

Cumulative effects have been analyzed for each environmental issue assessed in Section 4. In this EA, the cumulative effects analysis is focused on the incremental impacts of the Preferred Alternative taken in consideration with related activities, including past, present, and reasonably foreseeable future actions. Certain aspects of this product and its cultivation would be no different between the alternatives; those instances are described below. In this analysis, if there are no direct or indirect impacts identified for a resource area, then APHIS assumes there can be no cumulative impacts. Where it is not possible to quantify impacts, APHIS provides a qualitative assessment of potential cumulative impacts.

Stacked soybean varieties may contain more than one GE trait as the result of crossing two GE soybean plants. Under the Preferred Alternative, DAS-68416-4 soybean may be crossed with non-GE or GE soybean varieties that are no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. APHIS regulations at 7 CFR Part 340 do not provide for Agency oversight of GE soybean varieties no longer subject to the requirement of Part 340 and the plant pest provisions of the Plant Protection Act, or over stacked varieties combining these GE varieties, unless it can be positively shown that such stacked varieties were to pose a likely plant pest risk. DAS has indicated that it will likely develop a "stacked" hybrid with DAS-68416-4 soybean and other commercially available traits, expected to initially be GE glyphosate tolerance (DAS, 2010, 2011e, 2011f). In this process, the 2,4-D and glufosinate tolerance from DAS-68416-4 soybean will be combined with glyphosate tolerance from another soybean variety that is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. Such a stacked variety could provide growers with the option to combine several herbicides with different modes of action for control of weeds. Therefore, as part of the cumulative impacts analysis, APHIS will assume that DAS-68416-4 soybean could be combined with commercially available glyphosate-tolerant varieties of soybean as a reasonably foreseeable future action.

Nonregulated GE glyphosate-tolerant (e.g., Roundup Ready®) crop varieties have been in the market since 1996, when glyphosate-tolerant soybean became commercially available. The potential effects from the cultivation of glyphosate-tolerant crops, with a corresponding analysis of the implications of the use of glyphosate, have been thoroughly evaluated in other APHIS EAs since the 1993 introduction of the first glyphosate-tolerant crop product (see http://www.aphis.usda.gov/biotechnology/not_reg.html). Several of these evaluations included crops expressing tolerance to multiple herbicides. Specific crop examples include:

- Sugar Beet, 2011. Monsanto and KWS SAAT AG Glyphosate-tolerant Sugar Beet

(Petition No. 03-023-01p).
- Soybean, 2011. Monsanto Improved Fatty Acid Profile Soybean (which includes glyphosate tolerance) (Petition No. 09-201-01p).
- Alfalfa, 2011. Monsanto Glyphosate-tolerant Alfalfa (Petition 04-110-01p).
- Corn, 2009. Pioneer Glyphosate and Imadazolinone-tolerant Corn (Petition 07-152-01p).
- Cotton, 2009. Bayer Crop Science Glyphosate-tolerant Cotton (Petition 06-332-01p).
- Soybean, 2008. Pioneer Glyphosate and Acetolactate Synthase-tolerant Soybean (Petition No. 06-271-01p).
- Soybean, 2007. Monsanto Glyphosate-tolerant Soybean (Petition 06-178-01p).
- Cotton, 2005. Monsanto Glyphosate-tolerant Cotton (Petition 04-086-01p).
- Rapeseed 2001. Monsanto Glyphosate-tolerant Rapeseed (Petition 01-324-01p).
- Corn, 2000. Monsanto Glyphosate-tolerant Corn (Petitions No. 97-099-01p and 00-011-01p).
- Rapeseed 1998. Monsanto Glyphosate-tolerant Rapeseed (Petition 98-216-01p).
- Sugar Beet, 1998. Novartis Seeds and Monsanto Glyphosate-tolerant Sugar Beet (Petition No. 98-173-01p).
- Corn, 1997. Monsanto Glyphosate-tolerant Corn (Petition No. 97-099-01p).
- Corn, 1996. Monsanto Glyphosate-tolerant and European Corn Borer-resistant Corn (Petition No. 96-317-01p).
- Cotton, 1995. Monsanto Glyphosate-tolerant Cotton (Petition 95-045-01p).
- Soybean, 1993. Monsanto Glyphosate-tolerant Soybean (Petition 93-258-01p).

The first glyphosate-tolerant soybean became commercially available to growers in 1996 after Monsanto's Roundup Ready® Soybean (GTS 40-3-2) was determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act (see APHIS Petition File 93-258-01p at http://www.aphis.usda.gov/biotechnology/not_reg.html).

Other stacked varieties might also be developed at a later time which also derive tolerance to 2,4-D and glufosinate from the DAS-68416-4 soybean. Currently, all GE soybean varieties are herbicide-tolerant, namely to glyphosate, with a smaller (i.e., approximately 1.3% of planted acres in 2011) but growing number of glufosinate-tolerant varieties available since 2010 (DAS, 2011h). In addition to tolerance to glyphosate and glufosinate, other GE soybean traits no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act include lepidopteran resistance, high oleic acid content, and acetolactate synthase tolerance (see http://www.aphis.usda.gov/biotechnology/not_reg.html). Issues associated with potential future stacking, particularly cultivation of a stacked hybrid incorporating glyphosate resistance from a soybean variety previously determined to be nonregulated, are presented and discussed in the cumulative effects analyses where appropriate.

5.2 Cumulative Impacts: Acreage and Area of Soybean Production

Cumulative effects associated with a determination of nonregulated status of DAS-68416-4 soybean to acreage and areas of soybean production are unlikely. The Preferred Alternative is not expected to directly cause a change in agricultural acreage devoted to conventional or GE soybean cultivation in the U.S. and there are no anticipated changes to the availability of GE and non-GE soybean varieties on the market .

The potential future development and cultivation of a stacked soybean variety presenting tolerance to 2,4-D, glufosinate and glyphosate is not likely to change the current number of acres of soybean being treated with glyphosate, since currently more than 90% of soybean acres are planted with GE glyphosate-tolerant soybeans (USDA-ERS, 2011b). Additionally, the availability of a stacked variety of DAS-68416-4 soybean for commercial production is not expected to change the areas where soybean can be grown for soybean production in the U.S. since the agronomic characteristics of DAS-68416-4 soybean are essentially indistinguishable from other available soybean varieties. For these reasons, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to impact soybean acreage and areas of production.

5.3 Cumulative Impacts: Agronomic Practices

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides. DAS-68416-4 soybean would provide soybean growers with the option to use 2,4-D post-emergence in addition to using glufosinate, which would provide different modes of action to control glyphosate-resistant weeds. Similar to the current use of 2,4-D and glufosinate, the use of 2,4-D and glufosinate on soybean would be in accordance with the per application and per year rates approved by EPA. Studies conducted by DAS demonstrate that, in terms of agronomic characteristics and cultivation practices, DAS-68416-4 soybean is essentially indistinguishable from other soybean varieties currently grown (DAS, 2010). Consequently, impacts to cropping practices associated with the adoption of DAS-68416-4 soybean are not expected.

DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). If combined with a glyphosate tolerance trait, DAS-68416-4 soybean would enable growers to use a combination of herbicides with different modes of action on soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). The future development and cultivation of a stacked DAS-68416-4 soybean variety presenting the additional tolerance to glyphosate is not likely to increase the number of acres of soybean being treated with glyphosate, since, in 2011, more than 90% of soybean acres were planted with GE glyphosate-tolerant soybeans (USDA-ERS, 2011b). DAS-68416-4 soybean, either alone or combined with other traits, would likely replace these other GE herbicide-tolerant soybeans currently being cultivated. Therefore, it is expected that combining other herbicide tolerance traits with those of DAS-68416-4 soybean would not increase the overall number of acres that herbicide would be applied to. Herbicide use would be in accordance with per application an per year rates approved by EPA.

DAS Enlist™ Weed Control System includes a new choline salt-based formulation of 2,4-D (DAS, 2011a). This choline salt formulation is reported to present substantially lower volatility, improved stability at low temperatures, and lower odors than the amine and ester formulations of 2,4-D (DAS, 2011a). Additionally, the 2,4-D choline salt is reported to resolve many of the chemical incompatibilities currently associated with the mixing of 2,4-D amine and glyphosate potassium salts in tank mixes (DAS, 2011a). DAS plans to market a premix of 2,4-D choline salt and glyphosate for use with DAS-68416-4 soybean (DAS, 2011a). Tank mixes of glyphosate and 2,4-D are already in use for control of mixed weeds in the pre-plant stage in no-tillage weed control programs (Loux et al., 2011). DAS submitted a draft label for a new end-use formulation for 2,4-D with EPA in April 2011 (DAS, 2011a, 2011b) and will be submitting a new label for the 2,4-D and glyphosate premix. DAS has indicated that the premix application directions will be the same as those for DAS-40278-9 corn, since the DAS 2,4-D choline salt and glyphosate premix will be made to use on either DAS-68416-4 and DAS-40278-9 corn (DAS, 2012). APHIS assumes for the purposes of this analysis that, if and when the new premix becomes available, the mixture of herbicides will be used by growers consistently with the EPA-approved label.

Based on the above information, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to affect changes in tillage, crop rotation, or agronomic inputs.

5.4 Cumulative Impacts: Soybean Seed Production

Based on current trends, GE soybean are likely to continue to dominate soybean production. GE soybean varieties were grown on more than 94% of soybean acres in 2011 (USDA-ERS, 2011b). To the extent that growers see value in the traits offered by a stacked DAS-68416-4 soybean, this variety may replace existing soybean varieties. The availability of a stacked DAS-68416-4 soybean is not anticipated to change cultivation areas for soybean production in the U.S. Because changes in the agronomic practices and locations for soybean seed production are not expected, there are no cumulative effects identified for either GE or non-GE seed production with the potential commercial availability of a stacked DAS-68416-4 soybean.

5.5 Cumulative Impacts: Organic Soybean Production

A determination of nonregulated status of DAS-68416-4 soybean is not expected to change the market demands for GE soybean or soybean produced using organic methods. Data from USDA-ERS indicates that in 2011, 94% of all soybean grown in the U.S. were GE varieties (USDA-ERS, 2011b). In 2008, organic soybean varieties were grown on less than 1% of the 75.2 million acres planted with soybean in the U.S. (USDA-ERS, 2010b). Based upon information on recent trends, adding GE varieties to the market is not related to the ability of organic production systems to maintain their market share. Since 1994, nine GE soybean events or lines have been determined by APHIS to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act. Organic production of soybeans grew from 82,143 acres in 1997 to a maximum of 174,467 acres in 2001. Since 2001, the total acreage of organic soybean production has experienced a slight decline in growth over time, with 125,621 acres planted in 2008 (USDA-ERS, 2010b). The decline of organic soybean acreage has been attributed to high prices being paid for conventional soybean and high fuel costs (McBride and Greene, 2008) and not the adoption rate of GE soybean. Based on the trend in the cultivation of GE soybean, non-GE, and organic soybean varieties, and the corresponding production systems to maintain varietal integrity are likely to remain the same, APHIS has determined that there are no cumulative impacts to organic soybean production from a determination of nonregulated status of DAS-68416-4 soybean.

5.6 Cumulative Impacts: Soil Quality

No cumulative effects on soil quality have been identified associated with a determination of nonregulated status of DAS-68416-4 soybean. A determination of nonregulated status for DAS-68416-4 soybean would not change agronomic practices affecting the quality of soil cultivated in commercial soybean production. DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. If DAS-68416-4 soybean became commercially available and were stacked with other transgenic herbicide-tolerance traits, depending on the extent of its adoption, it may contribute to sustaining conservation tillage in U.S. soybean production that both directly and indirectly impacts soil quality. Stacking DAS-68416-4 soybean with glyphosate would enable use of a combination of robust rates of different herbicide modes of action to be applied to soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially lead to a reduction in crop residue and SOM (Towery and Werblow, 2010). This could subsequently decrease soil stability, soil structure, and infiltration and water holding capacity, as well as increase the potential for wind and water erosion (USDA-NRCS, 1996).

Use of herbicides on DAS-68416-4 soybean alone or stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean cultivars grown (USDA-ERS, 2011b). DAS-68416-4 soybean alone or stacked with other traits would likely replace other herbicide-tolerant soybeans currently being cultivated. The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment..

Based on the above information, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have a negative impact on soil resources; rather, there may be slight beneficial cumulative impacts to soil quality from sustaining conservation tillage rates in soybean production.

5.7 Cumulative Impacts: Water Resources

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides. No changes in water use or irrigation practices currently used in commercial soybean production are expected.

DAS has indicated its intention to stack DAS-68416-4 soybean with other nonregulated soybean varieties, particularly varieties expressing tolerance to the herbicide glyphosate. Some glyphosate-tolerant crops, also identified as "Roundup Ready®," have had nonregulated status since 1994 when glyphosate-tolerant soybean was determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act (see http://www.aphis.usda.gov/biotechnology/not_reg.html) (USDA-APHIS, 1994). Use of herbicides on DAS-68416-4 soybean alone or if stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to.. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean varieties produced (USDA-ERS, 2011b). DAS-68416-4 soybean, alone or stacked with other traits, would likely replace other herbicide-tolerant soybeans currently being cultivated.

Stacking DAS-68416-4 soybean with glyphosate-tolerance traits would enable the use of a combination of robust rates of different herbicide modes of action to be applied to soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially lead to increased sedimentation and agricultural chemical pollutant offloading to surface water from soil erosion (Towery and Werblow, 2010). Glyphosate is already used on soybean in both conventional and GE varieties and the impacts of glyphosate use on water resources are well documented. Although glyphosate is very soluble in water, it is strongly adsorbed to soils; consequently, glyphosate is unlikely to leach into groundwater or surface water runoff following application (Giesy et al., 2000; US-EPA, 1993). Relying on toxicological data, bioaccumulation and biodegradation studies, and acute and chronic tests on fish and other aquatic organisms, EPA has determined that "the potential for environmental effects of glyphosate in surface water is minimal" (US-EPA, 2002b).

The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

The potential future cultivation of a stacked soybean variety and the associated use of glyphosate in addition to 2,4-D is not expected to result in cumulative effects to water resources. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have a negative impact on water resources.

5.8 Cumulative Impacts: Air Quality

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides. As a result, no changes in air quality are aniticipated.

DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). If combined with a glyphosate tolerance trait, DAS-68416-4 soybean would enable growers to use a combination of herbicides with different modes of action on soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and

Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially impact conservation tillage. The future development and cultivation of a stacked DAS-68416-4 soybean variety presenting the additional tolerance to glyphosate is not likely to increase the number of acres of soybean being treated with glyphosate, since, in 2011, more than 90% of soybean acres were planted with GE glyphosate-tolerant soybeans (USDA-ERS, 2011b). DAS-68416-4 soybean, either alone or combined with other traits, would likely replace these other GE herbicide-tolerant soybeans currently being cultivated. Therefore, it is expected that combining other herbicide tolerance traits with those of DAS-68416-4 soybean would not increase the overall number of acres that herbicide would be applied to. Herbicide use would be in accordance with per application an per year rates approved by EPA.

DAS plans to market a premix of the new 2,4-D choline salt and glyphosate for use with DAS-68416-4 soybean (DAS, 2011a). DAS has developed a new choline salt 2,4-D formulation reportedly with lower volatility and odor than other forms of 2,4-D (DAS, 2011a), reducing its potential impact on air quality. Information from DAS indicates that the 2,4-D choline salt formulation has lower volatility and decreased drift than other 2,4-D formulations (DAS, 2011a). According to DAS, the decrease in volatility also results in lower odor associated with this product (DAS, 2011a). Technical information supporting this information has been submitted to the EPA as part of DAS' application for a new pesticide registration. Glyphosate is already used in soybean in both conventional and Roundup Ready® varieties. The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment. The potential future cultivation of a stacked soybean variety and the associated use of glyphosate in addition to 2,4-D is not expected to result in cumulative effects to air quality. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would have a negative impact on air quality.

5.9 Cumulative Impacts: Climate Change

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. Some agricultural practices, such as tillage, can contribute to climate change through releasing GHG emissions from soil and emissions from associated fuel-burning equipment (Brookes and Barfoot, 2006; CAST, 2009). DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). If combined with a glyphosate tolerance trait, DAS-68416-4 soybean would enable growers to use a combination of herbicides with different modes of action on soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially impact conservation tillage. The continued use of conservation tillage associated with GE crops may reduce GHG emissions as a result of increased carbon sequestration in soils, decreased fuel consumption, and the reduction of nitrogen soil amendments (Towery and Werblow, 2010).

Use of herbicides on DAS-68416-4 soybean alone or stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean cultivars grown (USDA-ERS, 2011b). DAS-68416-4 soybean alone or stacked with other traits would likely replace other herbicide-tolerant soybeans currently being cultivated.

APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an impact on climate change.

5.10 Cumulative Impacts: Animal Communities

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). If combined with a glyphosate tolerance trait, DAS-68416-4 soybean would enable growers to use a combination of herbicides with different modes of action on soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially impact conservation tillage. Reduced tillage improves habitat value through increased water quality, availability of waste grain, retention of cover in fields, and increased populations of invertebrates (Brady, 2007; Sharpe, 2010).

DAS-68416-4 soybean has been shown to have no toxic effects to animals. The FDA has completed its consultation on the safety of DAS-68416-4 soybean as animal feed (US-FDA, 2011a). FDA has previously evaluated the safety of soybean lines containing the PAT protein conferring glufosinate tolerance and no new evaluation is required (Appendix C) (US-FDA, 1998a, 1998b). DAS intends to stack DAS-68416-4 soybean with soybean lines containing the 5-enolpyruvylshikimate-3-phosphate synthase (*cp4 epsps*) gene encoding the CP4 EPSPS protein conferring glyphosate tolerance. FDA has previously evaluated the safety of the CP4 EPSPS protein for feed and found no toxic effects to animals (US-FDA, 1995).

Tank mixes of glyphosate and 2,4-D are already in use for control of mixed weeds in the pre-plant stage in no-tillage weed control programs (Loux et al., 2011). DAS plans to market a premix of 2,4-D choline salt and glyphosate for use with the stacked DAS-68416-4 soybean hybrid (DAS, 2011a). DAS has developed a new choline salt 2,4-D formulation that has a lower volatility and odor than other forms of 2,4-D (DAS, 2011a, 2011c). DAS also indicates that the 2,4-D choline salt formulation has decreased drift than other 2,4-D formulations (DAS, 2011a). Technical information supporting this information has been submitted to the EPA as part of DAS' application for a new pesticide registration (see Appendix B) (DAS, 2011g).

Glyphosate is already used in soybean in both conventional and Roundup Ready® varieties. The herbicide has been previously reviewed by EPA for impacts on non-target organisms and is currently being evaluated as part of the reregistration review process, scheduled to be completed in 2015 (US-EPA, 1993, 2009a, 2009d). The draft premix label and application rates for use on DAS-68416-4 have not yet been submitted to EPA. However, the premix application directions will be the same as those for DAS-40278-9 corn, since the DAS 2,4-D choline salt and glyphosate premix will be made to use on either DAS-68416-4 and DAS-40278-9 corn (DAS, 2012). APHIS assumes, for the purposes of this analysis, that if and when the new premix becomes available, that the mixture of herbicides will be used by growers consistent with the EPA label application rate. The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

DAS is developing a robust stewardship program that would include technological advancements in application and off-target movement of 2,4-D choline salt and the premix of 2,4-D and glyphosate for application to DAS-68416-4 soybean stacked with glyphosate resistance, including lower volatility and reduction in the amount of driftable spray droplets (DAS, 2011a, 2011d) reducing potential impacts to non-target organisms. DAS is developing several media venues to educate and facilitate adoption of the technology and decision management tools with incentives to ensure the proper use and stewardship of the traits and chemical technologies (DAS, 2010).

Use of herbicides on DAS-68416-4 soybean alone or stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean cultivars grown (USDA-ERS, 2011b). DAS-68416-4 soybean alone or stacked with other traits would likely replace other herbicide-tolerant soybeans currently being cultivated.

APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an adverse impact on animal communities.

5.11 Cumulative Impacts: Plants Communities

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). If combined with a glyphosate tolerance trait, DAS-68416-4 soybean would enable growers to use a combination of herbicides with different modes of action on soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially impact conservation tillage. Reduced tillage improves soil quality and reduces soil erosion, sustaining both crop and non-crop plants.

Tank mixes of glyphosate and 2,4-D are already in use for control of mixed weeds in the pre-plant stage in no-tillage weed control programs (Loux et al., 2011). DAS plans to market a premix of 2,4-D choline salt and glyphosate for use with the stacked DAS-68416-4 soybean hybrid (DAS, 2011a). According to DAS, the new choline salt 2,4-D formulation has lower volatility and odor than other forms of 2,4-D. Information from DAS indicates that the 2,4-D choline salt formulation has, in addition to the lower volatility, decreased drift than other 2,4-D formulations (DAS, 2011a). Technical information supporting this information has been submitted to the EPA as part of DAS' application for a new pesticide registration. Glyphosate is already used in soybean in both conventional and Roundup Ready® varieties. The herbicide has been previously reviewed by EPA for impacts on non-target plants and is currently being evaluated as part of the reregistration review process, scheduled to be completed in 2015 (US-EPA, 1993, 2009a, 2009d). The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

DAS-68416-4 soybean would provide alternatives to glyphosate in weed management systems, as 2,4-D and/or glufosinate will control the already glyphosate-resistant and hard to control broadleaf weeds. Integrated weed management programs that use herbicides from different groups, vary cropping systems, rotate crops, and use mechanical as well as chemical weed control methods will prevent the selection of herbicide-resistant weed populations (Green and Owen, 2011; Gunsolus, 2002; Powles, 2008; Sellers et al., 2011). DAS would require growers that purchase DAS-68416-4 soybean or the stacked variety also conferring tolerance to glyphosate to sign a grower agreement that provides the terms and conditions for the authorized use of the technology (DAS, 2011d). The grower agreement will include a provision requiring them to follow the product use guide and all EPA pesticide label requirements that mitigate the potential for the development of 2,4-D, glufosinate and glyphosate-resistant weeds. DAS is also developing a robust stewardship program that would include technological advancements in application and off-target movement of 2,4-D choline salt and the premix of 2,4-D and glyphosate for application to DAS-68416-4 soybean stacked with glyphosate resistance, including lower volatility and reduction in the amount of driftable spray droplets (DAS, 2011a, 2011d) reducing potential impacts to non-target plants. DAS is developing several media venues to educate and facilitate adoption of the technology and decision management tools with incentives to ensure the proper use and stewardship of the traits and chemical technologies (DAS, 2010).

Use of herbicides on DAS-68416-4 soybean alone or stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean cultivars grown (USDA-ERS, 2011b). DAS-68416-4 soybean alone or stacked with other traits would likely replace other herbicide-tolerant soybeans currently being cultivated.

APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an adverse impact on plant communities.

5.12 Cumulative Impacts: Gene Flow and Weediness

The reproductive characteristics of the DAS-68416-4 soybean are substantially equivalent to other GE and non-GE soybean varieties (DAS, 2010). Given the reproductive characteristics of soybean, the probability for cross-pollination is low (Caviness, 1966; Ray et al., 2003). While cross-pollination can occur between adjacent plants and adjacent rows, it is unlikely that DAS-68416-4 soybean or potential future varieties of DAS-68416-4 soybean stacked with other traits would be grown in the same fields as other soybean varieties. Methods commonly used to ensure seed purity such as isolation distances and rotation cycles that specify a minimum number of years between crops (Conner et al., 2003) would further minimize vertical gene transfer. Gene movement between sexually compatible soybean varieties would be no greater for a stacked DAS-68416-4 soybean than it is for other non-GE or GE cultivars. The potential for horizontal gene flow to other unrelated organisms would be highly unlikely (USDA-APHIS, 2012).

APHIS has evaluated the weediness characteristics of the DAS-68416-4 soybean and has concluded that it would not pose a plant pest risk (USDA-APHIS, 2012). Similarly, a soybean variety including DAS-68416-4 soybean herbicide-tolerance traits with glyphosate tolerance would not be expected to exhibit any weediness characteristics that would pose a plant pest risk. Soybeans seldom exhibit dormancy and require specific environmental conditions to grow the following year (OECD, 2000), although volunteer soybean has been known to occur in some of the warmer regions of the U.S. In addition, volunteer soybean do not compete well with other crops and are easily controlled with common agronomic practices. In spite of this, DAS-68416-4 soybean stacked with other herbicide-tolerant traits may exacerbate management of volunteer soybeans in regions in which they are prone, especially in crops with herbicide tolerance to the same mode(s) of action. Management of these soybeans may require the use of more narrow-spectrum herbicides (such as atrazine in maize), or more aggressive mechanical control methods. Similarly, the rotation of crops with tolerance to herbicides with differing modes of action used as part of an integrated system to control the development of herbicide-resistant weeds (Beckie and Owen, 2007) may aid in the control of volunteer stacked soybean varieties. While additional management practices for the control of volunteer stacked soybean varieties in rotation with other crops may be needed, these requirements are not expected to be anything beyond common agronomic practices.

APHIS has not identified any cumulative effects on gene movement and weediness that would occur from a determination of nonregulated status to DAS-68416-4 soybean.

5.13 Cumulative Impacts: Microorganisms

A determination of nonregulated status for DAS-68416-4 soybean is not expected to change any agronomic practices for the commercial production of soybean other than the application of certain herbicides. The factors that influence structure of the soil microbial community are complex. Similar to other GE crops, there is potential for both direct and indirect impacts to microorganisms from a determination of nonregulated status of DAS-68416-4 soybean, as well as the production of any potential varieties stacked with tolerance to multiple herbicides or other traits. Field studies conducted by DAS and reviewed by APHIS determined that there were no differences in agronomic performance between 2,4-D and glufosinate sprayed and non-sprayed

DAS-68416-4 soybean (DAS, 2010; USDA-APHIS, 2012), suggesting no meaningful changes to the microbial community that influence soybean growth and health.

The main factors affecting microbial population size and diversity include soil type (texture, structure, organic matter, aggregate stability, pH, and nutrient content), plant type (providers of specific carbon and energy sources into the soil), and agricultural management practices (crop rotation, tillage, herbicide and fertilizer application, and irrigation) (Garbeva et al., 2004). A determination of nonregulated status for DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. If DAS-68416-4 soybean became commercially available and were stacked with other transgenic herbicide-tolerance traits, depending on the extent of its adoption, it may contribute to sustaining conservation tillage. Stacking DAS-68416-4 soybean with glyphosate would enable use of a combination of robust rates of different herbicide modes of action to be applied to soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially lead to a reduction in crop residue and SOM (Towery and Werblow, 2010). Maintaining adequate residue in the first three inches of the surface provides for a cooler and moister environment, increasing substrates and food for microorganisms (USDA-NRCS, 1996).

Tank mixes of glyphosate and 2,4-D are already in use for control of mixed weeds in the pre-plant stage in no-tillage weed control programs (Loux et al., 2011). DAS plans to market a premix of 2,4-D choline salt and glyphosate for use with the stacked DAS-68416-4 soybean hybrid (DAS, 2011a). DAS has developed a new choline salt 2,4-D formulation that is reported to have lower volatility and odor than other forms of 2,4-D (DAS, 2011c). Information from DAS indicates that the 2,4-D choline salt formulation, in addition to lower volatility, has decreased drift than other 2,4-D formulations (DAS, 2011a). Technical information supporting this information has been submitted to the EPA as part of DAS' application for a new pesticide registration. Glyphosate is already used in soybean in both conventional and Roundup Ready® varieties. The herbicide has been previously reviewed by EPA for impacts on the environment and is currently being evaluated as part of the reregistration review process, scheduled to be completed in 2015 (US-EPA, 1993, 2009a, 2009d). The draft premix label and application rates for use on DAS-68416-4 have not yet been submitted to EPA. However, the premix application directions will be the same as those for DAS-40278-9 corn, since the DAS 2,4-D choline salt and glyphosate premix will be made to use on either DAS-68416-4 and DAS-40278-9 corn (DAS, 2012).

Investigations of the impact of glyphosate on microorganisms are mixed (Weaver et al., 2007). Haney et al. (2002) and Araujo et al. (2003) report that glyphosate is mineralized by microorganisms that leads to an increase in their population and activity, while Busse et al. (2001) and Weaver et al. (2007) found little evidence of changes to soil microorganism's population and activity and any declines recorded were small and not consistent throughout the season. It also has been reported that the use of glyphosate increases the colonization of soil borne fungal pathogens such as *Fusarium* spp. (Fernandez et al., 2009; Huber, 2010; Kremer and Means, 2009); however, peer reviewed research that report a direct correlation of glyphosate use to an increase in plant disease is limited and any connection to impacts on yield has not been established (Camberato et al., 2011). The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

Use of herbicides on DAS-68416-4 soybean alone or stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean cultivars grown (USDA-ERS, 2011b). DAS-68416-4 soybean alone or stacked with other traits would likely replace other herbicide-tolerant soybeans currently being cultivated.

Based on the above information, APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have a negative impact on microorganisms; rather, there may be slight beneficial cumulative impacts from sustaining conservation tillage rates in soybean production.

5.14 Cumulative Impacts: Biodiversity

A determination of nonregulated status of DAS-68416-4 soybean is not expected to result in changes in the current soybean cropping practices, with the exception of potential changes in the use of certain herbicides for weed management. DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). If combined with a glyphosate tolerance trait, DAS-68416-4 soybean would enable growers to use a combination of herbicides with different modes of action on soybean, an approach proposed to mitigate the future development of herbicide-resistant weeds (Duke and Powles, 2009). Based on individual grower needs, this approach may reduce the need to use more aggressive tillage to control glyphosate-resistant weeds (Owen, 2011), which could potentially impact conservation tillage. Reduced tillage improves habitat value through increased water quality, availability of waste grain, retention of cover in fields, and increased populations of invertebrates (Brady, 2007; Sharpe, 2010). Incorporation of herbicide tolerance in the crop may facilitate the grower adoption of conservation and no-till strategies, thereby improving soil characteristics enhancing soil fauna and flora (Towery and Werblow, 2010), increasing the flexibility of crop rotation, and facilitating strip cropping (Fernandez-Cornejo et al., 2002), all contributing to the health of the faunal and floral communities in and around soybean fields that promotes biodiversity (Palmer et al., No Date; Sharpe, 2010).

Tank mixes of glyphosate and 2,4-D are already in use for control of mixed weeds in the pre-plant stage in no-tillage weed control programs (Loux et al., 2011). DAS plans to market a premix of 2,4-D choline salt and glyphosate for use with the stacked DAS-68416-4 soybean hybrid (DAS, 2011a). DAS has developed a new choline salt 2,4-D formulation with lower volatility and odor than other forms of 2,4-D. Information from DAS indicates that the 2,4-D choline salt formulation has lower volatility and decreased drift than other 2,4-D formulations (DAS, 2011a). Technical information supporting this information has been submitted to the EPA as part of DAS' application for a new pesticide registration. Glyphosate is already used in soybean in both conventional and Roundup Ready® varieties. The herbicide has been previously reviewed by EPA for impacts on the environment and is currently being evaluated as part of the reregistration review process, scheduled to be completed in 2015 (US-EPA, 1993, 2009a, 2009d).

The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment.

DAS is developing a robust stewardship program that would include technological advancements in application and off-target movement of 2,4-D choline salt and the premix of 2,4-D and glyphosate for application to DAS-68416-4 soybean stacked with glyphosate resistance, including lower volatility and reduction in the amount of driftable spray droplets (DAS, 2011a, 2011d) reducing potential impacts to non-target organisms. DAS is developing several media venues to educate and facilitate adoption of the technology and decision management tools with incentives to ensure the proper use and stewardship of the traits and chemical technologies (DAS, 2010).

Use of herbicides on DAS-68416-4 soybean alone or stacked with other herbicide-tolerant or other traits is not expected to increase the overall number of acres that herbicide would be applied to. Herbicide-tolerant soybeans currently comprise 94% of U.S. soybean cultivars grown (USDA-ERS, 2011b). DAS-68416-4 soybean alone or stacked with other traits would likely replace other herbicide-tolerant soybeans currently being cultivated.

APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an adverse impact on biodiversity.

5.15 Cumulative Impacts: Human Health

A determination of nonregulated status of DAS-68416-4 soybean would have no adverse impact on human health. FDA has completed an early food safety consultation requested by DAS (NPC 000009) of the AAD-12 protein expressed in DAS-68416-4 soybean with no questions regarding the DAS conclusion that there are no food safety concerns with this soybean variety (Krieger, 2008; US-FDA, 2010, 2011a). Similarly, FDA consultation on the safety of the PAT protein (BNF No. 000055) conferring glufosinate tolerance was completed in 1998, concluding there is no material difference for food safety or nutrition with soybean varieties that express the PAT protein (US-FDA, 1998a, 1998b).

DAS has developed a new choline salt 2,4-D formulation that requires a new EPA active ingredient registration under Section 3 of FIFRA. They have submitted to EPA plant metabolism and crop residue data for application of the new 2,4-D to DAS-68416-4 soybean, as well as the proposed labeling for the new 2,4-D formulation (DAS, 2011a, 2011b, 2011g). This information is currently under review by the EPA for safety, efficacy, and environmental concerns associated with the use of this product. Worker safety is taken into consideration by EPA in the pesticide registration process and reregistration process. Pesticides are regularly reevaluated by EPA for each pesticide to maintain its registered status under FIFRA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the

DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). Glyphosate tolerance in crops is conferred via the CP4 EPSPS protein derived from *Agrobacterium* spp. As specified in 40 CFR §174.523, EPA has reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (US-EPA, 2012b). This exemption is based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. The lack of any documented reports of adverse effects since the introduction of other glyphosate crops in 1993 suggests the safety of its use.

DAS plans to market premixed 2,4-D choline salt and glyphosate for use on DAS-68416-4 soybean hybridized with nonregulated glyphosate-tolerant soybean varieties (DAS, 2011a). Glyphosate has been widely used on soybean since the first glyphosate-tolerant soybean variety in 1994 was determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act (Heiniger, 2000). The use of glyphosate herbicide does not appear to result in adverse effects on development, reproduction, or endocrine systems in humans and other mammals. Under present and expected use conditions, and when used in accordance with the EPA label, glyphosate does not pose a human health risk. Pesticide residue tolerances for glyphosate are listed in 40 CFR § 180.364 and include acceptable concentrations for soybean seeds (US-EPA, 2011b).

DAS has indicated that a premix of the 2,4-D choline salt and glyphosate would be marketed for use with DAS-68416-4 (DAS, 2012) The draft premix label and application rates for use on DAS-68416-4 have not yet been submitted to EPA. However, the premix application directions will be the same as those for DAS-40278-9 corn, since the DAS 2,4-D choline salt and glyphosate premix will be made to use on either DAS-68416-4 and DAS-40278-9 corn (DAS, 2012). APHIS understands that the EPA will consider potential human health and worker safety of the combined premix and include precautions and measures protecting human health and worker safety in the premix label instructions for use. As the 2,4-D choline salt and glyphosate formulation would be sold as a premix, applicator exposure would be minimized during the handling process, and its reduced volatilization and spray drift properties would enhance worker safety during application (DAS, 2011a).

The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. When used consistently with the EPA label, pesticides present minimal risk to human health and worker safety. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an adverse impact on human health.

5.16 Cumulative Impacts: Animal Feed

A determination of nonregulated status of DAS-68416-4 soybean would have no adverse impact on animal health. FDA has completed its consultation on DAS-68416-4 soybean (US-FDA, 2011a). Based on a review of composition and nutritional characteristics of DAS-68416-4 soybean, including the expression of gene products, the FDA has concluded that DAS-68416-4 soybean is not materially different in any respect relevant to feed safety compared to soybean varieties already on the market (US-FDA, 2011a).

A new DAS 2,4-D/choline salt herbicide formulation (GF-2654 TS) has been developed by DAS that requires a new EPA active ingredient registration under Section 3 of FIFRA. DAS has submitted to EPA plant metabolism and crop residue data for application of 2,4-D on DAS-68416-4 soybean, as well as the proposed labeling for the new 2,4-D formulation (DAS, 2011a, 2011b, 2011g). Proposed label restrictions for DAS's new formulation of 2,4-D for use with DAS-68416-4 soybean would prohibit the use of treated soybean for hay or grazing (DAS, 2011b), but soybean meal used for animal feed is allowable. This information is currently under review by the EPA for safety, efficacy, and environmental concerns associated with the use of this product (DAS, 2011g).

DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). Glyphosate tolerance in crops is conferred via the CP4 EPSPS protein that has been reviewed by EPA for safety, establishing a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities as specified in 40 CFR §174.523 (US-EPA, 2012b). This exemption is based on a safety assessment including rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. No reports of adverse effects since the commercial availability of other glyphosate crops in 1996 suggest the safety of its use.

Glyphosate has been widely used on soybean since the first glyphosate-tolerant soybean variety was determined to be no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act in 1994. The use of glyphosate herbicide does not appear to result in adverse effects on development, reproduction, or endocrine systems in mammals. Under present and expected use conditions, and when used in accordance with the EPA label, glyphosate does not pose a health risk to animals as an animal feed concern. Pesticide residue tolerances for glyphosate are listed in 40 CFR § 180.364 and include acceptable concentrations for soybean forage, hay, hulls, and seed (US-EPA, 2011b).

DAS plans to market a premix of 2,4-D choline salt and glyphosate for use with DAS-68416-4 soybean (DAS, 2011a). DAS will be submitting a new label to EPA for the 2,4-D and glyphosate premix. The premix application directions will be the same as those for DAS-40278-9 corn, since the DAS 2,4-D choline salt and glyphosate premix will be made to use on either DAS-68416-4 and DAS-40278-9 corn (DAS, 2012). APHIS understands that the EPA will consider potential impacts to animals from dietary and environmental exposure to the combined premix and include precautions and measures protecting animal health in the premix label instructions for use.

The total amount of the mix of herbicides that may be applied to DAS-68416-4 soybean or subsequent varieties derived from it would be used in accordance with per application an per year rates approved by EPA. EPA's process ensures that each registered pesticide continues to meet the highest standards of safety to protect human health and the environment. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an adverse impact on animal health.

5.17 Cumulative Impacts: Domestic Economic Environment

There are potential implications of the change in herbicide use as a result of a determination of non-regulated status of DAS-68416-4 soybean, particularly with regard to the management of glyphosate-resistant weeds. DAS has indicated its intention to develop a stacked hybrid with DAS-68416-4 soybean and commercially available soybean varieties expressing glyphosate tolerance (DAS, 2010, 2011e, 2011f). This stacked soybean has the potential to improve grower management strategies for control of glyphosate-resistant weeds and also improve grower economics.

DAS-68416-4 soybean stacked with glyphosate tolerance would enable farmers to choose 2,4-D, glyphosate, and a mixture of the two for post-emergence weed control, and the additional ability to apply glufosinate. This herbicide management strategy is anticipated to sustain the long-term viability of the glyphosate-tolerant cropping system and preserve the benefits it provides to growers, the agricultural industry, and society (DAS, 2011f). The adoption of such a diverse weed management strategy, incorporating several herbicides with alternative modes of action, may initially cost more than the conventional single-herbicide approach, but these costs are offset by an increase in yields in those fields where the weed pressure has been reduced (Weirich et al., 2011).

As part of an economic impacts analysis, DAS compared alternative herbicide application strategies and application rates (DAS, 2011f). This analysis evaluated weed control strategies in glyphosate-tolerant soybean where glyphosate-resistant and inherently hard to control weeds had emerged. In this 2009 study, DAS compared the current herbicide strategies for weed control in conventional and glyphosate-tolerant soybean, and compared them against projected herbicide programs in glyphosate-tolerant soybean alone and a glyphosate-tolerant soybean stacked with the DAS-68416-4 soybean tolerance traits (DAS, 2011f). DAS based this analysis on inputs from grower surveys and university agronomists for soybean growers in Arkansas, Georgia, Illinois, Iowa, and Minnesota (DAS, 2011f). Market costs were calculated and normalized to a cost per acre for each strategy (DAS, 2011f). DAS found that the projected pounds per acre of herbicides required to control glyphosate-resistant weeds was lower with the Enlist™ Weed Control System than the alternatives (DAS, 2011f). Table 19 provides a summary of the results of this research.

The results of this study would suggest that with the adoption of the Enlist™ Weed Control System, growers would potentially apply fewer pounds of active ingredient per acre to control glyphosate-tolerant weeds, with a corresponding lower cost. The reader is cautioned to note that DAS' analysis was based upon a projected 2,4-D application rate of 0.71 lbs ae/A, which is approximately halfway between the per application rates of 0.5-1.0 lbs ae/A currently proposed for use in the Enlist™ Weed Control System, and was also based on 2009 prices for 2,4-D (DAS, 2011f). Trials conducted by DAS indicate a premix based upon 0.71 lbs ae/A of 2,4-D with 0.75 lbs ae/A of glyphosate achieved high levels of weed control (DAS, 2011f). The projected costs used by DAS in framing the cost comparison also do not consider any change in costs associated with the new 2,4-D formulation or any technology fees associated with this weed control system (DAS, 2011f). A grower adopting this Enlist™ Weed Control System would need to consider the comparative costs in balance with market demands in determining whether to adopt this new weed control strategy.

Based on these factors, no net negative cumulative effects on domestic economics have been identified associated with the cultivation of DAS-68416-4 soybean. If growers adopt the stacked variety and take advantage of the weed management strategy incorporating herbicides with different modes of action to control glyphosate-resistant weeds, local farm economics may be positively impacted. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action that would have an adverse impact on the domestic economic environment.

Table 19. **Summary of projected application rates and corresponding cost per acre comparing current soybean weed management strategies with three potential future strategies.**

Strategy	State									
	Arkansas		Georgia		Illinois		Iowa		Minnesota	
	Avg. Rate[1]	Avg. Cost[2,3]	Avg. Rate[1]	Avg. Cost[2,3]	Avg. Rate[1]	Avg. Cost[2,3]	Avg. Rate[1]	Avg. Cost[2,3]	Avg. Rate[1]	Avg. Cost[2,3]
Top Five Herbicide Programs in Glyphosate-Tolerant Soybean	1.59	$16.29	1.45	$18.92	1.64	$19.21	1.28	$16.05	1.32	$16.36
Projected Herbicide Programs in Glyphosate-Tolerant Soybean Stacked with DAS-68416-4 soybean Traits	1.87	$19.60	2.17	$19.84	2.22	$16.52	1.84	$15.23	1.90	$17.40
Top Five Herbicide Programs in Conventional Soybean[4,5]	N/A	N/A	N/A	N/A	0.84	$23.00	0.84	$23.00	0.84	$23.00
Projected Herbicide Programs in Glyphosate-Tolerant Soybean without DAS-68416-4 soybean Traits	1.97	$44.34	1.53	$28.64	1.68	$31.49	1.68	$31.49	1.68	$31.49

Source: (DAS, 2011f)

Notes:

1. Average Rate expressed in pounds of acid equivalent per acre (lbs ae/A), combining all herbicide strategies employed.
2. Average costs are based on costs per pound per acre of herbicides, normalized to reflect the percent of the acres treated in the survey area.
3. Note that the costs used to make this comparison were based on 2009 pricing for 2,4-D, and do not reflect the retail cost of the new formulation of 2,4-D or associated technology fees.
4. This data was developed assuming an application of 2,4-D at 0.71 lbs ae/A, which is less than that currently sought in the proposed registration and label use.
5. Data for this strategy in Arkansas and Georgia not reported.

5.18 Cumulative Impacts: Trade Economic Environment

A determination of nonregulated status of DAS-68416-4 soybean, including subsequent stacked herbicide tolerant varieties, are not expected to adversely impact the trade economic environment and may have a positive impact through increased yields in soybean areas affected by glyphosate-resistant weeds. Current and historic economic evidence indicates that herbicide-tolerant soybean technology has the potential to increase domestic production at lower cost. This trend of lower production costs could enhance international soybean trade by making U.S. soybean and soybean products more competitive in the global market.

The subsequent development and global adoption of these stacked varieties of DAS-68416-4 soybean could provide another herbicide-tolerant management choice for growers. As the value and benefits of these products are realized, particularly where glyphosate-resistant weeds have emerged, DAS-68416-4 soybean and subsequent stacked varieties may have potential for export as a seed product.

APHIS has determined that there are no past, present, or reasonable foreseeable actions that in aggregate with effects of the proposed action would negatively impact the trade economic environment.

6 THREATENED AND ENDANGERED SPECIES

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

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

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 critical habitat. To facilitate APHIS' ESA consultation process, APHIS met with the USFWS from 1999 to 2003 to discuss factors relevant to APHIS's regulatory authority and effects analysis for petitions for nonregulated status, and developed a process for conducting an effects determination consistent with the Plant Protection Act (PPA) of 2000 (Title IV of Public Law 106-224). This process is described in a decision tree document, which is presented in Appendix E. APHIS uses this process to help fulfill its obligations and responsibilities under Section 7 of the ESA for biotechnology regulatory actions.

APHIS' regulatory authority over GE organisms under the PPA 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). APHIS does not have authority to regulate the use of any herbicide, including 2,4-D, glufosinate, or glyphosate. After completing a plant pest risk analysis, if APHIS determines that DAS-68416-4 soybean does not pose a plant pest risk, then DAS-68416-4 soybean 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 the article is no longer regulated. As part of its EA analysis, APHIS is analyzing the potential effects of DAS-68416-4 soybean on the environment including any potential effects to threatened and endangered species and critical habitat. As part of this process, APHIS thoroughly reviews the GE product information and data related to the organism (generally a plant species, but may also be other genetically engineered organisms). For each transgene/transgenic plant, the following information, APHIS considers the following information, data, and questions:

- A review of the biology and taxonomy 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 impacts;
- 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 threatened or endangered species (TES) of plants or a host of any TES; 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 of DAS-68416-4 soybean may have, if any, on Federally-listed TES and species proposed for listing, as well as designated critical habitat and habitat proposed for designation. Based upon the scope of the EA and production areas identified in the Affected Environment section of the EA, APHIS obtained and reviewed the USFWS list of TES species (listed and proposed) for each state where soybean is commercially produced from the USFWS Environmental Conservation Online System (ECOS; as accessed 1/10/12 at http://ecos.fws.gov/tess_public/pub/stateListingAndOccurrence.jsp). Prior to this review, APHIS considered the potential for DAS-68416-4 soybean to extend the range of soybean production and also the potential to extend agricultural production into new natural areas. DAS' studies demonstrate that agronomic characteristics and cultivation practices required for DAS-68416-4 soybean are essentially indistinguishable from practices used to grow other soybean varieties, including other herbicide-tolerant varieties (DAS, 2010; USDA-APHIS, 2012). Although DAS-68416-4 soybean may be expected to replace other varieties of soybean currently cultivated, APHIS does not expect the cultivation of DAS-68416-4 soybean to result in new soybean acres to be planted in areas that are not already devoted to agriculture. Accordingly, the issues discussed herein focus on the potential environmental consequences of the determination of nonregulated status of DAS-68416-4 soybean on TES species in the areas where soybean are currently grown.

APHIS focused its TES review on the implications of exposure to the AAD-12 and PAT proteins in soybean, the interaction between TES and DAS-68416-4 soybean, including the potential for sexual compatibility and the ability to serve as a host for a TES (see Subsection 6.1, Potential Effects of DAS-68416-4 soybean on TES); and potential impacts of the use of 2,4-D and glufosinate herbicides to non-target organisms and the natural environment (see Subsection 6.2. Potential Effects of 2,4-D and Glufosinate Herbicides).

6.1 Potential Effects of DAS-68416-4 Soybean on TES

Threatened and Endangered Plant Species

The agronomic and morphologic characteristics data provided by DAS were used in the APHIS analysis of the weediness potential for DAS-68416-4 soybean, and further evaluated for the potential to impact TES. Agronomic studies conducted by DAS tested the hypothesis that the weediness potential of DAS-68416-4 soybean is unchanged with respect to conventional soybean (DAS, 2010). No differences were detected between DAS-68416-4 soybean and nontransgenic soybean in growth, reproduction, or interactions with pests and diseases, other than the intended effect of herbicide tolerance (DAS, 2010; USDA-APHIS, 2012). Soybean possesses few of the characteristics of successful weeds, and have been cultivated around the globe without any report that it is a serious weed or that it forms persistent feral populations (USDA-APHIS, 2010b). Soybean cannot survive in the majority of the country without human intervention, and it is easily controlled if volunteers appear in subsequent crops (see Section 2.1.2 Agronomic Practices and 2.3.3 Gene Flow and Weediness discussion). The expression of the AAD-12 protein providing the herbicide tolerance trait in DAS-68416-4 soybean is unlikely to appreciably improve seedling establishment or increase weediness potential. APHIS has concluded the determination of nonregulated status of DAS-68416-4 soybean does not present a plant pest risk, does not present a risk of weediness, and does not present an increased risk of gene flow when compared to other currently cultivated soybean varieties (USDA-APHIS, 2012).

APHIS evaluated the potential of DAS-68416-4 soybean to cross with a listed species. As previously discussed in the analysis of Gene Movement and Weediness and Plants, APHIS has determined that there is no risk to unrelated plant species from the cultivation of DAS-68416-4 soybean. Soybean is highly self-pollinating and can only cross with other members of *Glycine* subgenus *Soja*. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR; in the U.S. there are no *Glycine* species found outside of cultivation and the potential for outcrossing is minimal (OECD, 2000). After reviewing the list of threatened and endangered plant species in the U.S. states where soybean is grown, APHIS determined that DAS-68416-4 soybean would not be sexually compatible with any listed threatened or endangered plant species proposed for listing, as none of these listed plants are in the same genus nor are known to cross pollinate with species of the genus *Glycine*.

Based on agronomic field data, literature surveyed on soybean weediness potential, and no sexually compatibility of TES with soybean, APHIS has concluded that DAS-68416-4 soybean will have no effect on threatened or endangered plant species.

Threatened and Endangered Animal Species

Threatened and endangered animal species that may be exposed to the gene products in DAS-68416-4 soybean would be those TES that inhabit soybean fields and feed on DAS-68416-4 soybean. To identify potential effects on threatened and endangered animal species, APHIS evaluated the risks to threatened and endangered animals from consuming DAS-68416-4 soybean. Soybean commonly is used as a feed for many livestock. Additionally, wildlife may use soybean fields as a food source, consuming the plant or insects that live on the plants; although, TES generally are found outside of agricultural fields. Few if any TES are likely to use soybean fields because they do not provide suitable habitat. Only whooping crane (*Grus americana*), sandhill crane (*Grus canadensis pulla*), piping plover (*Charadrius melodus*), interior least tern (*Sterna antillarum*), and Sprague's pipit (*Anthus spragueii;* a candidate species) occasionally feed in farmed sites (USFWS, 2011a). These bird species may visit soybean fields during migratory periods, but would not be present during normal farming operations (Krapu et al., 2004; USFWS, 2011a). In a study of soybean consumption by wildlife in Nebraska, results indicated that soybeans do not provide the high energy food source needed by cranes and waterfowl (Krapu et al., 2004). The Delmarva fox squirrel (*Sciurus niger cinereus*), which inhabits mature forests of mixed hardwoods and pines, may be found adjacent to agricultural areas of the Delmarva Peninsula (USFWS, 2011b). The squirrel forages for food in woodlots and openings, such as farm fields, with a diet that mainly includes acorns, nuts/seeds of hickory, beech, walnut, and loblolly pine. They also feed on tree buds and flowers, fungi, insects, fruit, and seeds in the spring and mature, green pine cones in the summer and early fall (USF&WS, 1999). The Louisiana black bear (*Ursus americanus luteolus*), occurring in Louisiana, Mississippi, and Texas (Johnsen et al., 2005), may occasionally forage on soybean; however, other crops such as corn, sugarcane, and winter wheat are preferred by the species (MSU, No Date).

DAS has presented data on the food and feed safety of DAS-68416-4 soybean, evaluating the agronomic and morphological characteristics of DAS-68416-4 soybean, including compositional and nutritional characteristics, safety evaluations and toxicity tests, as compared to a conventional hybrid soybean variety (DAS, 2010). Compositional elements compared included moisture, protein, fat, carbohydrates, ash, minerals, dietary fiber, essential and non-essential amino acids, fatty acids, vitamins, and antinutrients (DAS, 2010; Herman et al., 2011b). As discussed in Section 4.6.1.2, the data collected indicate there is no difference in the composition and nutritional quality of DAS-68416-4 soybean compared with conventional soybean varieties, apart from the presence of the AAD-12 and PAT proteins. The results presented by DAS show that the incorporation of the *aad-12* and *pat* genes and the accompanying expression of the AAD-12 and PAT proteins in DAS-68416-4 soybean does not result in any biologically-meaningful differences between DAS-68416-4 soybean and non-transgenic hybrids.

The AAD-12 protein is expressed in DAS-68416-4 soybean through the incorporation of the *aad-12* gene which was derived from the gram-negative soil bacterium *Delftia acidovorans. Delftia acidovorans* (previously identified as *Pseudomonas acidovorans* and *Comamonas acidovorans*) can be found in soil, fresh water, activated sludge, and clinical specimens (DAS, 2010; Tamaoka et al., 1987; Von Gravenitz, 1985; Wen et al., 1999). The bacteria *D. acidovorans* can be used to transform ferulic acid into vanillin and related flavor metabolites

(DAS, 2010; Ramachachandra Rao and Ravishankar, 2000; Shetty et al., 2006; Toms and Wood, 1970) and has a safe history of use in the food processing industry.

The *pat* gene expressing the PAT protein in DAS-68416-4 soybean was derived from *Streptomyces viridochromogenes*, a gram-positive soil bacterium. The *pat* gene produced in DAS-68416-4 soybean is equivalent to that produced in other transgenic crops that have been previously deregulated by USDA (e.g., USDA 1996, USDA 2001, USDA 2004, USDA 2005). The food and feed safety of PAT has been assessed in these products and shown to present no significant food or feed safety risk.

The FDA has concluded its review of DAS' submittal of safety and nutritional data for DAS-68416-4 soybean (US-FDA, 2011b). DAS conducted safety evaluations based on Codex Alimentarius Commission procedures to assess any potential adverse effects to humans or animals resulting from environmental releases and consumption of DAS-68416-4 soybean (DAS, 2010; FAO, 2009; US-FDA, 2011c). These safety studies included evaluating protein structure and function, including homology searches of the amino acid sequences with comparison to all known allergens and toxins, an *in vitro* digestibility assay of the proteins, an acute oral toxicity feeding study in mice, and a feeding study in broiler chickens (DAS, 2010; Herman et al., 2011a; Herman et al., 2011b; US-FDA, 2011c). DAS-68416-4 soybean AAD-12 protein was determined to have no amino acid sequence similar to known allergens, lacked toxic potential to mammals, and was degraded rapidly and completely in gastric fluid (DAS, 2010; US-FDA, 2011c). At this time, the FDA considers the consultation on DAS-68416-4 soybean to be complete (US-FDA, 2011a). A copy of the FDA consultation is provided in Appendix C.

Additionally, FDA previously evaluated the safety and nutritional data for soybean containing the PAT protein and considered the consultation complete (Appendix C) (US-FDA, 1998a, 1998b). EPA, after reviewing the acute toxicity and digestibility of the PAT protein, determined "there is a reasonable certainty that no harm will result from aggregate exposure to the U. S. population, including infants and children, to the PAT protein and the genetic material necessary for its production (US EPA, 1997)." Based on their evaluation, EPA approved a tolerance exemption for the PAT protein and the genetic material necessary for its production in all plants.

Because there is no toxicity or allergenicity potential with DAS-68416-4 soybean, there would be no direct or indirect toxicity or allergenicity impacts on wildlife species that feed on soybean or the associated biological food chain of organisms. Consultations with FDA were successfully completed for both the AAD-12 and PAT proteins (Appendix C), which demonstrated a lack of toxicity and allergenicity of DAS-68416-4 soybean for human and animal consumption (US-FDA, 2011c). Therefore, based on these analyses, APHIS concludes that, although unlikely, consumption of DAS-68416-4 soybean plant parts (seeds, leaves, stems, pollen, or roots) would have no effect on any listed threatened or endangered animal species or animal species proposed for listing.

APHIS considered the possibility that DAS-68416-4 soybean could serve as a host plant for a threatened or endangered species. A review of the species list reveals that there are no members of the genus *Glycine* that serve as a host plant for any threatened or endangered species.

In addition to evaluating DAS' comparisons of DAS-68416-4 soybean with the non-transgenic near-isoline hybrid variety (Maverick) for potential agronomic and morphological differences, APHIS also considers the EPA and FDA regulatory assessments in making its determination of the potential impacts of a determination of nonregulated status of the new agricultural product. As discussed above in Animal and Plant Communities (Subsection 4.4) and Public Health (Subsection 4.5), DAS-68416-4 soybean would be the first commercially available food crop expressing the AAD-12 protein. In that regard, DAS has submitted food and feed safety and nutritional assessments for DAS-68416-4 soybean to the FDA. DAS also has submitted information to the EPA in support of exemptions from pesticide residue tolerance and the registration review for the use of 2,4-D on DAS-68416-4 soybean. The EPA review is discussed below in Subsection 6.2.

APHIS expects DAS-68416-4 soybean to replace some to all of the presently available glyphosate tolerant soybean varieties, but APHIS does not expect that DAS-68416-4 soybean will cause new soybean acres to be planted in areas that are not already devoted to agriculture. TES generally are found outside of agricultural fields. Combining the above information, cultivation of DAS-68416-4 soybean and its progeny is expected to have no effect on TES nor is it expected to adversely modify designated critical habitat compared to current agricultural practices. Based on this analysis, there is no apparent potential for significant impact on non-target organisms from DAS-68416-4 soybean, including beneficial organisms and threatened or endangered species, if APHIS were to make a determination of non-regulated status for the petition in whole. If APHIS chooses the no action alternative, there would also be no impact on non-target organisms.

After reviewing the possible effects of allowing the environmental release of DAS-68416-4 soybean, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. APHIS also considered the potential effect of a determination of nonregulated status of DAS-68416-4 soybean on designated critical habitat or habitat proposed for designation, and could identify no differences from effects that would occur from the production of other soybean varieties. Soybean is not considered a particularly competitive plant species and has been selected for domestication and cultivation under conditions not normally found in natural settings (US-EPA, 2010b). Soybean is not sexually compatible with, or serves as a host species for, any listed species or species proposed for listing. Consumption of DAS-68416-4 soybean 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 of DAS-68416-4 soybean, and the corresponding environmental release of this soybean variety will have no effect on listed species or species proposed for listing, and would not affect designated habitat or habitat proposed for designation. Because of this no-effect determination, consultation under Section 7(a)(2) of the Act or the concurrences of the USFWS or NMFS are not required.

6.2 Potential Impacts of the Use of 2,4-D and Glufosinate Herbicides

APHIS met with USFWS officials on June 15, 2011, to discuss whether APHIS has any obligations under the ESA regarding analyzing the effects of herbicide use associated with all GE crops on TES. As a result of these joint discussions, USFWS and APHIS have agreed that it is not necessary for APHIS to perform an ESA effects analysis on herbicide use associated with GE crops currently planted because EPA has both regulatory authority over the labeling of pesticides and the necessary technical expertise to assess pesticide effects on the environment under FIFRA. APHIS has no statutory authority to authorize or regulate the use of 2,4-D and glufosinate, or any other herbicide, by soybean growers. Under APHIS' current Part 340 regulations, APHIS only has the authority to regulate DAS-68416-4 soybean or any GE organism as long as APHIS believes it may pose a plant pest risk. For GE organisms, APHIS has no regulatory jurisdiction over any other risks associated with GE organisms including risks resulting from the use of herbicides or other pesticides on those organisms. Nevertheless, APHIS is aware that there may be potential environmental impacts resulting from the use of 2,4-D and glufosinate on DAS-68416-4 soybean, including potential impacts on TES and critical habitat, based on assessments performed by the EPA and as available in the peer reviewed scientific literature. APHIS is providing the available information of potential environmental impacts resulting from 2,4-D and glufosinate use on DAS-68416-4 soybean, below.

EPA Endangered Species Protection Program (ESPP)

In 1988, Congress enacted Public Law 100-478 (October 7, 1988) to in part address the relationship between ESA and EPA's pesticide labeling program (Section 1010), which required EPA to conduct a study, and report to Congress, on ways to implement EPA's endangered species pesticide labeling program in a manner that both complies with ESA and allows people to continue production of agricultural food and fiber. This law provided a clear sense that Congress wanted EPA to fulfill its obligation to conserve listed species, while at the same time consider the needs of agriculture and other pesticide users (70 FR 211 2005-11-02).

In 1988, EPA established the ESPP to meet its obligations under the ESA. EPA's Endangered Species Protection Program Web site[8] describes the EPA assessment process for endangered species. Some of the elements of that process, as reported on the website, are summarized below. The goal of EPA's ESPP is to carry out its FIFRA responsibilities in compliance with the ESA, without placing unnecessary burden on agriculture and other pesticide users consistent with Congress' intent.

EPA is responsible for reviewing pesticide information and data to determine whether a pesticide product may be registered for a particular use, including those uses associated with the approval of biotechnology products. As part of that determination, the Agency assesses whether listed endangered or threatened species or their designated critical habitat may be affected by use of the pesticide product. All pesticide products that EPA determines "may affect" a listed species or its designated critical habitat may be subject to the ESPP. If limitations on pesticide use are necessary to protect listed species in areas where a pesticide may be used, the information is related through Endangered Species Protection Bulletins. Bulletins identify the species of concern and the pesticide active ingredient that may affect the listed species. They also provide a description of the measures necessary to protect the species and contain a county-level map showing the geographic area(s) associated with the protection measures, depending on the susceptibility of the species. Bulletins are enforceable as part of the

[8] http://www.epa.gov/espp/

product label (http://www.epa.gov/oppfead1/endanger/basic-info.htm; last accessed on April 2011 and last updated by EPA on February 25, 2011).

EPA TES Evaluation Process

EPA evaluates listed species and their critical habitat concerns in connection with its actions under FIFRA. EPA's review of the pesticide under FIFRA is independent of APHIS' review and regulatory decisions under 7 CFR 340. EPA does not require data or analyses conducted by APHIS to complete its reviews. EPA evaluates extensive toxicity, ecological effects data, and environmental fate, transport and behavior data, most of which is required under FIFRA data requirements, to assess and determine how a pesticide will move through and break down in the environment. Risks to various taxa, e.g., birds, fish, invertebrates, plants and mammals are routinely assessed and used in EPA's determinations of whether a pesticide may be licensed for use in the U.S.

EPA's core pesticide risk assessment and regulatory processes address non-target species, not just threatened and endangered species. EPA has developed a comprehensive risk assessment process modeled after, and consistent with, EPA's numerous guidelines for environmental assessments (http://www.epa.gov/oppfead1/endanger/consultation/ecorisk-overview.pdf). The result of an assessment, which may go through several refinements, is to determine whether the potential effects of a pesticide's registration to a listed species will result in either a "no effect" or "may affect" determination. EPA consults with the USFWS and/or the National Marine Fisheries Service (NMFS) on determinations that "may affect" a listed species or adversely modify its critical habitat (http://www.epa.gov/oppfead1/endanger). As a result of either an assessment or consultation, EPA may seek to require changes to the use conditions specified on the label of the product. When such changes are necessary only in specific geographic areas rather than nationwide to ensure protection of the listed species, EPA implements these changes through geographically-specific Endangered Species Protection Bulletins, otherwise, these changes are applied to the label for all uses of the pesticide.

Ecological Risks of 2,4-D and Glufosinate

The EPA conducts a pesticide registration review program pursuant to FIFRA Section 3(g) whereby the safety of each registered pesticide active ingredients is reviewed every 15 years to determine whether it continues to meet the FIFRA standard for registration. EPA is currently conducting reregistration review for glufosinate (US-EPA, 2007c), scheduled to be completed in 2012. In addition, EPA completed a reregistration review, referred to as a Reregistration Eligibility Document (RED), analysis for 2,4-D in 2005 (US-EPA, 2005d) and is scheduling registration review to be completed in 2015..

The 2,4-D has been used as an herbicide since the mid-1940s (US-EPA, 2005d). Currently over 600 end-use products are registered for use on over 300 distinct agricultural and residential sites, and there are over 100 tolerances for 2,4-D listed in the CFR (US-EPA, 2005d). As part of the reregistration analysis for 2,4-D (US-EPA, 2005d) completed in 2005, EPA considered human health risk and ecological risks associated with potential exposure to 2,4-D through multiple pathways (US-EPA, 2005d). The effects associated with 2,4-D are summarized in the RED fact sheet for the herbicide (US-EPA, 2005a). The RED for 2,4-D required registrants of

2,4-D to provide proximity information on Federally protected freshwater fish, invertebrates and vascular plants, estuarine/marine invertebrates, birds, mammals, and non-target terrestrial plants to 2,4-D use sites, and certain additional toxicity and environmental persistence studies that have since been completed (US-EPA, 2005c).

For terrestrial species, laboratory studies indicate that the ecological toxicity of 2,4-D is: (1) moderate to practically non-toxic to birds and does not exceed the agency's level of concern; (2) slightly toxic to small mammals on an acute oral basis; (3) practically non-toxic to the honey bee; and (4) toxic to terrestrial plants. The ecological risk quotients exceeded the Level of Concern for most organisms including non-target TES. However, as noted in EPA's risk characterization, many of the assumption used in the modeling of exposures were considered conservative, as such, risks to many organisms may be overestimated (US-EPA, 2005a, 2005c). Among the effects noted for terrestrial animals ws the potential for alteration of habitat and reduction of vegetative food supply from the effects of spray drift and runoff.

EPA determined that risks could be mitigated by modifying the approved label application rates and spray droplet size (US-EPA, 2005d). Similar concerns and mitigation practices were identified in the EPA's recent Pesticide Effects Determination evaluating the potential impacts of the use of 2,4-D on the Federally Threatened California Red-legged Frog and Alameda Whipsnake (US-EPA, 2009e). Note that the EPA has requested initiation of formal consultation under Section 7 of the Endangered Species Act to address the potential effects of 2,4-D on these two species (US-EPA, 2009e). The EPA's formal consultation request was based on the potential for direct and indirect effects due to decreases in prey items as well as potential habitat effects for all labeled uses except citrus and potato (US-EPA, 2009e).

The EPA is also currently undertaking a separate consultation with the NMFS on potential detrimental effects of 2,4-D on endangered and threatened Pacific salmonids (see http://www.epa.gov/oppfead1/endanger/litstatus/biop4-march2011.pdf). A draft biological opinion was published by the NMFS on March 1, 2011 (NOAA-NMFS, 2011) which concluded that the continued use of 2,4-D is likely to jeopardize the continued existence of 28 ESU and adversely modify or destroy critical habitats for 26 of these ESUs for these endangered and threatened salmonids. However, this determination is based on use patterns of aquatic applications and restoration activities with a lack of restrictions on where and when direct water applications can occur which would not be an authorized use per the EPA approved label for 2,4-D. While 2,4-D is potentially mobile, it degrades rapidly in soil. Dissipation studies indicate that more than 95% of 2,4-D moves less than six inches in soil from the point of application, somewhat more (12-18 inches) in sandy soils with heavy amounts of applied water (Senseman, 2007). The EPA has solicited public comments on the NMFS report as part of the process.

While these consultations are underway, EPA has allowed 2,4-D to remain on the market and is approved for continued use in accordance with all label requirements. The EPA is currently reviewing the petitioner's applications for label changes for the new use of 2,4-D on DAS-68416-4 soybean. The EPA's label review would be conducted consistent with the requirements that EPA consider potential impacts to all non-target species associated with these new uses. The proposed change in the use of 2,4-D on the DAS-68416-4 soybean is for terrestrial use only, and label restrictions on application and control of non-target spray drift and runoff would be employed; as such, the Preferred Alternative may avoid adverse impacts to aquatic TES species (DAS, 2011b).

The EPA is currently conducting a registration review for glufosinate (US-EPA, 2007c). Assessments of the toxicity of glufosinate on Federally protected species conducted by EPA indicated a relatively low risk to animals but high risk to plants. On an acute exposure basis, it is considered practically nontoxic to birds, mammals, and insects; slightly non-toxic to freshwater fish, slightly toxic to estuarine/marine fish; moderately toxic to freshwater and estuarine/marine invertebrates; and toxic to terrestrial and aquatic plants (US-EPA, 2008c). Non-target exposure for plants typically results from runoff or drift. While animals can also be affected from runoff and drift, ingestion is often the most important exposure pathway. The EPA has determined that the use of glufosinate "may affect" TES and, as part of the registration review, is currently conducting a comprehensive ecological risk assessment, including an endangered species assessment, for all glufosinate-ammonium uses to make a final determination of effects (US-EPA, 2008c). The EPA's Final Work Plan for Registration Review for glufosinate (US-EPA, 2008a) states that:

> "The planned ecological risk assessment will allow the Agency to determine whether glufosinate-ammonium use has "no effect" or "may affect" federally listed threatened or endangered species (listed species) or their designated critical habitat. If the assessment indicates that glufosinate-ammonium "may affect" a listed species or its designated critical habitat, the assessment will be refined. The refined assessment will allow the Agency to determine whether the use of glufosinate-ammonium is "likely to adversely affect" the species or critical habitat or "not likely to adversely affect" the species or critical habitat. When an assessment concludes that a pesticide's use "may affect" a listed species or its designated critical habitat, the Agency will consult with the U.S. Fish and Wildlife Service and National Marine Fisheries Service (the Services), as appropriate."

Submittals to this analysis can be found at www.Regulations.gov under docket designation EPA-HQ-OPP-2008-0190. Labeled uses of glufosinate are approved pending the outcome of the EPA's ecological risk analysis. DAS is not proposing any change in the currently permitted use rate of glufosinate on GE soybean.

EPA has approved the continued use of these two herbicides consistent with current label restrictions pending the outcome of the ecological risk assessments being conducted as part of the TES consultations for 2,4-D and the registration review of glufosinate.

There are legal precautions in place to reduce the possibility of exposure and adverse impacts to TES from application of 2,4-D and glufosinate to DAS-68416-4 soybean. These precautions include the EPA pesticide label restrictions and best practice guidance provided by DAS (for 2,4-D and DAS-68416-4 soybean) and Bayer (the manufacturer and label registrant for glufosinate). EPA will consider potential TES impacts as part of the label changes currently being considered for those changes in use provided by DAS-68416-4 soybean. Adherence to these label use restrictions by the pesticide applicator will ensure that the use of either herbicide will not adversely affect TES or critical habitat.

As discussed in Subsections 4.2.2, Cropping Practices, and 4.4, Animal and Plant Communities, DAS has announced its intention to market a new formulation of 2,4-D based on a choline salt (DAS, 2010, 2011a). The new formulation of 2,4-D is chemically identified as 2,4-dichlorophenoxyacetic acid (2-hydroxyethyl) trimethylammonium salt (DAS, 2011a). DAS has submitted a draft label and required supporting information and data to EPA for this new 2,4-D formulation for use with DAS-68416-4 soybean (DAS, 2011b, 2011g). Technical information supporting this pesticide registration package, including chemical and physical characteristics, environmental fate and effect, and toxicity data, are not publicly available. APHIS understands that the EPA will consider each of these characteristics in conducting its review, and that appropriate label use restrictions will be considered such that this new formulation will not adversely affect non-target species, including TES or critical habitat, by reducing potential exposures. Approved label application rates, and corresponding precautions and label use restrictions, have not yet been published by the EPA. APHIS assumes, for the purposes of this TES impacts analysis, that the new choline salt formulation of 2,4-D will not be used on DAS-68416-4 soybean or its progeny until a new pesticide use registration and corresponding label have been published by the EPA. APHIS also assumes that EPA's label for this new formulation will establish use precautions and restrictions so as to reduce potential exposures to listed species or species proposed for listing, designated critical habitat or habitat proposed for designation.

DAS has also announced its intention to market DAS-68416-4 soybean as a stacked variety by combining this trait via conventional hybridization techniques with other nonregulated varieties (DAS, 2011f). The initial stacked variety will combine the DAS-68416-4 soybean variety with a glyphosate-tolerant variety no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act, providing the grower with the option to combine several herbicides with different modes of action for control of weeds. As noted above for the use of 2,4-D and glufosinate, the label use restrictions and best management practices in place for the use of glyphosate are intended to reduce the possibility of exposure of TES to this herbicide.

EPA considered these potential effects as part of their review process and label use restrictions for glyphosate tolerant crops imposed under authority of FIFRA. To mitigate potential adverse effects to TES, EPA has imposed specific label use restrictions for glyphosate use when applied with aerial equipment including "The product should only be applied when the potential for drift to adjacent sensitive areas (e.g., residential areas, bodies of water, known habitat for threatened or endangered species, non-target crops) is minimal (e.g., when wind is blowing away from the sensitive areas)."

7 CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS, AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

7.1 Executive Orders with Domestic Implications

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

- ***EO 12898 (US-NARA, 2010), "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations,"*** 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,"*** acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children.

The No Action and Preferred Alternatives were analyzed with respect to EO 12898 and EO 13045. Neither alternative is expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

Available mammalian toxicity data associated with the AAD-12 and PAT proteins establish the safety of DAS-68416-4 soybean and its products to humans, including minorities, low-income populations, and children who might be exposed to them through agricultural production and/or processing. No additional safety precautions would need to be taken.

Based on the information submitted by the applicant and assessed by APHIS, DAS-68416-4 soybean is agronomically, phenotypically, and biochemically comparable to conventional soybean except for the introduced AAD-12 and PAT proteins. The information provided in the petition indicates that the two proteins, AAD-12 and PAT, expressed in DAS-68416-4 soybean are not expected to be allergenic, toxic, or pathogenic in mammals (USDA-APHIS, 2012). Also, FDA has completed biotechnology consultations on both proteins in the context of other food and feeds and indicated that they had no questions (US-FDA, 1998a, 2011a).

Human toxicity has also been thoroughly evaluated by the EPA in its development of pesticide labels for both herbicides (US-EPA, 2005c, 2008b). Pesticide labels include use precautions and restrictions intended to protect workers and their families from exposures. APHIS assumes that growers will adhere to these herbicide use precautions and restrictions. As discussed in Subsection 4.5, Human Health, the potential use of 2,4-D and glufosinate on DAS-68416-4 soybean at the proposed application rates would be no more than that currently approved for other crops and found by the EPA not to have adverse impacts to human health when used in accordance with label instructions. It is expected that EPA and USDA ERS would monitor the use of DAS-68416-4 soybean to determine impacts on agricultural practices, such as chemical use, as they have done previously for herbicide-tolerant products.

Based on these factors, a determination of nonregulated status of DAS-68416-4 soybean is not expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

The following executive order addresses Federal responsibilities regarding the introduction and effects of invasive species:

> ***EO 1311 (US-NARA, 2010),"Invasive Species,"*** states that Federal agencies take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause.

Soybean is not listed in the U.S. as a noxious weed species by the Federal government (USDA-NRCS, 2011c) nor is it listed as an invasive species by major invasive plant data bases. Cultivated soybean seed does not usually exhibit dormancy and requires specific environmental conditions to grow as a volunteer the following year (OECD, 2000). Any volunteers that may become established do not compete well with the planted crop and are easily managed using standard weed control practices. Soybean does not possess characteristics such as the tolerance for a variety of habitat conditions, rapid growth and reproduction, aggressive competition for resources, and the lack of natural enemies or pests (USDA-APHIS, 2012) that would make it a successful invasive plant. Non-engineered soybean, as well as other herbicide-tolerant soybean varieties, are widely grown in the U.S. Based on historical experience with these varieties and the data submitted by the applicant and reviewed by APHIS, DAS-68416-4 soybean plants are sufficiently similar in fitness characteristics to other soybean varieties currently grown and are not expected to become weedy or invasive.

The following executive order requires the protection of migratory bird populations:

> ***EO 13186 (US-NARA, 2010), "Responsibilities of Federal Agencies to Protect Migratory Birds,"*** states that 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.

Migratory birds may be found in soybean fields. While soybean does not meet the nutritional requirements for many migratory birds (Krapu et al., 2004), they may forage for insects and weed seeds found in and adjacent to soybean fields. As discussed in Subsection 4.4.1, Animal Communities, data submitted by the applicant has shown no difference in compositional and nutritional quality of DAS-68416-4 soybean compared with other GE corn or non-GE soybean, apart from the presence of the AAD-12 and PAT proteins. DAS-68416-4 soybean is not expected to be allergenic, toxic, or pathogenic in mammals. Both AAD-12 and PAT proteins have a history of safe consumption in the context of other food and feeds (DAS, 2010). Additionally, the FDA has completed its food safety consultation on the AAD-12 protein in DAS-68416-4 soybean and the PAT protein (US-FDA, 1998a, 1998b, 2011a). Based on APHIS' assessment of DAS-68416-4 soybean, it is unlikely that a determination of nonregulated status of DAS-68416-4 soybean would have a negative effect on migratory bird populations.

The environmental effects associated with 2,4-D are summarized in the EPA RED fact sheet for the herbicide (US-EPA, 2005a). Testing indicates that ecological toxicity of 2,4-D is moderate to practically non-toxic to birds and does not exceed the agency's LOC. On an acute exposure basis, glufosinate is considered practically nontoxic to birds (US-EPA, 2008b). Based on these factors, it is unlikely that the determination of nonregulated status of DAS-68416-4 soybean would have a negative effect on migratory bird populations.

7.2 International Implications

EO 12114 (US-NARA, 2010), "Environmental Effects Abroad of Major Federal Actions," requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories, and possessions that result from actions being taken.

APHIS has given this EO careful consideration and does not expect a significant environmental impact outside the U.S. in the event of a determination of nonregulated status of DAS-68416-4 soybean. All existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new soybean cultivars internationally apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR part 340.

Any international trade of DAS-68416-4 soybean subsequent to a determination of nonregulated status would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC) (IPPC, 2010). The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control" (IPPC, 2010). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds.

The IPPC establishes a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (172 countries as of March 2010). In April 2004, a standard for Pest Risk Analysis (PRA) of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11, Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for genetically engineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity of LMOs, which include those modified through biotechnology. The Protocol came into force on September 11, 2003, and 160 countries are Parties to it as of December 2010 (CBD, 2010). Although the U.S. is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S. exporters will still need to comply with those regulations that importing countries which are Parties to the Protocol have promulgated to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol and the required documentation.

LMOs imported for food, feed, or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11, Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the U.S. Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (NBII, 2010). These data will be available to the Biosafety Clearinghouse.

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines, and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the U.S., and within the OECD. NAPPO has completed three modules of the Regional Standards for Phytosanitary Measures (RSPM) No. 14, *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (NAPPO, 2009).

APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico, and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including Argentina, Brazil, Japan, China, and Korea.

7.3 Impacts on Unique Characteristics of Geographic Areas

A determination of nonregulated status of DAS-68416-4 soybean is not expected to impact unique characteristics of geographic areas such as park lands, prime farmlands, wetlands, wild and scenic areas, or ecologically critical areas.

DAS has presented results of agronomic field trials for DAS-68416-4 soybean. The results of these field trials demonstrate that there are no differences in agronomic practices between DAS-68416-4 soybean and non-GE hybrids. The common agricultural practices that would be carried out in the cultivation of DAS-68416-4 soybean are not expected to deviate from current practices, including the use of EPA-registered pesticides. The product is expected to be deployed on agricultural land currently suitable for production of soybean and replace existing varieties, and is not expected to increase the acreage of soybean production.

There are no proposed major ground disturbances; no new physical destruction or damage to property; no alterations of property, wildlife habitat, or landscapes; and no prescribed sale, lease, or transfer of ownership of any property. This action is limited to a determination of nonregulated status of DAS-68416-4 soybean. This action would not convert land use to nonagricultural use and, therefore, would have no adverse impact on prime farmland. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on agricultural lands planted to DAS-68416-4 soybean, including the use of EPA-registered pesticides. The Applicant's adherence to EPA label use restrictions for all pesticides is expected to mitigate potential impacts to the human environment.

With regard to pesticide use, a determination of nonregulated status of DAS-68416-4 soybean is likely to result in changes to the use of 2,4-D on soybean. The potential changes in herbicide use, including application rates and annual maximum allowable applications, are discussed in Subsection 4.2.2, Agronomic Practices. DAS has submitted an application to EPA to provide for this change in use for 2,4-D on DAS-68416-4 soybean(there is no expected change in glufosinate use from the currently approved application rate for soybeans). APHIS assumes that any new EPA label would provide for label use restrictions intended to mitigate potential impacts to the human environment, including potential impacts to unique geographic areas. As noted above, APHIS further assumes that the grower will closely adhere to EPA label use restrictions for all pesticides.

Potential impacts to geographic areas have been considered by the EPA in its evaluation of these two herbicides. In 2005, the EPA completed a reregistration analysis for 2,4-D which considered human health risk and ecological risks associated with potential exposure to 2,4-D in multiple pathways (US-EPA, 2005c). Although some risks were identified, the EPA determined that these risks could be mitigated by modifying the approved label application rates and spray droplet size (US-EPA, 2005c). Similar concerns and mitigation practices were identified in the EPA's recent Pesticide Effects Determination evaluating the potential impacts of the use of 2,4-D on the Federally Threatened California Red-legged Frog and Alameda Whipsnake (US-EPA, 2009e). Note that the EPA has requested initiation of formal consultation under Section 7 of the Endangered Species Act to address the potential effects of 2,4-D on these two species (US-EPA, 2009e). The EPA's formal consultation request was based on the potential for direct and indirect effects due to decreases in prey items, as well as potential habitat effects, for all labeled uses except citrus and potato (US-EPA, 2009e).

The EPA is also currently undertaking a separate consultation with the NMFS on potential detrimental effects of 2,4-D on endangered and threatened pacific salmonids (see http://www.epa.gov/oppfead1/endanger/litstatus/biop4-march2011.pdf). A draft biological opinion was published by the NMFS on March 1, 2011 (NOAA-NMFS, 2011) which concluded that the continued use of 2,4-D is likely to jeopardize the continued existence of 28 ESU and adversely modify or destroy critical habitats for 26 of these ESUs for these endangered and threatened salmonids. The EPA has solicited public comments on the NMFS report as part of the process.

While these consultations are underway, EPA has allowed 2,4-D to remain on the market and it is approved for continued use in accordance with all label requirements.

Glufosinate-ammonium was first registered for home use with the EPA in 1993 (OSTP, 2001). It is currently labeled under the trade name Ignite® 280 SL by Bayer CropScience LP (Bayer CropScience, 2011). The EPA is currently conducting a reregistration review for glufosinate with a forthcoming final decision scheduled in 2013 (US-EPA, 2008b). The Agency plans to conduct a comprehensive ecological risk assessment, including an endangered species assessment, for all glufosinate-ammonium uses. The Agency has requested additional aquatic nonvascular plant data to evaluate the extent of risk to aquatic plants imposed by the application of glufosinate-ammonium. The EPA's Final Work Plan for Registration Review (US-EPA, 2008a) states:

> "The planned ecological risk assessment will allow the Agency to determine whether glufosinate-ammonium use has "no effect" or "may affect" federally listed threatened or endangered species (listed species) or their designated critical habitat. If the assessment indicates that glufosinate-ammonium "may affect" a listed species or its designated critical habitat, the assessment will be refined. The refined assessment will allow the Agency to determine whether use of glufosinate-ammonium is "likely to adversely affect" the species or critical habitat or "not likely to adversely affect" the species or critical habitat. When an assessment concludes that a pesticide's use "may affect" a listed species or its designated critical habitat, the Agency will consult with the U.S. Fish and Wildlife Service and National Marine Fisheries Service (the Services), as appropriate."

Submittals to this analysis can be found at the Regulations.gov website under docket designation EPA-HQ-OPP-2008-0190. Labeled uses of glufosinate are approved pending the outcome of the EPA's ecological risk analysis.

Based on these findings, including the assumption that label use restrictions are in place to protect unique geographic areas and that those label use restrictions are adhered to, a determination of nonregulated status of DAS-68416-4 soybean is not expected to impact unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas.

7.4 National Historic Preservation Act (NHPA) of 1966 as Amended

The NHPA of 1966 and its implementing regulations (36 CFR 800) require 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.

APHIS' proposed action, a determination of nonregulated status of DAS-68416-4 soybean is not expected to adversely impact cultural resources on tribal properties. Any farming activity that may be taken by farmers on tribal lands would only be conducted at the tribe's request;

thus, the tribes would have control over any potential conflict with cultural resources on tribal properties.

APHIS' Preferred Alternative would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would it likely cause any loss or destruction of significant scientific, cultural, or historical resources. This action is limited to a determination of non-regulated status of DAS-68416-4 soybean.

APHIS' proposed action is not an undertaking that may directly or indirectly cause alteration in the character or use of historic properties protected under the NHPA. In general, common agricultural activities conducted under this action do not have the potential to introduce visual, atmospheric, or noise elements to areas in which they are used that could result in effects on the character or use of historic properties. For example, there is potential for increased noise on the use and enjoyment of a historic property during the operation of tractors and other mechanical equipment close to such sites. A built-in mitigating factor for this issue is that virtually all of the methods involved would only have temporary effects on the audible nature of a site and can be ended at any time to restore the audible qualities of such sites to their original condition, with no further adverse effects. Additionally, these cultivation practices are already being conducted throughout the soybean production regions. The cultivation of DAS-68416-4 soybean is not expected to change any of these agronomic practices that would result in an adverse impact under the NHPA.

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Appendix A Draft Label GF-2654 TS Submitted to U.S. EPA

DRAFT
Under Review by US EPA

Summary

Dow AgroSciences (DAS) has submitted a petition to the United States Department of Agriculture (USDA)'s Animal and Plant Health Inspection Service (APHIS) for the determination of nonregulated status for DAS-68416-4 soybeans, which provides tolerance to the herbicides 2,4-dichlorophenoxy acetic acid (2,4-D) and glufosinate.

This information in this draft label was submitted to the United States Environmental Protection Agency (EPA) supporting DAS' April 2011 application to EPA to register the use of a new end-use formulation of 2,4-D on DAS-68416-4 soybean.

DRAFT
Under Review by US EPA

1. GF-2654 TS "Draft" Label

GF-2654 TS

EPA Reg. No. 62719-XXX

Registration Notes:

Proposed Section 3 label.

(Base label):

GF-2654 TS

Herbicide

For control of broadleaf weeds in soybeans containing DAS-68416-4

Group	4	HERBICIDE

Active Ingredient:
2,4-Dichlorophenoxyacetic acid,
choline salt 56.3%
Other Ingredients 43.7%
Total..100.0%

2,4-dichlorophenoxyacetic acid - 38.4% - 3.8 lb/gal

Keep Out of Reach of Children

DANGER PELIGRO

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle. (If you do not understand the label, find someone to explain it to you in detail.)

Precautionary Statements

Hazards to Humans and Domestic Animals

Corrosive • Causes Irreversible Eye Damage • Harmful If Swallowed, Inhaled Or Absorbed Through The Skin

Do not get in eyes, on skin, or on clothing. Avoid breathing vapor or spray mist. Wash thoroughly with soap and water after handling.

Personal Protective Equipment (PPE)

Some materials that are chemical-resistant to this product are made of any waterproof material. If you want more options, follow the instructions for category A on an EPA chemical resistance category selections chart.

All mixers, loaders, applicators and handlers must wear:

- Long-sleeved shirt and long pants
- Shoes plus socks
- Chemical-resistant gloves
- Protective eyewear
- Chemical resistant apron when mixing or loading, cleaning up spills or equipment, or otherwise exposed to the concentrate

See engineering controls for additional requirements.

Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

Engineering Controls

When handlers use closed systems or enclosed cabs in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

User Safety Recommendations

Users should:

- Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.
- Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

First Aid

If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.

If on skin or clothing: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for treatment advice.

If swallowed: Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison control center or doctor. Do not give anything by mouth to an unconscious person.

If inhaled: Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice.

Have the product container or label with you when calling a poison control center or doctor, or going for treatment.

Note to Physician: Probable mucosal damage may contraindicate the use of gastric lavage.

Environmental Hazards

This product is toxic to fish and aquatic invertebrates. For terrestrial uses: Do not apply directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark. Drift or runoff may adversely affect aquatic invertebrates and non-target plants. Drift and runoff may be hazardous to aquatic organisms in water adjacent to treated areas. Do not contaminate water when disposing of equipment washwaters or rinsate.

This chemical has properties and characteristics associated with chemicals detected in groundwater. The use of this chemical in areas where soils are permeable, particularly where the water table is shallow, may result in groundwater contamination. Application around a cistern or well may result in contamination of drinking water or groundwater.

Agricultural Use Requirements
Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. Refer to the label booklet under "Agricultural Use Requirements" in the Directions for Use section for information about this standard.

(Storage and Disposal for rigid containers 5 gal or less)

Storage and Disposal
Do not contaminate water, food, or feed by storage or disposal.
Pesticide Storage: Keep container tightly closed when not in use. If exposed to subfreezing temperatures, the product should be warmed to at least 40°F and mixed thoroughly before using.
Pesticide Disposal: Pesticide wastes are toxic. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal law and may contaminate groundwater. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.
Container Handling: Nonrefillable container. Do not reuse or refill this container.
Triple rinse or pressure rinse container (or equivalent) promptly after emptying. **Triple rinse** as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container 1/4 full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. **Pressure rinse** as follows: Empty the remaining contents into application equipment or a mix tank and continue to drain for 10 seconds after the flow begins to drip. Hold container upside down over application equipment or mix tank or collect rinsate for later use or disposal. Insert pressure rinsing nozzle in the side of the container, and rinse at about 40 psi for at least 30 seconds. Drain for 10 seconds after the flow begins to drip. Then offer for recycling if available or puncture and dispose of in a sanitary landfill, or by incineration, or by other procedures allowed by state and local authorities.

(Storage and Disposal for refillable rigid containers larger than 5 gal)

Storage and Disposal
Do not contaminate water, food, or feed by storage or disposal.
Pesticide Storage: Keep container tightly closed when not in use. If exposed to subfreezing temperatures, the product should be warmed to at least 40°F and mixed thoroughly before using.
Pesticide Disposal: Pesticide wastes are toxic. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal law and may contaminate groundwater. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.
Container Handling: Refillable container. Refill this container with pesticide only. Do not reuse this container for any other purpose.
Cleaning the container before final disposal is the responsibility of the person disposing of the container. Cleaning before refilling is the responsibility of the refiller. To clean the container before final disposal, empty the remaining contents from this container into application equipment or a mix tank. Fill the container about 10% full with water and, if possible, spray all sides while adding water. If practical, agitate vigorously or recirculate water with the pump for two minutes. Pour or pump rinsate into application equipment or rinsate collection system. Repeat this rinsing procedure two more times. Then offer for recycling if available, or puncture and dispose of in a sanitary landfill, or by incineration, or by other procedures allowed by state and local authorities.

(Storage and Disposal for nonrefillable rigid containers larger than 5 gal)

Storage and Disposal

Do not contaminate water, food, or feed by storage or disposal.
Pesticide Storage: Keep container tightly closed when not in use. If exposed to subfreezing temperatures, the product should be warmed to at least 40°F and mixed thoroughly before using.
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Refer to label booklet for Directions for Use.

Notice: Read the entire label. Use only according to label directions. **Before using this product, read Warranty Disclaimer, Inherent Risks of Use, and Limitation of Remedies at end of label booklet. If terms are unacceptable, return at once unopened.**

In case of emergency endangering health or the environment involving this product, call 1-800-992-5994.

Agricultural Chemical: Do not ship or store with food, feeds, drugs or clothing.

EPA Reg. No. 62719-XXX EPA Est. _________

®Trademark of Dow AgroSciences LLC
Produced for
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

Net Contents _____

(cover, shipping container):

GF-2654 TS

Herbicide

For control of broadleaf weeds in soybeans containing DAS-68416-4

Active Ingredient:
2,4-Dichlorophenoxyacetic acid,
choline salt 56.3%
Other Ingredients 43.7%
Total...100.0%

2,4-dichlorophenoxyacetic acid - 38.4% - 3.8 lb/gal

Keep Out of Reach of Children

DANGER PELIGRO

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle. (If you do not understand the label, find someone to explain it to you in detail.)

Agricultural Use Requirements
Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. Refer to the label booklet under "Agricultural Use Requirements" in the Directions for Use section for information about this standard.

Refer to inside of label booklet for Directions for Use.

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Net Contents _____

(Page 1 through end):

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Hazards to Humans and Domestic Animals

DANGER

Corrosive • Causes Irreversible Eye Damage • Harmful If Swallowed, Inhaled Or Absorbed Through The Skin

Do not get in eyes, on skin, or on clothing. Avoid breathing vapor or spray mist. Wash thoroughly with soap and water after handling.

Personal Protective Equipment (PPE)

Some materials that are chemical-resistant to this product are made of any waterproof material. If you want more options, follow the instructions for category A on an EPA chemical resistance category selections chart.

All mixers, loaders, other applicators and handlers must wear:

- Long-sleeved shirt and long pants
- Shoes plus socks
- Chemical-resistant gloves
- Protective eyewear
- Chemical resistant apron when mixing or loading, cleaning up spills or equipment, or otherwise exposed to the concentrate

See engineering controls for additional requirements.

Follow manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

Engineering Controls

When handlers use closed systems or enclosed cabs in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

User Safety Recommendations

Users should:

- Wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.
- Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

First Aid

If in eyes: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice.
If on skin or clothing: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for treatment advice.
If swallowed: Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison control center or doctor. Do not give anything by mouth to an unconscious person.
If inhaled: Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice.

Have the product container or label with you when calling a poison control center or doctor, or going for treatment.

Note to Physician: Probable mucosal damage may contraindicate the use of gastric lavage.

Environmental Hazards

This product is toxic to fish and aquatic invertebrates. For terrestrial uses: Do not apply directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark. Drift or runoff may adversely affect aquatic invertebrates and non-target plants. Drift and runoff may be hazardous to aquatic organisms in water adjacent to treated areas. Do not contaminate water when disposing of equipment washwaters or rinsate.

This chemical has properties and characteristics associated with chemicals detected in groundwater. The use of this chemical in areas where soils are permeable, particularly where the water table is shallow, may result in groundwater contamination. Application around a cistern or well may result in contamination of drinking water or groundwater.

Directions for Use

It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Read all Directions for Use carefully before applying.

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application. For any requirements specific to your state or tribe, consult the agency responsible for pesticide regulation.

Agricultural Use Requirements

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This Standard contains requirements for the protection of agricultural workers on farms, forests, nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted-entry interval. The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 48 hours.

PPE required for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil, or water, is:

- Coveralls
- Chemical-resistant gloves made of any waterproof material
- Shoes plus socks
- Protective eyewear

Storage and Disposal

Do not contaminate water, food, or feed by storage or disposal.

Pesticide Storage: Keep container tightly closed when not in use. If exposed to subfreezing temperatures, the product should be warmed to at least 40°F and mixed thoroughly before using.

Pesticide Disposal: Pesticide wastes are toxic. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal law and may contaminate groundwater. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.

Nonrefillable containers 5 gallons or less:

Container Handling: Nonrefillable container. Do not reuse or refill this container.

Triple rinse or pressure rinse container (or equivalent) promptly after emptying. **Triple rinse** as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container 1/4 full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. **Pressure rinse** as follows: Empty the remaining contents into application equipment or a mix tank and continue to drain for 10 seconds after the flow begins to drip. Hold container upside down over application equipment or mix tank or collect rinsate for later use or disposal. Insert pressure rinsing nozzle in the side of the container, and rinse at about 40 psi for at least 30 seconds. Drain for 10 seconds after the flow begins to drip. Then offer for recycling if available or puncture and dispose of in a sanitary landfill, or by incineration, or by other procedures allowed by state and local authorities.

Refillable containers larger than 5 gallons:

Container Handling: Refillable container. Refill this container with pesticide only. Do not reuse this container for any other purpose.

Cleaning the container before final disposal is the responsibility of the person disposing of the container. Cleaning before refilling is the responsibility of the refiller. To clean the container before final disposal, empty the remaining contents from this container into application equipment or a mix tank. Fill the container about 10% full with water and, if possible, spray all sides while adding water. If practical, agitate vigorously or recirculate water with the pump for two minutes. Pour or pump rinsate into application equipment or rinsate collection system. Repeat this rinsing procedure two more times. Then offer for recycling if available, or puncture and dispose of in a sanitary landfill, or by incineration, or by other procedures allowed by state and local authorities.

Nonrefillable containers 5 gallons or larger:
Container Handling: Nonrefillable container. Do not reuse or refill this container.
Triple rinse or pressure rinse container (or equivalent) promptly after emptying. **Triple rinse** as follows: Empty the remaining contents into application equipment or a mix tank. Fill the container 1/4 full with water. Replace and tighten closures. Tip container on its side and roll it back and forth, ensuring at least one complete revolution, for 30 seconds. Stand the container on its end and tip it back and forth several times. Turn the container over onto its other end and tip it back and forth several times. Empty the rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Repeat this procedure two more times. **Pressure rinse** as follows: Empty the remaining contents into application equipment or a mix tank and continue to drain for 10 seconds after the flow begins to drip. Hold container upside down over application equipment or mix tank or collect rinsate for later use or disposal. Insert pressure rinsing nozzle in the side of the container, and rinse at about 40 psi for at least 30 seconds. Drain for 10 seconds after the flow begins to drip. Then offer for recycling if available, or puncture and dispose of in a sanitary landfill, or by incineration, or by other procedures allowed by state and local authorities.

Product Information

GF-2654 TS herbicide is intended for broadleaf weed control in soybeans containing the DAS-68416-4 trait.

Apply GF-2654 TS as a water spray when target weeds are actively growing. Application under drought conditions may give poor results. Generally, the lower dosages specified on this label will be satisfactory for young, succulent growth of susceptible weed species. For less susceptible species and under conditions where control is more difficult, use higher specified rates. Deep-rooted perennial weeds such as Canada thistle and field bindweed usually require repeated applications for satisfactory control. Consult your State Agricultural Experiment stations or Extension Service Weed Specialists for recommendations from this label that best fit local conditions.

Use Precautions and Restrictions

Be sure that use of GF-2654 TS conforms to all application regulations.

Chemigation: Do not apply this product through any type of irrigation system.

Do not aerially apply this product.

Use of this product in certain portions of California, Oregon, and Washington is subject to the January 22, 2004 Order for injunctive relief in Washington Toxics Coalition et al. v. EPA, C01-0132C, (W.D. W.A.). For further information, please refer to EPA website: http://www.epa.gov/espp/litstatus/wtc/index.htm.

Herbicide Resistance Management
2,4-D choline, the active ingredient in this product, is a Group 4 herbicide (synthetic auxin). Some naturally occurring weed biotypes that are tolerant (resistant) to 2,4-D may exist due to genetic variability in a weed population. Where resistant biotypes exist, the repeated use of herbicides with the same mode of action can lead to the selection for resistant weeds. Certain agronomic practices reduce the likelihood

that resistant weed populations will develop and can be utilized to manage weed resistance once it occurs.

Proactively implementing diversified weed control strategies to minimize selection for weed populations resistant to one herbicide or more is recommended. A diversified weed management program may include the use of multiple herbicides with different modes of action and overlapping weed spectrum with or without tillage operations and/or other cultural practices. Research has demonstrated that using the labeled rate and directions for use is important to delay the selection for resistance.

To aid in the prevention of developing 2,4-D resistant weeds, Dow AgroSciences recommends the following practices:

Herbicide Selection:

- Rotate the use of GF-2654 TS with non-auxin (non-Group 4) herbicides.
- Utilize a broad spectrum soil-applied herbicide as a foundation treatment.
- Utilize tank mixes of herbicides or sequential applications of herbicides with alternative modes of action.
- Avoid using more than two applications of a Group 4 herbicide, such as GF-2654 TS, within a single growing season unless mixed with another mode of action herbicide with overlapping spectrum.
- Apply full rates of GF-2654 TS at the specified time (correct weed size) to minimize escapes of tolerant weeds.

Crop Selection and Cultural Practices:

- Incorporate additional weed control practices whenever possible, such as mechanical cultivation, delayed planting, crop rotation, and weed-free crop seeds, as part of an integrated weed control program.
- Do not allow weed escapes to produce seeds, roots or tubers.
- Thoroughly clean plant residues from equipment before leaving fields suspected to contain resistant weeds.
- Scout fields after application to detect weed escapes or shifts in weed species.
- If resistance is suspected, treat weed escapes with an alternate mode of action or cultivation.
- Report any incidence of repeated non-performance of this product against a particular weed species to the local retailer, county extension agent, or Dow AgroSciences representative.

Because the presence of herbicide resistance in weed populations is difficult to detect prior to use, Dow AgroSciences accepts no liability for any losses that may result from the failure of this product to control weeds resistant to this mode of action. Incidents of non-performance should be reported to the local retailer, county extension agent, or Dow AgroSciences representative.

Spray Drift Management

A variety of factors including weather conditions (e.g., wind direction, wind speed, temperature, relative humidity) and method of application can influence pesticide drift. The applicator must evaluate all factors and make appropriate adjustments when applying this product.

Droplet Size

When applying sprays that contain GF-2654 TS as the sole active ingredient, or when applying sprays that contain GF-2654 TS mixed with other active ingredients, use drift reducing nozzle tips in accordance with manufacturer recommendations that produce a droplet size classification of coarse or coarser (ASABE S-572 standard).

Wind Speed

Do not apply at wind speeds greater than 15 mph. Only apply this product if the wind direction favors on-target deposition and there are no sensitive areas (including residential areas, bodies of water, known habitat for nontarget species, nontarget crops) within 250 feet downwind.

Temperature Inversions
If applying at wind speeds less than 3 mph, the applicator must determine if: a) conditions of temperature inversion exist, or b) stable atmospheric conditions exist at or below nozzle height. Do not make applications into areas of temperature inversions or stable atmospheric conditions.

Susceptible Plants
Do not apply under circumstances where spray drift may occur to food, forage, or other plantings that might be damaged or crops thereof rendered unfit for sale, use or consumption. Susceptible crops include cotton, okra, flowers, fruit trees, grapes (in growing stage), fruit trees (foliage), soybeans that do not contain the DAS-68416-4 trait, ornamentals, sunflowers, tomatoes, beans, other vegetables, and tobacco. Small amounts of spray drift that may not be visible may injure susceptible broadleaf plants.

Other State and Local Requirements
Applicators must follow all state and local pesticide drift requirements regarding application of 2,4-D herbicides. Where states have more stringent regulations, they must be observed.

Equipment
All ground application equipment must be properly maintained and calibrated using appropriate carriers or surrogates.

Groundboom Application
Use the minimum boom height based upon the nozzle manufacturer's recommendations.

Mixing Directions

GF-2654 TS – Alone
Mix GF-2654 TS only with water unless otherwise directed on this label. Add about half of the water to the mixing tank, then add GF-2654 TS with agitation, and finally the rest of the water with continuing agitation. **Note:** Adding oil, wetting agent, or other surfactant to the spray mixture may increase effectiveness on weeds, but also may reduce selectivity to crops resulting in crop damage.

GF-2654 TS - Tank Mix
When tank mixing, read and follow the label of each tank mix product used for precautionary statements, directions for use, weeds controlled, and geographic and other restrictions. Use in accordance with the most restrictive label limitations and precautions. Do not exceed any active ingredient's maximum use rates when tank mixing. Do not tank mix this product with any product containing a label prohibition against tank mixing with 2,4-D.

Tank Mix Compatibility Testing: A jar test is recommended prior to tank mixing to ensure compatibility of this product and other pesticides. Use a clear glass quart jar with lid and mix the tank mix ingredients in their relative proportions. Invert the jar containing the mixture several times and observe the mixture for approximately 1/2 hour. If the mixture balls-up, forms flakes, sludges, jels, oily films or layers, or other precipitates, it is not compatible and the tank mix combination should not be used.

Mixing with Liquid Micronutrient Fertilizer: This product may be combined with liquid micronutrient fertilizer suitable for foliar application to accomplish broadleaf weed control and fertilization of soybean in a single operation. Use liquid micronutrient fertilizer at rates recommended by the supplier. Chelated forms of micronutrient fertilizers are recommended to reduce potential for precipitate formation in the spray tank. Test for mixing compatibility as described above before mixing in a spray tank. Always read and follow directions for use and mixing instructions provided on micronutrient product label.

Sprayer Clean-Out
To avoid injury to desirable plants, thoroughly clean equipment used to apply this product before re-use or applying other chemicals.

1. Rinse and flush application equipment thoroughly after use at least three times with water. Dispose of all rinse water by applying to treatment area or applying to non-cropland area away from water supplies.
2. During the second rinse, add 1 quart of household ammonia for every 25 gallons of water or use commercially available tank cleaner solution. Circulate the solution through the entire system so that all internal surfaces are contacted (15 to 20 minutes). Let the solution stand for several hours, preferably overnight.
3. Flush the solution out of the spray tank through the boom.
4. Rinse the system twice with clean water, recirculating and draining each time.
5. Remove nozzles and screens and clean separately.
6. If equipment is to be used to apply another pesticide or agricultural chemical to a 2,4-D susceptible crop, additional steps may be required to remove all traces of 2,4-D, including cleaning of disassembled parts and replacement of hoses or other fittings that may contain absorbed 2,4-D.

Application Directions

Apply with calibrated ground equipment using sufficient spray volume to provide adequate coverage of target weeds or as otherwise directed in specific use directions. For best results in a broadcast application, use a spray volume of 10 gallons or more per acre for ground equipment. Where states have regulations which specify minimum spray volumes, they must be observed. In general, increase spray volume as crop canopy, height and weed density increase in order to obtain adequate spray coverage. **Do not apply less than 5 gallons total spray volume per acre.**

Application Rate
The lower dosages given will be satisfactory for young, succulent growth of sensitive weed species. For less sensitive species and under conditions where control is more difficult, the higher dosages will be needed.

Application Timing
Apply GF-2654 TS when weeds are young and actively growing.

Weeds Controlled

1.1 Annual or Biennial Weeds

bittercress, smallflowered
buttercup, smallflowered[1]
carpetweed
cocklebur, common
coffeeweed
copperleaf, Virginia
galinsoga
horseweed (marestail)
jimsonweed
knotweed[1]
kochia
lambsquarters, common
lettuce, prickly[1]
mallow, little[1]
mallow, Venice[1]
marshelder
morningglory, ipomea species
mustards (except blue mustard)
nightshade, black[1]
nightshade, hairy[1]
pennycress, field
pepperweed[1]
pigweed, palmer[1]
pigweed, redroot[1]
pigweed, smooth[1]
purslane, common
pusley, Florida
ragweed, common
ragweed, giant
rape, wild
rocket, yellow
salsify, common[1]
salsify, western[1]
shepherdspurse
sicklepod
smartweed (annual species)[1]
sowthistle, annual
Spanishneedles
sunflower
sweetclover
tansymustard
thistle, Russian (tumbleweed)[1]
velvetleaf
waterhemp, common

1.2 Perennial Weeds

alfalfa[1]
artichoke, Jerusalem[1]
bindweed (hedge, field and European)[1]
blue lettuce
clover, red[1]
coffeeweed
cress, hoary[1]
dandelion[1]
docks[1]
dogbanes[1]
eveningprimrose, cutleaf
garlic, wild[1]
ivy, ground[1]
onion, wild[1]
sowthistle, perennial
thistle, Canada[1]

[1]May require application to small weeds, repeat applications, and/or use of higher specified rates of this product.

Specific Use Directions

Agricultural Use Requirements for Crops: For the following crop use, follow PPE and re-entry instructions in the Agricultural Use Requirements section of this label.

Soybeans Containing DAS-68416-4

The directions are for use on soybeans containing the DAS-68416-4 trait. This is a patented gene that provides tolerance to 2,4-D choline herbicides. Information on crop varieties containing DAS-68416-4 may be obtained from your seed supplier.

Application Timing	GF-2654 TS (pint/acre)	Use Directions
preplant (burndown) preemergence	1 – 2.1	Use the high rate in the rate range for less susceptible weeds, weeds in advanced stages of development, or under less favorable growing conditions. **Preplant:** Apply anytime prior to and up through soybean planting to control emerged broadleaf weed seedlings or existing cover crops. **Preemergence:** Apply anytime after planting but before soybean emerges to control broadleaf weed seedlings or existing cover crops.
postemergence annual or perennial broadleaf weeds		Apply when weeds are small and soybean growth stage is no later than R2 (full flowering stage). Make one to two applications with a minimum of 12 days between applications.

Restrictions:
- These directions are only for soybeans identified as containing DAS-68416-4.
- **Preharvest Interval:** Do not apply within 30 days of harvest.
- Do not graze treated soybean.
- Do not harvest for forage or hay.
- Do not apply more than one preemergence application and no more than two postemergence applications per use season.
- Do not apply more than 6.3 pints of GF-2654 TS (3 lb of acid equivalent) per acre per use season.
- Do not apply GF-2654 TS to soybeans later than the R2 stage.

2. Terms and Conditions of Use

If terms of the following Warranty Disclaimer, Inherent Risks of Use and Limitation of Remedies are not acceptable, return unopened package at once to the seller for a full refund of purchase price paid. To the extent permitted by law, otherwise, use by the buyer or any other user constitutes acceptance of the terms under Warranty Disclaimer, Inherent Risks of Use and Limitation of Remedies.

Warranty Disclaimer

Dow AgroSciences warrants that this product conforms to the chemical description on the label and is reasonably fit for the purposes stated on the label when used in strict accordance with the directions, subject to the inherent risks set forth below. TO THE EXTENT PERMITTED BY LAW, Dow AgroSciences MAKES NO OTHER EXPRESS OR IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE OR ANY OTHER EXPRESS OR IMPLIED WARRANTY.

Inherent Risks of Use

It is impossible to eliminate all risks associated with use of this product. Crop injury, lack of performance, or other unintended consequences may result because of such factors as use of the product contrary to label instructions (including conditions noted on the label, such as unfavorable temperatures, soil conditions, etc.), abnormal conditions (such as excessive rainfall, drought, tornadoes, hurricanes), presence of other materials, the manner of application, or other factors, all of which are beyond the control of Dow AgroSciences or the seller. To the extent permitted by law, all such risks shall be assumed by buyer.

Limitation of Remedies

To the extent permitted by law, the exclusive remedy for losses or damages resulting from this product (including claims based on contract, negligence, strict liability, or other legal theories), shall be limited to, at Dow AgroSciences' election, one of the following:

1. Refund of purchase price paid by buyer or user for product bought, or
2. Replacement of amount of product used.

To the extent permitted by law, Dow AgroSciences shall not be liable for losses or damages resulting from handling or use of this product unless Dow AgroSciences is promptly notified of such loss or damage in writing. To the extent permitted by law, in no case shall Dow AgroSciences be liable for consequential or incidental damages or losses.

The terms of the Warranty Disclaimer, Inherent Risks of Use and Limitation of Remedies cannot be varied by any written or verbal statements or agreements. No employee or sales agent of Dow AgroSciences or the seller is authorized to vary or exceed the terms of the Warranty Disclaimer or Limitation of Remedies in any manner.

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EPA accepted __/__/__

Storage and Disposal

Do not contaminate water, food, or feed by storage or disposal.

Pesticide Storage: Keep container tightly closed when not in use. If exposed to subfreezing temperatures, the product should be warmed to at least 40°F and mixed thoroughly before using.

Pesticide Disposal: Pesticide wastes are toxic. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal law and may contaminate groundwater. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance.

Container Handling: Nonrefillable container. Do not reuse or refill this container.

Triple rinse or pressure rinse container (or equivalent) promptly after emptying. **Triple rinse** as follows: Empty the remaining contents into application equipment or a mix tank. Fill the container 1/4 full with water. Replace and tighten closures. Tip container on its side and roll it back and forth, ensuring at least one complete revolution, for 30 seconds. Stand the container on its end and tip it back and forth several times. Turn the container over onto its other end and tip it back and forth several times. Empty the rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Repeat this procedure two more times. **Pressure rinse** as follows: Empty the remaining contents into application equipment or a mix tank and continue to drain for 10 seconds after the flow begins to drip. Hold container upside down over application equipment or mix tank or collect rinsate for later use or disposal. Insert pressure rinsing nozzle in the side of the container, and rinse at about 40 psi for at least 30 seconds. Drain for 10 seconds after the flow begins to drip. Then offer for recycling if available, or puncture and dispose of in a sanitary landfill, or by incineration, or by other procedures allowed by state and local authorities.

Refer to label booklet for Directions for Use.

Notice: Read the entire label. Use only according to label directions. **Before using this product, read Warranty Disclaimer, Inherent Risks of Use, and Limitation of Remedies at end of label booklet. If terms are unacceptable, return at once unopened.**

In case of emergency endangering health or the environment involving this product, call 1-800-992-5994.

Agricultural Chemical: Do not ship or store with food, feeds, drugs or clothing.

EPA Reg. No. 62719-XXX EPA Est. _________

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Produced for
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

Net Contents _____

Appendix B Supporting Submission by DAS to U.S. EPA for Application for New Registration for GF-2654 TS

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054

MRID No. 48449800

308/2E
April 15, 2011

Dow AgroSciences

Document Processing Desk (**APPL/E-SUB/REGFEE**)
Office of Pesticide Programs (7504P)
U. S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Attention: Kathryn Montague/PM-23 (7505P)

GF-2654 TS (AI: 2,4-D)
EPA REGISTRATION NUMBER: 62719-XXX
APPLICATION FOR NEW REGISTRATION - SECTION 3

Dow AgroSciences is respectfully submitting an application for new registration of GF-2654 TS, which is an end-use herbicide product for control of broadleaf weeds on soybean containing DAS-68416-4. Soybean containing DAS-68416-4 is also referred in this dossier as AAD-12 Soybean and also as DHT Soybean. As part of this new registration, we are citing the following studies submitted to EPA on September 29, 2010:

MRID No.	Study Title
48234601	Group A
48234602	Determination of color
48234603	Acute Oral Toxicity
48234604	Acute Dermal Toxicity
48234605	Acute Inhalation Toxicity
48234606	Primary Eye Irritation
48234607	Primary Skin Irritation
48234608	Local Lymph Node Assay
48260701	Dissociation in water

We believe this registration action qualifies as a ***PRIA action R320, $11,996, New product; new physical form; requires data review in science divisions.*** Complimentary copy of the Pay.Gov. Payment Confirmation has been included (Pay.gov Tracking ID: 2532R621; Agency Tracking ID: 74191837251)

Dow AgroSciences is submitting this submission electronically (e-PRISM.xml New Section 3 for **GF-2654 TS**).

Contents of Submission

Volume No.	**Administrative Contents**
Volume 1	▪ Transmittal document (this letter) ▪ Complimentary Copy: Pay Gov Payment Confirmation ▪ EPA Form 8570-1, Application for Pesticide, ▪ EPA Form 8570-34, Certification with Respect to Citation of Data

KATHRYN MONTAGUE/PM-23
GF-2654 TS (AI: 2,4-D)
EPA REGISTRATION NUMBER: 62719-XXX
APPLICATION FOR NEW REGISTRATION - SECTION 3
April 15, 2011

Page 2

- EPA Form 8570-35, Data Matrix – Agency Copy (15 Pages)
- EPA Form 8570-35, Data Matrix – Public File Copy (15 Pages)
- Basic Confidential Statement of Formula entitled GF-2654 TS dated April 15, 2011 (1 page)
- Alternate #1 Confidential Statement of Formula entitled GF-2654 TS dated April 15, 2011 (1 page)
- Label entitled 062719-XXXXX 20110411 GF-2654 TS-XXX 12Apr11d.pdf (13 Pages plus Registration Notes)
- CD containing e-PRISM.xml – New Section 3

Volume Number	MRID No.	Contents	
Volume #2 (860.1300)	48449801	Title: A Nature of the Residue Study with [14C]-2,4-D DMA Applied to AAD-12 Soybeans	
		Author: L. K. Graper; J. L. Balcer; K. P. Smith; P. S. Hogan Study ID: 090051	Report Date: March 25, 2011
		Pages: 1-190	(1 PDF Copy)
Volume #3 (860.1500, 860.1520)	48449802	Title: Magnitude of the Residue of 2,4-D in/on Herbicide Tolerant Soybeans Containing the Aryloxyalkanoate Dioxygenase-12 (AAD-12) Gene	
		Author: John F. Culligan Study ID: ARA-09-15-11	Report Date: January 17, 2011
		Pages: 1-338	(1 PDF Copy)
Volume #4	48449803	Title: A Risk Assessment Overview of 2,4-D Treated Soybean Containing the DAS AAD-12 Trait	
		Author: C.B. Cleveland; T.C. Blewett	Report Date: April 13, 2011
		Study ID: 110297	
		Pages: 1-42	(1 PDF Copy)

If you require additional information, please contact Kerri Hipsky, Registration Assistant for this product, at 317-337-7827; (kahipsky@dow.com) or Cindy Loy, Regulatory Specialist at 317-337-4655 (caloy@dow.com).

Sincerely,

Diego Fonseca
Regulatory Leader – Regulatory Affairs
317-337-4693
317-337-4649 (FAX)
dfonseca@dow.com

Enclosures

DF/kh

Appendix C FDA Biotechnology Consultations

Early Food Safety Evaluation
For
Aryloxyalkanoate Dioxygenase-12 (AAD-12) Protein

Submitted to:

FDA, Center for Food Safety and Nutrition, Office of Food Additives

Part II. As Described in the Guidance for Industry published in the Federal Register, Vol. 71, No. 119, June 26, 2006

Submitted by:

Mycogen Seeds c/o Dow AgroSciences LLC

Mark S Krieger, Ph.D.
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268
Telephone: 317-337-3458
Fax: 317-337-4649

Submission Date: 2008 December 15

Total Pages: 13

No Confidential Business Information (CBI)

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List of Figures

List of Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
aad-12	gene encoding for AAD-12 protein
AAD-12	aryloxyalkanoate dioxygenase-12 protein
BSA	bovine serum albumin
C. acidovorans	*Comamonas acidovorans*, alternative name used in literature for *D acidovorans*
D. acidovorans	*Delftia acidovorans*
DCP	2,4-dichlorophenol
MCPA	4-chloro-2-methylphenoxyacetic acid
P. acidovorans	*Pseudomonas acidovorans*, alternative name used in literature for *D acidovorans*
SdpA	alternative name used in literature for AAD-12 protein
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SGF	simulated gastric fluid

1. Name, Description and Function of Protein

Aryloxyalkanoate dioxygenase-12 (AAD-12) is an α-ketoglutarate-dependent dioxygenase enzyme from *Delftia acidovorans*, a gram-negative bacterium present in soil, fresh water, activated sludge, and clinical specimens (von Gravenitz 1985, Tamaoka *et al.* 1987, Wen *et al.*, 1999). This protein was previously identified in the literature as SdpA, but this nomenclature does not indicate the broader functionality that has recently been discovered for this and related proteins (Dow AgroSciences *et al.,* 2005). Therefore the nomenclature AAD-12 is considered to be the most accurate description of the protein.

The amino acid sequence of the native enzyme was modified by the insertion of an alanine at position number 2. The additional alanine codon (GCT; underlined in Figure 1) encodes part of an *NcoI* restriction enzyme recognition site (CCATGG) spanning the ATG translational start codon. This additional codon serves the dual purpose of facilitating subsequent cloning operations and improving the sequence context surrounding the ATG start codon to optimize translation initiation. The proteins encoded by the native and plant-optimized (v1) coding regions are 99.3% identical, differing only at amino acid number 2.

Figure 1. Amino Acid Sequence of AAD-12 Protein (293 amino acids)

```
001 MAQTTLQITP TGATLGATVT GVHLATLDDA GFAALHAAWL QHALLIFPGQ
051 HLSNDQQITF AKRFGAIERI GGGDIVAISN VKADGTVRQH SPAEWDDMMK
101 VIVGNMAWHA DSTYMPVMAQ GAVFSAEVVP AVGGRTCFAD MRAAYDALDE
151 ATRALVHQRS ARHSLVYSQS KLGHVQQAGS AYIGYGMDTT ATPLRPLVKV
201 HPETGRPSLL IGRHAHAIPG MDAAESERFL EGLVDWACQA PRVHAHQWAA
251 GDVVVWDNRC LLHRAEPWDF KLPRVMWHSR LAGRPETEGA ALV
```

2. Information as to Whether Protein Has Been Safely Consumed in Foods

The *aad-12* gene, which codes for the AAD-12 protein, is derived from *Delftia acidovorans*, a ubiquitous bacterium in soil and water (von Gravenitz, 1985). *Deftia acidovorans*, which has previously been identified as *Pseudomonas acidovorans* and *Comamonas acidovorans*, is a non glucose-fermenting, gram-negative, non spore-forming rod.

D. acidovorans can be used to transform ferulic acid into vanillin and related flavor metabolites (Toms and Wood, 1970; Shetty et al, 2006). This utility has led to a history of safe use of use for *D. acidovorans* in the food processing industry. For example, US Patent 5,128,253 "Bioconversion process for the production of vanillin" was issued on July 7, 1992 to Kraft General Foods. This patent demonstrates the use of a variety of microorganisms, including *Pseudomonas acidovorans*, for the transformation of ferulic acid to vanillin.

3. Identity and Sources of Introduced Genetic Material

Identity: *aad*-12 v1: Synthetic, plant-optimized version of an aryloxyalkanoate dioxygenase gene from *Delftia acidovorans*.

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Delftia acidovorans (previously designated *Pseudomonas acidovorans* prior to 1987, and *Comamonas acidovorans* from 1987-1999) are strictly aerobic, gram-negative bacteria that are present in soil, fresh water, activated sludge, and clinical specimens (von Gravenitz, 1985, Tamaoka *et al.* 1987, Wen *et al.*, 1999). *Delftia acidovorans*, like many other soil dwelling bacteria, has evolved the ability to use herbicides as one of many carbon sources for growth, affording the bacteria a competitive advantage in soil.

To obtain high expression of heterologous genes in plants, it is generally preferred to re-engineer the genes so that they are more efficiently expressed in plant cells (Dow AgroSciences *et al.*, 2005). The synthetic version of the gene was produced in order to modify the G+C codon bias to a level more typical for plant DNA ("plant-optimized") to improve expression of the AAD-12 protein in plants. The native and plant-optimized DNA sequences are 79.7% identical.

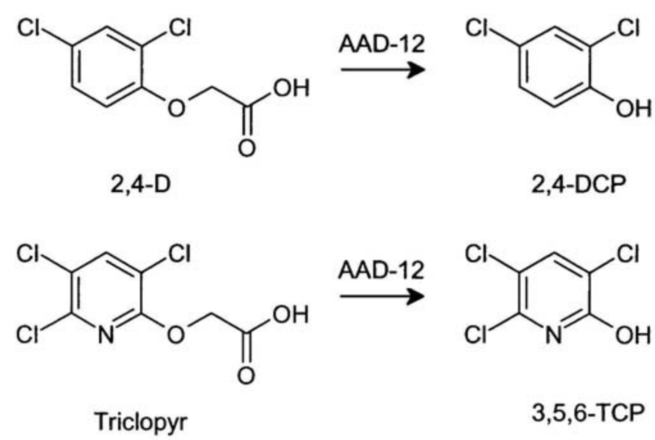
4. Description of Intended Effect of the Protein

Expression of the AAD-12 protein in transgenic crops provides tolerance to the phenoxy acid herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) by catalyzing the conversion of 2,4-D to 2,4-dichlorophenol (DCP) (Müller *et al.*, 1999; Westendorf *et al.*, 2002 and 2003; Dow AgroSciences *et al.*, 2005), a herbicidally inactive compound.

AAD-12 is also able to degrade related achiral phenoxyacetate herbicides such as MCPA ((4-chloro-2-methylphenoxy)acetic acid) and pyridyloxyacetate herbicides such as triclopyr and fluroxypyr to their corresponding inactive phenols.

AAD-12 has enantiomeric selectivity for the (S)-enantiomers of the chiral phenoxy acid herbicides (*e.g.*, dichlorprop and mecoprop), but does not catalyze degradation of the (R)-enantiomers. It is the R-enantiomers in this class of chemistry that are herbicidally active, therefore AAD-12 does not provide tolerance to commercially-available chiral phenoxy acid herbicides.

Figure 2. Degradation Reaction of Representative Phenoxyacetate (2,4-D) and Pyridyloxyacetate (Triclopyr) Herbicides Catalyzed by AAD-12.



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2,4-DCP, as a metabolite of 2,4-D inactivation, has been well studied, characterized and assessed by regulatory agencies. Prior to 1993, U.S. EPA tolerances for 2,4-D consisted of 2,4-D residues in plants, processed food/feed and fish as well as 2,4-D and 2,4-DCP residues in animal commodities (meat, milk, poultry and eggs). In 1993, U.S. EPA Health Effects Division (HED) Metabolism Committee changed the tolerances to 2,4-D *per se* for all commodities in order to harmonize with Codex maximum residue levels (MRLs). In 2003, U.S. EPA HED Metabolism Assessment Review Committee (MARC) indicated that minor plant metabolites such as hydroxylated metabolites and 2,4-DCP are not expected to be significantly more toxic than the parent (US EPA, 2003). Therefore, MARC concluded that for plants, parent 2,4-D, both free and conjugated, determined as the acid, are the residues of concern for risk assessment and tolerance expression. MARC also upheld the earlier HED Metabolism Committee decision to delete 2,4-DCP from the livestock tolerance expression for 2,4-D, i.e., 2,4-DCP is not of concern for either the tolerance expression or for risk assessment at the levels expected in livestock tissues and considering the likely lower toxicity of 2,4-DCP compared to 2,4-D. Dropping 2,4-DCP from the tolerance expression for livestock tissues allows for harmonization of U.S. tolerances with international MRLs established by Codex and other trading partners such as Japan and Europe who never included 2,4-DCP in any tolerance expression (US EPA, 2007).

3,5,6-TCP, as a metabolite of triclopyr inactivation, has been well studied, characterized and assessed by regulatory agencies (US EPA, 1998). The EPA's HED Metabolism Committee concluded that the residue to be regulated in grass and rice commodities and milk, poultry and eggs is triclopyr *per se*. The residues to be regulated in meat and meat byproducts are the combined residues of triclopyr and the metabolite 3,5,6-trichloro-2-pyridinol. 3,5,6-TCP is included in the residue definition for meat and meat byproducts (of cattle, goats, hogs, horses, and sheep) because the use of triclopyr on pasture grasses results in measurable levels of triclopyr in the forage and hay fed to animals. Triclopyr is metabolized *in situ* to 3,5,6-TCP, therefore 3,5,6-TCP is included in the residue definition for meat and meat byproducts.

5. Assessment of Amino Acid Similarity to Known Allergens and Toxins

5.1. Amino Acid Sequence Homology of the Protein to Known Allergens

The AAD-12 protein was evaluated for amino-acid sequence similarity to known allergens based on 8-contiguous amino-acid identity or 35% or greater identity over any 80 amino-acid segment (Codex, 2003). No matches were found in the FARRP (Food Allergy Research and Resource Program) version 7.00 allergen database (Gendel, 1998; Herman, 2007).

5.2. Amino Acid Sequence Homology to Known Toxins

The AAD-12 protein was evaluated for amino-acid sequence similarity to known toxins by conducting a global sequence similarity search against the GenBank non-redundant protein dataset. The search identified 618 similar proteins, which can be broken down into a few major subclasses. The largest subclass, containing 474 proteins, was identified as tauD or taurine dioxygenases. These are proteins involved in the degradation of taurine (Eichorn et al., 2007). The next largest class, with 138 members, was clavaminic acid synthetases or "CAS-like"(Zhang et al., 2000). There were 2 TolC proteins (XX) which are known efflux pumps (Koronakis et al., 2000). The last four proteins were: 1) a (S)-2-(2,4-dichlorophenoxy)propionate, 2-oxoglutarate dioxygenase (Schleinitz et al., 2005); 2) a pvcB protein which is a known CAS-like protein (see accession page of NP_968348); 3) a inosine-uridine preferring nucleoside hydrolase (Gopaul et al., 1996); and 4) a hypothetical protein with no functional annotation. None of these protein classes are known toxins (Larriunua and Herman, 2007).

6. Overall Stability and Resistance to Enzymatic Degradation

6.1. Lability to Pepsin in Simulated Gastric Fluid (SGF)

The digestibility of the AAD-12 protein was tested *in vitro* using Simulated Gastric Fluid (SGF).

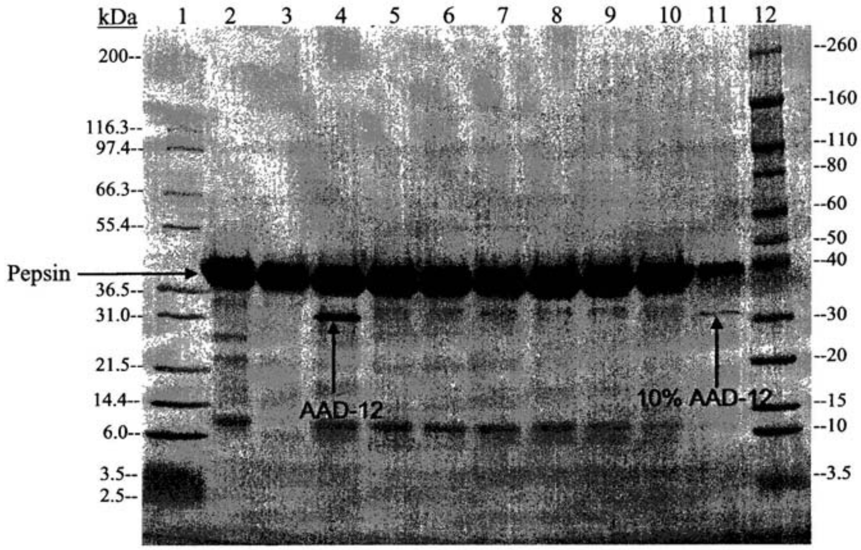
Microbially-produced (in *Pseudomonas fluorescens)* AAD-12 protein at a concentration of approximately 0.074 mM was incubated in SGF (0.32% w/v pepsin at pH 1.2; U.S. Pharmacopeia). Bovine serum albumin (BSA) and β-lactoglobulin A were included as positive and negative controls at equimolar concentration to AAD-12.

The digestions were performed for time intervals of approximately 30 seconds, 1, 2, 4, 8 and 16 minutes in a water bath set to 37 °C. At each specified incubation interval, 100 µL of the reaction mixture was removed and added to tubes containing stop solution (40 µL of 200 mM sodium carbonate, pH ~11.0). The stopped reactions were then placed on ice until all of the time points were sampled for the three proteins. Zero time point samples (neutralized control) were prepared by neutralizing a 2.85-mL aliquot of SGF with 1.2 mL 200 mM sodium carbonate, and then adding 150 µL of the respective protein to the solution.

Aliquots of the neutralized and digested proteins were mixed with equal volumes of Laemmli sample buffer and heated for 5 minutes at ~95 °C. Additionally, aliquots of all samples and the AAD-12 neutralized control were initially diluted in PBST to a concentration appropriate for western blot analysis, then mixed with equal volumes of the Laemmli sample buffer preparation and heated. The samples were analyzed with SDS-PAGE and western blot analyses.

AAD-12 was not detectable at the 30-second time point as demonstrated by both SDS-PAGE (Figure 3, lane 5) and western blot (Figure 4, lane 5) analyses. Additionally, both SDS-PAGE (Figure 3, lane 11) and western blot (Figure 4, lane 11) demonstrated that 10% of the initial AAD-12 protein was readily detectable and that no AAD-12 was detectable at or beyond the 30-second time point. These results demonstrate that AAD-12 protein is rapidly hydrolyzed in SGF (Embrey and Schafer, 2008).

Figure 3. SDS-PAGE Analysis of AAD-12 (M.W. ~32 kDa) Protein Subjected to Digestion in Simulated Gastric Fluid.



Lane	Sample	Amount Loaded
1	Invitrogen Mark 12 MW markers	10µL
2	SGF Reagent Blank, 0 minute incubation	40 µL
3	SGF Reagent Blank, >16 minute incubation	40 µL
4	Neutralized AAD-12 digestion	~1.68 µg
5	30-second AAD-12 digestion	~1.68 µg
6	1-minute AAD-12 digestion	~1.68 µg
7	2-minute AAD-12 digestion	~1.68 µg
8	4-minute AAD-12 digestion	~1.68 µg
9	8-minute AAD-12 digestion	~1.68 µg
10	16-minute AAD-12 digestion	~1.68 µg
11	10% Neutralized AAD-12 digestion	~0.17 µg
12	Invitrogen Novex Sharp Prestained MW markers	10µL

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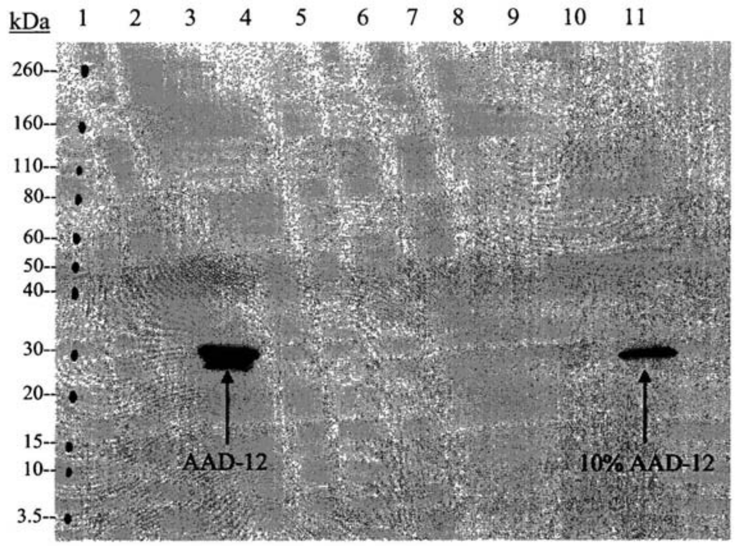
Figure 4. Western Blot Analysis of AAD-12 Protein Subjected to Digestion in Simulated Gastric Fluid.



Lane	Sample	Amount Loaded
1	Invitrogen Novex Sharp Prestained MW markers	10μL
2	SGF Reagent Blank, 0 minute incubation	40 μL
3	SGF Reagent Blank, >16 minute incubation	40 μL
4	Neutralized AAD-12 digestion	~0.17μg
5	30-second AAD-12 digestion	~0.17μg
6	1-minute AAD-12 digestion	~0.17μg
7	2-minute AAD-12 digestion	~0.17μg
8	4-minute AAD-12 digestion	~0.17μg
9	8-minute AAD-12 digestion	~0.17μg
10	16-minute AAD-12 digestion	~0.17μg
11	10% Neutralized AAD-12 digestion	~0.017μg

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7. Other Relevant Information

7.1. Acute Oral Toxicity

The human diet contains many protein components and in general the consumption of food proteins, including many uncharacterized proteins, does not raise safety concerns (FAO/WHO, 1996). In general, when a protein is toxic, it acts via acute mechanisms and at very low dose levels (Jones and Maryanski, 1991; Sjoblad *et al.*, 1992, Hammond and Fuchs, 1998, Pariza and Johnson, 2001). Therefore an acute oral mouse toxicity study of the AAD-12 protein was conducted as it represents the mostly likely route of human exposure.

AAD-12 protein was purified from a *Pseudomonas fluorescens* expression host strain using anion exchange and hydrophobic interaction chromatography. The AAD-12 protein was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to determine molecular weight and western blotting to determine immunoreactivity. The purity of the AAD-12 protein relative to total proteins in was >99%.

Five fasted Crl:CD1(ICR) mice per sex were simultaneously given 5666 mg/kg body weight of the test material (containing 2000 mg/kg AAD-12) as a 20% suspension in 0.5% aqueous methylcellulose. The total volume administered was 28.3 ml/kg given in two fractional oral gavage doses of 14.2 ml/kg, approximately 1 hour apart. Animals were observed for signs of toxicity daily for 15 days after dosing.

All animals survived until euthanization on Day 15. No clinical signs of toxicity were observed in any of the test animals. Nine out of the ten animals gained or maintained weight by test day 2. All animals gained weight by test day 8 and 15. A cyst in the cortex of the kidney was observed in one female mouse, which was interpreted to be a spontaneous alteration unassociated with test material administration. No other gross lesions were observed at necropsy.

The acute oral LD_{50} of AAD-12 in male and female mice is greater than 2000 mg/kg (5666 mg/kg of test substance at 35.3% purity) (Wiescinski and Golden, 2008).

8. Conclusions

The AAD-12 protein shows no significant homology to known toxins or allergens. The AAD-12 protein is rapidly degraded in simulated gastric fluid, and did not cause acute toxicity in mice at the highest levels tested (2000 mg/kg).

Based on the data and information presented in this submission, the presence of the AAD-12 protein in food or feed should be of no significant concern.

7. Other Relevant Information

7.1. Acute Oral Toxicity

The human diet contains many protein components and in general the consumption of food proteins, including many uncharacterized proteins, does not raise safety concerns (FAO/WHO, 1996). In general, when a protein is toxic, it acts via acute mechanisms and at very low dose levels (Jones and Maryanski, 1991; Sjoblad *et al.*, 1992, Hammond and Fuchs, 1998, Pariza and Johnson, 2001). Therefore an acute oral mouse toxicity study of the AAD-12 protein was conducted as it represents the mostly likely route of human exposure.

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U.S. Food and Drug Administration

Food

NPC 000009: Agency Response Letter

CFSAN/Office of Food Additive Safety

May 19, 2010

Mark S. Krieger, Ph.D.
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054

Re: NPC 00009; aryloxyalkanoate dioxygenase-12, AAD-12

Dear Dr. Krieger:

This letter is in response to Dow AgroSciences LLC's (Dow) early food safety evaluation of the protein, aryloxyalkanoate dioxygenase-12, AAD-12, expressed in a new plant variety under development for food use, which you submitted to the Food and Drug Administration (FDA) on December 15, 2008, under FDA's guidance to industry, "Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (71 FR 35688 June 21, 2006, and available on the FDA home page at http://www.fda.gov - follow the hyperlinks from the "Food" topic to the "Biotechnology" program area). As usec in the guidance and in this letter, the term "food" refers to both human food and animal feed. All materials relevant to this evaluation have been placed in a file designated NPC 00009. This file will be maintained in the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

In cases of inadvertent low level presence in the food supply of a new food plant variety, FDA believes that any food or feed safety concern would be limited to the safety of the new protein(s) in that plant (generally, the potential allergenicity and toxicity of the new protein(s)). Based on Dow's early food safety evaluation, it is our understanding that Dow has concluded that AAD-12 protein would not raise food safety concerns when it is in a new food plant variety that is present at low levels in the food supply. We have completed our evaluation of your submission, and we have no questions at this time regarding Dow's conclusion.

Sincerely yours,

Antonia Mattia, Ph.D.
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

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Biotechnology Consultation Note to the File BNF No. 000124

Biotechnology Consultation - Note to the File
Biotechnology Notification File BNF No. 000124

Date: November 8, 2011

Subject: DAS-68416-4, herbicide tolerant soybean

Keywords: Soybean; *Glycine max*; herbicide tolerance; DAS-68416-4 soybean; *aad-12* gene; aryloxyalkanoate dioxygenase-12 protein (AAD-12) protein from *Delftia acidovorans*; 2,4-dichlorophenoxyacetic acid (2,4-D); *pat* gene; PAT protein from *Streptomyces viridochromogenes*; glufosinate; Dow AgroSciences LLC (Dow); NPC 00009

Purpose

This document summarizes our evaluation of Biotechnology Notification File (BNF) No. 000124. In a submission dated December 22, 2009, Dow AgroSciences (Dow) submitted a safety and nutritional assessment of bioengineered herbicide tolerant soybean, transformation event DAS-68416-4 soybean (hereafter referred to as DAS-68416-4 soybean). Dow provided additional information on April 15, 2010, and on August 23 and 24, 2011. FDA evaluated the information in Dow's submissions to ensure that regulatory and safety issues regarding human food and animal feed derived from the new plant variety have been resolved prior to commercial distribution.

In our evaluation of BNF No. 000124, we considered all information provided by Dow as well as publicly available information and information in the agency's files. Here we discuss the outcome of the consultation, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effect

The intended technical effects of the modifications in DAS-68416-4 soybean are to confer herbicide tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate. To accomplish this objective, Dow introduced both the *aad-12* gene that encodes the aryloxyalkanoate dioxygenase-12 (AAD-12) protein, which confers tolerance to 2,4-D,[1] and the *pat* gene that encodes the phosphinothricin *N*-acetyl transferase (PAT) protein, which confers tolerance to glufosinate.

Regulatory Considerations

The purpose of this evaluation is to assess whether the developer has introduced a substance requiring premarket approval or raised other regulatory issues under the Federal Food, Drug, and Cosmetic Act (FD&C Act).

The Environmental Protection Agency (EPA) regulates herbicides under the FD&C Act and the Federal Insecticide, Fungicide, and Rodenticide Act. Under EPA regulations, the herbicide residues and metabolic by-products in DAS-68416-4 soybean, resulting from the detoxification of the applied herbicides by the expression products, are considered pesticide residues. In its submission to FDA, Dow indicated that it has submitted a regulatory package to EPA for the use of 2,4-D on DAS-68416-4 soybean.

Genetic Modification and Characterization

Parental Variety

Dow transformed the recipient soybean line Maverick (a publicly available soybean line) to obtain DAS-68416-4 soybean.

Transformation Plasmid and Methods

Dow described the transformation plasmid. The pDAB4468 plasmid contained two gene expression cassettes within the transfer DNA (T-DNA), the *aad-12* expression cassette and the *pat* expression cassette. The *aad-12* expression cassette contained the *aad-12* gene under the control of the AtUbi10 promoter region from *Arabidopsis thaliana* and the AtuORF23 termination region from *Agrobacterium tumefaciens*. The *aad-12* expression cassette was flanked at both ends by matrix attachment regions (MARs) from *Nicotiana tabacum*. According to Dow, the MARs were included to potentially increase consistency of *aad-12* gene expression in transgenic plants. The *pat* expression cassette contained the *pat* gene under the control of the CsVMV promoter from the cassava vein mosaic virus and the AtuORF1 terminator region from *A. tumefaciens*. The spectinomycin resistance gene *SpecR*, present in the intact pDAB4468 plasmid, is not expected to be present in the transformed cells because it was outside the T-DNA, which is delimited by T-DNA right and left border sequences.

To generate DAS-68416-4 soybean, Dow used *Agrobacterium*-mediated transformation. Soybean seeds were germinated on basal media and cotyledonary nodes were isolated and infected with the disarmed *A. tumefaciens* strain EHA101, carrying the binary vector pDAB4468. Following transformation, removal of *Agrobacterium* was achieved through supplementation of shoot initiation, shoot elongation, and rooting media with cefotaxime, timentin, and vancomycin. Transgenic shoots were selected based on tolerance to glufosinate, transferred to rooting medium for root development, and then transferred to soil mix for acclimatization of plantlets. Transgenic plants (T0) were confirmed by molecular analysis of the *pat* and *aad-12* genes. T0 plants were allowed to self-fertilize, giving rise to T_1 seed.

Characterization, Inheritance, and Stability of the Introduced DNA

Dow characterized the insert in DAS-68416-4 soybean using restriction enzyme digestion of genomic DNA followed by Southern blot analysis. Dow concluded that the results of this analysis demonstrate that DAS-68416-4 soybean contains a single intact copy of the *aad-12* and *pat* expression cassettes at a single site of insertion in the soybean genome. Dow also confirmed that no DNA sequences outside of the T-DNA border regions (i.e., vector backbone sequences) from the plasmid pDAB4468 were detected in DAS-68416-4 soybean. Therefore, the *SpecR* gene from pDAB4468 was not inserted into the soybean genome.

Dow studied the inheritance of the *aad-12* gene in DAS-68416-4 soybean within a single segregating generation. Based on the results of Chi square analysis of the AAD-12 phenotypic segregation ratio, Dow concluded that the transgene insert displayed a Mendelian inheritance pattern consistent with that for a single dominant trait. Dow confirmed the genetic equivalence of the transgenic DNA in the tested population through restriction enzyme digestion and Southern blot analysis. As all of the AAD-12 expression positive plants displayed identical hybridization patterns, Dow concluded that the individual plants contained the same insert and were equivalent to one another.

Dow assessed the stability of the DNA insert across 4 generations of DAS-68416-4 soybean through restriction enzyme digestion of genomic DNA followed by Southern blot analysis. Samples were first tested using an AAD-12 lateral flow strip test kit to confirm AAD-12 expression positive plants prior to analysis by Southern blot. Dow concluded that the results across all DAS-68416-4 soybean samples were, as expected, for an intact, single copy insert whose integration and inheritance is stable across multiple generations.

Protein Characterization

Identity, Function, and Characterization

DAS-68416-4 soybean was genetically engineered to express the arylalkanoate dioxygenase (AAD-12) protein and the phosphinothricin acetyltransferase (PAT) protein. The AAD-12 protein renders the plant tolerant to several classes of herbicides, including aryloxyalkanoate herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D); achiral phenoxyacetate herbicides such as MCPA ((4-chloro-2-methylphenoxy) acetic acid); and pyridyloxyacetate herbicides such as triclopyr and fluroxypyr. The PAT protein confers tolerance to the herbicide glufosinate.

According to Dow, the AAD-12 protein is encoded by the *aad-12* gene isolated from the Gram-negative soil bacterium *D. acidovorans*, which has a history of safe use in food processing. The DNA sequence of the *aad-12* gene used by Dow was codon-optimized for expression in plants; the plant-expressed *aad-12* gene encodes a protein that is identical to the native enzyme, except for one amino acid. The plant-expressed AAD-12 protein contains an additional alanine at position number 2, is 293 amino acids in length, and has an approximate molecular weight of 32 kDa.

The PAT protein is encoded by the *pat* gene isolated from the Gram-positive soil bacterium *S. viridochromogenes*. Dow stated that the *pat* gene has been widely used both as a selectable marker during development and to confer herbicide tolerance in the field in submissions previously reviewed by the FDA; Dow referred to BNFs 000055, 000073, 000081, 000085, and 000092. The DNA sequence of the *pat* gene used by Dow was codon-optimized for expression in plants; the plant-expressed *pat* gene encodes a protein that is identical to the native enzyme. The PAT protein is 183 amino acids in length and has an approximate molecular weight of 21 kDa.

Protein Expression Levels

Dow conducted field expression studies of the AAD-12 and PAT proteins in DAS-68416-4 soybean. Protein levels were measured and reported for leaf (V5 and V10 stages), root and forage (R3 stage) and grain (R8 stage) samples collected from plants from six field locations in North America. DAS-68416-4 soybean was subjected to one of four herbicide conditions (unsprayed; sprayed with 2,4-D; sprayed with glufosinate; and sprayed with both 2,4-D and glufosinate) designed to replicate maximum label application rate for commercial practices. The samples were analyzed using enzyme-linked immunosorbent assay (ELISA). Dow reports that the average levels of AAD-12 protein in various plant tissues at various growth stages ranged from ca. 15 (R3 stage root) to 66 nanograms (V5 stage leaf tissue) per milligram of tissue (dry weight). The average levels of PAT protein in various plant tissues at various growth stages ranged from ca. 2 (R3 stage root) to 12 nanograms (V10 stage leaf tissue) per milligram of tissue (dry weight). Expression values for the AAD-12 and PAT proteins were similar among the untreated and herbicide-treated samples. The AAD-12 and the PAT proteins were not detected in the non-transgenic, control tissues sampled across the six locations.

Safety Assessment of Potential for Toxicity and Allergenicity of the Introduced Proteins

Safety Assessment of the AAD-12 Protein

To obtain sufficient quantities of the AAD-12 protein for conducting safety assessment studies, Dow produced the AAD-12 protein using a *Pseudomonas fluorescens* protein expression system. Dow confirmed the identity and biochemical equivalency of the microbe-derived and plant-derived AAD-12 proteins using several analytical techniques.[2] Based on the results of these studies, Dow concluded that DAS-68416-4 soybean-expressed AAD-12 protein is biochemically equivalent to *P. fluorescens*-expressed AAD-12 protein. The *P. fluorescens*-expressed AAD-12 protein was used subsequently for *in vitro* and *in vivo* studies, including simulated gastric fluid (SGF), heat lability, and acute oral toxicity studies.

To assess the potential for toxicity of the AAD-12 protein, Dow conducted a homology search of the AAD-12 amino acid sequence as well as an acute oral toxicity study in mice. Dow conducted the bioinformatic analyses using a global sequence similarity search against the GenBank non-redundant protein database (updated February 18, 2011) and, although several significant homologies were identified, Dow concluded that the search did not reveal meaningful homologies between known toxins and the AAD-12 protein. Dow reported the results of an acute oral toxicity study in which a single dose of 2000 milligrams[3] of AAD-12 protein per kilogram of body weight was administered to 10 mice, 5 per sex.[4] Dow stated that there were no treatment-related gross pathological observations. On the basis of the results of the homology search and the toxicity study, Dow concluded that the AAD-12 protein is unlikely to cause toxic effects in humans.

To assess the potential for allergenicity of the AAD-12 protein, Dow considered the potential for allergenicity of the donor organism as well as the similarity of the AAD-12 sequence to known allergens, digestibility in simulated gastric fluid, and heat lability. Dow compared the amino acid sequence of the AAD-12 protein to the amino acid sequences of known allergens in the Food Allergy Research and Resource Program Database (FARRP, version 11) and reported that the AAD-12 protein had no meaningful homology to known allergens, where meaningful is defined as (1) greater than 35 percent identity over 80-amino-acid stretches (sliding windows) or (2) shared contiguous stretches of eight or greater amino acids.[5] Further, Dow reported that the AAD-12 protein was rapidly digested (less than 30 seconds) in simulated gastric fluid and that AAD-12 enzymatic activity was eliminated under all heating conditions. Dow concluded that the AAD-12 protein is considered to have a low risk of allergenicity.

Safety Assessment of the PAT Protein

Dow produced the PAT protein using a *P. fluorescens* protein expression system and confirmed the identity and biochemical equivalency of the microbe-derived and plant-derived PAT proteins using Western analysis techniques.[6] Based on the results of these studies, Dow concluded that DAS-68416-4 soybean-expressed PAT protein has the same molecular weight and immunoreactivity as the native PAT protein. Dow states that PAT protein produced in DAS-68416-4 soybean was shown to be equivalent to that produced in other transgenic crops.

Dow explained that the potential for toxicity and allergenicity of the PAT protein was assesses through bioinformatics analysis of the PAT amino acid sequence, as well as through review of data pertaining to the toxicity and digestibility of the PAT protein. Dow determined the amino acid sequence similarity of the PAT protein with respect to known or putative toxins and allergens using a BLASTp search against the GenBank non-redundant protein database (updated February 18, 2011) and using the Food Allergy Research and Resource Program Database (FARRP, version 11), respectively. Dow stated that these bioinformatic analyses revealed no meaningful homologies to known or putative allergens or toxins for the PAT amino acid sequence. On the basis of the low level of its expression and the results of the overall safety assessment of the PAT protein, Dow concluded that the PAT protein is unlikely to cause allergenic or toxic effects in humans or animals. In support of its conclusion, Dow cited EPA's review of data on the acute toxicity and digestibility of the PAT protein and conclusion that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to the PAT protein.[7]

Potential Endogenous Plant Substrates of AAD-12 Dioxygenase Activity

Dow screened the AAD-12 protein for the ability to utilize endogenous plant substrates. Dow selected potential substrates based on chemical structure, similar physiological function to known substrates, and abundance within metabolic pathways in plants. The potential substrates were screened using a dioxygenase-coupled *in vivo* enzyme assay. Dow reported that the compounds tested were not oxidized upon incubation with the AAD-12 protein and, based on these results, concluded that there is no indication that the AAD-12 protein has enzymatic activity on endogenous plant substrates.

Potential New Proteins

To assess the potential for new open reading frames or for disruption of endogenous coding sequences resulting from the insertion of the DNA fragment containing the *aad-12* and *pat* expression cassettes, Dow determined the genomic DNA sequence of the border flanking the transgenic insert in DAS-68416-4 soybean. Dow concluded that this analysis confirmed that the transgenic DNA insertion neither created novel open reading frames nor interrupted any genomic open reading frames.

Food and Feed Use

Dow stated that DAS-68416-4 soybean will be grown for the same commercial uses as current transgenic and non-transgenic commercial soybean varieties.

Soybean (*Glycine max*) is grown around the world for a variety of food, feed, and industrial uses. Soybean seeds are processed into oil and meal. Soybean oil is rich in polyunsaturated fatty acids and is commonly used as a salad and cooking oil and in the production of margarine and other food ingredients. A small fraction of soybean meal is further processed into soy flours and soy proteins for a variety of food uses. Traditional foods prepared from soybeans include tofu, miso, soymilk, tempeh, and soy sauce.

The preponderance of soybean meal is used in animal feed, primarily in poultry, swine, and beef and dairy cattle diets. Soybean meal is processed in moist heat to inactivate trypsin inhibitors and lectins, which are antinutrients occurring in raw soybeans.

Composition

Scope of Analysis

Dow analyzed the composition of forage and grain from transgenic DAS-68416-4 soybean and compared it with Maverick, the non-transgenic, recipient variety (hereafter referred to as the control), which has the same genetic background as the transgenic line, but does not contain the DAS-68416-4 event.

Study Design - Compositional analyses

Dow conducted a study to obtain compositional data for forage and grain samples from DAS-68416-4 soybean and the control. Plants were grown at six test sites within the soybean-producing regions in North America using a randomized complete block design. DAS-68416-4 soybeans were subjected to one of four herbicide treatment conditions: (1) unsprayed, (2) sprayed with glufosinate; (3) sprayed with 2,4-D; and (4) sprayed with both glufosinate and 2,4-D. Herbicide treatments were applied according to current agricultural practices. The compositional analysis included key nutrients and antinutrients.[8] Forage samples were collected at the R3 growth stage and grain samples were collected at maturity.

Dow performed statistical analyses on composition data obtained for DAS-68416-4 soybean and control samples using values calculated from analytical data obtained from individual sites and data aggregated from all sites. Dow used paired t-tests to compare data from DAS-68416-4 soybean and control samples grown under the four herbicide treatment conditions, to identify statistical differences. Dow also used an F-test to identify the presence of any overall treatment effects. Dow reported the composition data analyses by providing mean values, p-values, and p-values adjusted using a False Discovery Rate (FDR) procedure[9] for DAS-68416-4 soybean and control samples. A significance level of $p < 0.05$ was chosen for both analyses. Dow compared the results of its compositional analyses with values reported in published literature.[10]

Results of Analyses - Compositional analysis of soybean forage

Dow reported the results of compositional analysis for crude protein, crude fat, ash, moisture, carbohydrates (by difference), acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus in forage. Although a statistically significant overall treatment effect by F-test was observed for crude fat, no statistically significant differences by the paired t-test, in any of the four treatment groups, were observed between DAS-68416-4 soybean and the control in the levels of the analyzed components. Further, based on the summary of results across all locations reported by Dow, the mean levels for crude protein, crude fat, moisture, and carbohydrates were within the combined literature range.[11]

Dow reported that certain mean levels of ash, ADF, and NDF summarized across all locations were outside the literature values. When compared with the combined literature range, the mean levels reported for DAS-68416-4 soybean were consistent with those of the control. In drawing its conclusions, Dow considered that Maverick is a commercial non-transgenic line that has a history of safe use. Based on the analytical results, Dow concluded that forage from DAS-68416-4 soybean was substantially equivalent to that of the non-transgenic soybean.

Results of Analyses - Compositional analysis of soybean grain

Proximates and Fiber

Dow reported the results of compositional analyses for proximates (crude protein, crude fat, ash, moisture, and carbohydrate) and fiber (ADF, NDF, and total dietary fiber (TDF)) in grain. No statistically significant differences by the paired t-test, in any of the four treatment groups, were observed between DAS-68416-4 soybean and the control in levels of crude fat, ADF, and TDF.[12] Statistically significant differences by the paired t-test, in one or more of the four treatment groups, were observed for crude protein, ash, moisture, carbohydrates, and NDF. Statistically significant overall treatment effects by F-test were reported for crude protein and carbohydrates. However, the mean levels for crude protein, crude fat, ash, moisture, carbohydrates, and TDF were within the combined literature range.

Dow reported that certain mean levels of ADF and NDF were outside the literature values but further noted that the mean levels reported for DAS-68416-4 soybean were consistent with those of the control when compared with the literature range.

Minerals

Dow reported that the compositional analyses of 13 minerals in grain revealed no statistically significant differences between control and DAS-68416-4 soybean for calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, phosphorus, selenium, and zinc.[13] Statistically significant differences by the paired t-test, in all four treatment groups, as well as a statistically significant overall treatment effect by F-test were observed for potassium. With the exception of magnesium and potassium, the mean levels for the analyzed minerals were within the combined literature range.[14]

Dow reported that certain mean levels of magnesium and potassium were outside the literature values. Dow noted that all four of the mean magnesium levels and one of the mean potassium levels from DAS-68416-4 soybean were lower than the combined literature range. Dow further noted that these mean levels from DAS-68416-4 soybean were closer to the literature range compared with the mean magnesium and potassium levels from the control, which were also lower than the combined literature range. Based on the data, Dow concluded that DAS-68416-4 soybean is compositionally equivalent to its conventional counterpart.

Amino Acids

Dow reported that the compositional analyses of 18 amino acids in grain revealed no statistically significant differences between control and DAS-68416-4 soybean for cysteine, methionine, proline, tyrosine, and tryptophan. Statistically significant differences by the paired t-test, in one or more of the four treatment groups, were observed for alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, phenylalanine, serine, threonine, and valine. Statistically significant overall treatment effects by F-test were reported for alanine, arginine, glutamic acid, glycine, histidine, leucine, lysine, phenylalanine, and threonine. However, the mean levels for the analyzed amino acids were within the combined literature range.

Fatty Acids

Dow reported the results of compositional analyses of 22 fatty acids in grain, 12 of which were below the limit of quantitation. Of the 10 fatty acids that were quantifiable, no statistically significant differences by the paired t-test, in any of the four treatment groups, were observed between DAS-68416-4 soybean and the control in levels of palmitic, heptadecanoic, stearic, linoleic, and eicosenoic acids. Statistically significant differences by the paired t-test, in one or more of the four treatment groups, were observed for palmitoleic, oleic, linolenic, arachidic, and behenic acids. Statistically significant overall treatment effects by F-test were reported for palmitoleic, oleic, linolenic, and arachidic acids. The mean levels for the analyzed fatty acids were within the combined literature range.[15]

Vitamins

Dow reported the compositional analyses of 10 vitamins in grain, 4 of which were below the limit of quantitation. Of the 6 vitamins that were quantifiable, no statistically significant differences by the paired t-test, in any of the four treatment groups, were observed between DAS-68416-4 soybean and the control in levels of vitamin B_1, vitamin B_2, vitamin B_3, vitamin B_5, vitamin B_6, vitamin C, vitamin E, and delta-tocopherol. Statistically significant differences by the paired t-test, in two of the four treatment groups, as well as a statistically significant overall treatment effect by F-test were observed between DAS-68416-4 soybean and the control in levels of folic acid and gamma-tocopherol. However, the mean level of folic acid was within the combined literature range and the relative differences between DAS-68416-4 soybean and the control in levels of folic acid and gamma-tocopherol were small.[16]

Dow reported that the mean levels of vitamin B_2 were higher than the combined literature range; however, these were not statistically different from the mean level of vitamin B_2 reported for the control.

Isoflavones

Dow reported the compositional analyses of both the free and glycoside forms of isoflavones in grain. Dow reported that the levels of free forms (daidzein, genistein, and glycitein) were below the limit of quantitation in all or nearly all samples, but that the levels of glycoside forms (daidzin, genistin, and glycitin) were quantifiable. Statistically significant differences by the paired t-test, in one of the four treatment groups, were observed for daidzin, genistin, and glycitin. A statistically significant overall treatment effect by F-test was reported for glycitin. However, the mean levels for the 3 glycosides[17] were within the combined literature ranges for their respective total aglycone-equivalents.

Antinutrients

Dow reported that the compositional analyses of antinutrients in grain revealed no statistically significant differences (by paired t-test) or overall treatment effects (by F-test) between DAS-68416-4 soybean and the control for lectin, phytic acid, raffinose, stachyose, and trypsin inhibitor. Furthermore, the mean levels for the analyzed anti-nutrients were within the combined literature ranges.

Dow concludes that the compositional assessment supports the conclusion that DAS-68416-4 soybean is substantially equivalent to the non-transgenic control and to conventional soybean varieties. Dow bases its conclusion on compositional analysis, which shows that the various components analyzed in DAS-68416-4 soybean were either statistically indistinguishable from the non-transgenic control, less than 13% different from the non-transgenic control, or within the literature range for conventional soybean varieties.

Endogenous Allergens

To assess endogenous allergenicity of the DAS-68416-4 soybean, Dow evaluated IgE-binding capacity to extracts of DAS-68416-4 soybean and the control using one dimensional IgE immunoblot and ELISA inhibition using pooled sera from clinically-reactive soy allergic patients. Dow concluded that immunoblot and ELISA inhibition data demonstrate that the genetic modification used to generate DAS-68416-4 soybean did not alter endogenous allergenicity compared with that of the control.

Conclusion

FDA evaluated Dow's submission to determine whether DAS-68416-4 soybean raises any safety or regulatory issues with respect to the intended modification or with respect to the food and feed itself. Based on the information provided by the company and other information available to the agency, FDA did not identify any issues under the FD&C Act that would require further evaluation at this time.

Dow has concluded that its herbicide tolerant soybean variety, DAS-68416-4 soybean, and the food and feed derived from it are as safe as conventional soybean varieties, and with the exception of the herbicide tolerance traits, are not materially different in composition or other relevant parameters from other soybean varieties now grown, marketed and consumed in the United States. At this time, based on Dow's data and information, the agency considers Dow's consultation on DAS-68416-4 soybean to be complete.

Carrie McMahon, Ph.D.

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[1]Dow submitted its evaluation of the potential for allergenicity and toxicity of the AAD-12 protein, which FDA designated as New Protein Consultation No. NPC 000009 under FDA's Guidance to Industry: "Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use." FDA responded that it had no questions regarding Dow's conclusions.

[2]The analytical techniques discussed in the submission include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western hybridization analysis, glycosylation analysis, mass determination of tryptic and Asp-N peptides by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray-ionization liquid chromatography/mass spectrometry (LCMS), followed by N- and C-terminal amino acid sequence analysis.

[3]Dose adjusted for purity.

[4]In its discussion. Dow referred to Test No. 423 of the OECD Guideline for Testing of Chemicals: Acute Oral Toxicity - Acute Toxic Class

Method." (2001).

[5]These criteria can be found in the guidelines for the evaluation of the potential allergenicity of introduced proteins, published in 2003 by the Codex Alimentarius Commission.

[6]The analytical techniques discussed in the submission include SDS-PAGE, lateral flow strips, and Western hybridization analysis.

[7]EPA subsequently established an exemption from the requirement of a tolerance for residues of PAT protein when used as a plant-pesticide inert ingredient in or on all raw agricultural commodities (62 FR 17717; April 11, 1997).

[8]In its discussion, Dow referred to the OECD (2001) *Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients.*

[9]According to Dow, FDR procedures account for multiplicity due to the large number of comparisons made in the compositional analysis. Dow states that "the p-values were adjusted using FDR to improve discrimination of true differences among treatments from random effects (false positives)."

[10]Dow used a combined literature range for its comparison, in which values from Version 3.0 of the publicly available International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2006) were combined with values, where available, from the OECD guidelines (2001) . The database is maintained by ILSI and can be accessed at http://www.cropcomposition.org/.

[11]No literature ranges were reported for calcium and phosphorus.

[12]Dow noted that the values for cholesterol were all below the limit of quantitation and that no literature values were reported.

[13]Dow noted that the majority of values for sodium were below the limit of quantitation; therefore, no statistical analysis was performed.

[14]No literature values were reported for selenium, sodium, and zinc.

[15]No literature values were reported for capric, pentadecanoic, pentadecenoic, gamma-linolenic, eicostrienoic, and arachidonic acids.

[16]Dow reported that literature values were unavailable for beta-carotene, vitamin B_3, vitamin B_5, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, beta-tocopherol, and gamma-tocopherol.

[17]The mean values for each of the 3 glycone-equivalents are equal to the respective total aglycone-equivalents since there were essentially no detectable free aglycones in the samples.

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Biotechnology Consultation Agency Response Letter BNF No. 000124

See FDA's memo on BNF No. 000124[1] for further details

CFSAN/Office of Food Additive Safety

Laura Tagliani
Global Regulatory Leader
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

November 14, 2011

Dear Ms. Tagliani:

This is in regard to Dow AgroSciences LLC's (Dow's) consultation with the Food and Drug Administration (FDA) (Center for Veterinary Medicine and Center for Food Safety and Applied Nutrition) on genetically engineered soybean, DAS-68416-4 soybean. According to information Dow has provided, DAS-68416-4 soybean is engineered to express the aryloxyalkanoate dioxygenase-12 (AAD-12) protein, and the phosphinothricin N-acetyl transferase (PAT) protein. The AAD-12 and PAT proteins confer tolerance to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate, respectively. All materials relevant to this notification have been placed in a file designated BNF 000124. This file will be maintained in the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

As part of bringing the consultation regarding this product to closure, Dow submitted a summary of its safety and nutritional assessment of the genetically engineered soybean on December 22, 2009. Dow submitted additional information on April 15, 2010, and on August 23 and 24, 2011. These communications informed FDA of the steps taken by Dow to ensure that this product complies with the legal and regulatory requirements that fall within FDA's jurisdiction. Based on the safety and nutritional assessment Dow has conducted, it is our understanding that Dow has concluded that food and feed derived from DAS-68416-4 soybean are not materially different in composition, safety, and other relevant parameters from soybean-derived food and feed currently on the market, and that the genetically engineered DAS-68416-4 soybean does not raise issues that would require premarket review or approval by FDA.

It is Dow's responsibility to obtain all appropriate clearances, including those from the Environmental Protection Agency and the United States Department of Agriculture, before marketing food or feed derived from DAS-68416-4 soybean.

Based on the information Dow has presented to FDA, we have no further questions concerning food and feed derived from DAS-68416-4 soybean at this time. However, as you are aware, it is Dow's continuing responsibility to ensure that foods marketed by the firm are safe, wholesome, and in compliance with all applicable legal and regulatory requirements. A copy of the text of this letter responding to BNF 000124, as well as a copy of the text of FDA's memorandum summarizing the information in BNF 000124, is available for public review and copying via the FDA Completed Consultations on Bioengineered Foods page at www.fda.gov/bioconinventory.

Sincerely,

Dennis M. Keefe, Ph.D.
Director
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

U.S. Food and Drug Administration

Food

Biotechnology Consultation Note to the File BNF No. 000055

April 21, 1998

Subject: BNF0055 - Glufosinate-tolerant Soybean Lines

Keywords:

Soybean, *Glycine max*, Glufosinate, Liberty®, LibertyLink® soybeans, phosphinothricin acetyltransferase (PAT), *pat* gene, *Streptomyces viridochromogenes*, beta-lactamase (bla) gene

Background

The new soybean varieties that are the subject of this consultation contain the *pat* gene from *Streptomyces viridochromogenes*. The *pat* gene encodes the phosphinothricin acetyltransferase (PAT) enzyme, which confers resistance to glufosinate ammonium herbicides. AgrEvo first provided some background information or the new soybean varieties (derived from events A2704-12 and A5547-127) on March 21, 1998. AgrEvo then submitted a full summary of safety and nutritional assessment of the new soybean varieties on March 31, 1998. AgrEvo noted that the mode of action of the enzyme phosphinothricin acetyltransferase and information on its safety have been submitted to FDA as part of previous food and feed safety assessment of other glufosinate-tolerant crops.

Intended Effect and Food/Feed Use

The intended technical effect of the genetic modification of the new soybean lines is to confer tolerance to the herbicide glufosinate. AgrEvo notes that the food and fee uses of the new soybean varieties are no different than those of varieties currently on the market. Soybeans or processed products derived from soybeans are used for both human and animal food. In addition to oil for human consumption and defatted meal for animal feed, whole soybeans are used to produce such items as soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and traditional soy foods such as miso, soy milk, soy sauce and tofu. Soybean protein products also have food and feed uses.

Molecular Alterations and Characterization

Both new soybean varieties were produced by the particle bombardment method of generating transformants using a plasmid with a pUC backbone and containing the *pat* gene with the Cauliflower Mosaic Virus 35S promoter and terminator. The plasmid was linearized using a restriction enzyme prior to use in the transformation process in order to destroy the beta-lactamase (*bla*) gene found in the plasmid backbone.

The *pat* gene that has been introduced into the new soybean varieties is a synthetic version of the *S. viridochromogenes* gene. Since the bacterial *pat* gene has a high G:C content, which is atypical for plants, AgrEvo synthesized a gene with a modified nucleotide sequence using codons preferred by plants. AgrEvo notes, however, tha while the nucleotide sequence has been altered, the amino acid sequence of the enzyme remains unchanged.

AgrEvo states that subsequent analysis of the transformants shows that two copies of the *pat* gene integrated into the genome of transformant A2704-12 in a head-to-tail configuration and that one copy of 3' *bla* sequences and one copy of 5' *bla* sequences are integrated in between the two *pat* genes. The integrated parts of the *bla* gene do not reconstitute an intact *bla* gene because the sequences integrated into the genome in an inverted orientation with respect to each other. In transformant A5547-127, one copy of 3' *bla* sequences, and one copy of 5' *bla* sequences are integrated downstream, and upstream, respectively, of a single copy of the *pat* gene that integrated into the plant genome. The 5' and 3' *bla* sequences do not reconstitute an intact *bla* gene because of the intervening *pat* gene. For both transformation events, examination of progeny shows that the *pat* locus is inherited in Mendelian fashion.

Safety of Expressed Protein

Only one protein, namely the phosphinothricin acetyltransferase enzyme, is expressed in the new soybean varieties. The *bla* gene is not expressed; expression is not expected because the gene is not present in the soybean genome in an intact form. Expression would not be expected even if an intact gene was present because a bacterial promoter was used with the gene. In any case, AgrEvo stated that Northern blot analysis it conducted showed that no *bla* messenger RNA is made in the transgenic soybeans.

AgrEvo states that it measured PAT protein levels in whole and processed plant fractions using enzyme-linked immmunosorbent assays (ELISA) in order to determine whether the PAT protein would be a major constituent in human or livestock diets. AgrEvo states that using the largest amounts of PAT detected and the smallest level of crude protein reported in the literature for forage, hay and seed, PAT protein represents 0.0031%, 0.0015%, and 0.0005% of the crude protein in forage, hay and seed in event A2704-12, respectively. The corresponding percentages for event A5547-127 are 0.0174%, 0.0034%, and 0.0031% for forage, hay and seed respectively.

AgrEvo states that, as expected, there was no PAT protein detected in refined oil, food grade oil, and crude lecithin for both events. No PAT activity was detected in the meal fractions or the soy isolates from event A2704-12 although the protein was detected in the hull fraction. For event A5547-127, immunoreactive PAT protein was detected in all processed fractions except refined oil, food grade oil, and crude lecithin.

AgrEvo notes that ELISA detects both active and inactive PAT protein and that, while activity assays were not done to determine if any of the immunoreactive PAT is enzymatically active, processing temperatures are sufficiently high to inactivate the enzyme. AgrEvo adds that regardless of the level present in whole and processed fractions, PAT is not likely to be toxic or allergenic. AgrEvo notes that biochemical characterization, and allergenic and toxicity potential of the PAT protein have been addressed in previous submissions to FDA (BNFs 0023, 0029, and 0046). In addition, AgrEvo states that PAT protein showed no evidence of toxicity in rats fed the protein at dietary concentrations of 50,000 ppm for 14 days. AgrEvo also fed broilers diets prepared from the two new soybean varieties as well as nontransgenic soybean and reported that evaluation of body weight, body weight gain, feed intake and carcass characteristics showed no differences between chickens fed diets usinc seed from A2704-12 or A5547-127 and commercial varieties.

Compositional Analysis

Antinutrients

AgrEvo analyzed for stachyose, raffinose and phytic acid in seed, and for trypsin inhibitor and lectins in seed, non-toasted soy meal, and toasted soy meal. AgrEvo also measured phytoestrogen levels in seed, as well as non-toasted and toasted soy meal (AgrEvo noted that although phytoestrogens such as the isoflavones daidzein, genistein, and glycitein have been implicated in adverse effects on the reproductive system in animals fed large amounts of soybean meal, they are not universally accepted as anti-nutrients, and that they have also been reported as having beneficial anti-cancer effects). AgrEvo stated that the antinutrient content of the new soybeans does not differ significantly from that of non-transgenic soybeans.

Allergens

AgrEvo also analyzed whether the genetic modification might have altered the allergenic potential of seed derived from the new soybean varieties. AgrEvo screened soybean seed extracts from lines derived from both A2704-12 and A5547-127 events as well as their non-transgenic counterparts against a panel of sera from 16 soy-allergic individuals using Radioallergosorbent Test (RAST). In other studies, pooled sera were used in RAST inhibition assays and in immunoblotting. AgrEvo reports tha the results of these studies demonstrate that there is no qualitative or quantitative difference in endogenous soybean allergen content between the two transgenic soybean varieties and their non-transgenic counterparts.

Nutrients

AgrEvo conducted the following analyses for lines derived from transformation events A2704-12 and A5547-127: 1) proximate analysis (moisture, crude protein, crude fat, ash, acid detergent fiber, neutral detergent fiber, carbohydrate) in hay, forage, seed, hulls, and toasted and non-toasted defatted soy meal; 2) minerals (calcium, phosphorus, and potassium) in seed; 3) fatty acid analysis in seed and food grade oil; and 4) amino acid analysis in seed, soy isolates, and non-toasted as well as toasted soy meal.

AgrEvo reported that there were no nutritionally significant differences in proximates or mineral contents between the new soybean varieties and current commercial varieties and that values obtained were similar to values established for soybeans. In addition, no statistically significant differences were observed for amino acid leve between soybeans derived from event A5547-127 and non-transgenic soybeans. Statistical differences were seen in the levels of some amino acids between soybeans derived from event A2704-12 and the non-transgenic counterpart with the levels in the new variety being higher. AgrEvo states that although differences were noted, the amino acid profiles for both soybean varieties and the non-transgenic varieties were qualitatively and quantitatively similar to those reported by the US Departmen of Agriculture (USDA) for soybeans with glutamic acid being present at the highest level and tryptophan being present at the lowest level. Similarly, when the fatty aci profile of oil derived from the new soybean varieties was compared to the fatty acid profile of oil derived from non-transgenic soybean, differences were observed for some fatty acids. However, AgrEvo notes that the qualitative and quantitative profiles of total lipids and fatty acids of the transgenic soybeans as well as non-transgeni soybeans are similar to profiles reported for soybeans by USDA.

AgrEvo notes that the statistically significant differences in some amino acids and fatty acids could be due to factors such as growing conditions, maturity at harvest, post harvest storage and processing, and the inherent variability of nutrient content. The differences were not observed consistently in samples obtained from all growing regions. AgrEvo concludes that the differences observed are not meaningful from a nutritional point of view.

Conclusions

AgrEvo has concluded that its transgenic glufosinate-tolerant soybean lines are not materially different in terms of food safety and nutritional profile from soybean varieties currently on the market. At this time, based on AgrEvo's description of its data and analyses, the Agency considers AgrEvo's consultation on soybean lines from transformation events A2704-12 and A5547-127 to be complete.

Nega Beru, Ph.D

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Biotechnology Consultation Agency Response Letter BNF No. 000055

See FDA's memo on BNF No. 000055 [1] for further details

May 15, 1998

Sally L. Van Wert, Ph.D.
Manager Regulatory Affairs - Biotechnology
AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808

Dear Dr. Van Wert:

This is in regard to AgrEvo's consultation with the Food and Drug Administration (FDA) (Center for Veterinary Medicine an Center for Food Safety and Applied Nutrition) on its glufosinate-tolerant soybean lines A2704-12 and A5547-127. According to AgrEvo, the new soybean varieties have been rendered tolerant to glufosinate ammonium herbicides througl expression of a modified phosphinothricin acetyltransferase (*pat*) gene from *Streptomyces viridochromogenes*.

As part of bringing the consultation regarding these varieties to closure, you submitted a summary safety and nutritional assessment of the genetically modified soybean varieties on March 31, 1998. This communication informed FDA of the steps taken by AgrEvo to ensure that these products comply with the legal and regulatory requirements that fall within FDA's jurisdiction. Based on the safety and nutritional assessment AgrEvo has conducted, it is our understanding that AgrEvo has concluded that the new soybean varieties are not materially different in composition, safety, or any other relevant parameters from soybean lines currently on the market and that they do not raise issues that would require premarket review or approval by FDA. All materials relevant to this notification have been placed in a file designated BNF0055 that will be maintained in the Office of Premarket Approval.

Based on the information AgrEvo has presented, we have no further questions concerning these new soybean varieties at this time. However, as you are aware, it is AgrEvo's continued responsibility to ensure that foods marketed by the firm ar safe, wholesome and in compliance with all applicable legal and regulatory requirements.

Sincerely yours,

Alan M. Rulis, Ph.D.
Director
Office of Premarket Approval
Center for Food Safety

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NPC 000009: Agency Response Letter

CFSAN/Office of Food Additive Safety

May 19, 2010

Mark S. Krieger, Ph.D.
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054

Re: NPC 00009; aryloxyalkanoate dioxygenase-12, AAD-12

Dear Dr. Krieger:

This letter is in response to Dow AgroSciences LLC's (Dow) early food safety evaluation of the protein, aryloxyalkanoate dioxygenase-12, AAD-12, expressed in a new plant variety under development for food use, which you submitted to the Food and Drug Administration (FDA) on December 15, 2008, under FDA's guidance to industry, "Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (71 FR 35688 June 21, 2006, and available on the FDA home page at http://www.fda.gov - follow the hyperlinks from the "Food" topic to the "Biotechnology" program area). As usec in the guidance and in this letter, the term "food" refers to both human food and animal feed. All materials relevant to this evaluation have been placed in a file designated NPC 00009. This file will be maintained in the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

In cases of inadvertent low level presence in the food supply of a new food plant variety, FDA believes that any food or feed safety concern would be limited to the safety of the new protein(s) in that plant (generally, the potential allergenicity and toxicity of the new protein(s)). Based on Dow's early food safety evaluation, it is our understanding that Dow has concluded that AAD-12 protein would not raise food safety concerns when it is in a new food plant variety that is present at low levels in the food supply. We have completed our evaluation of your submission, and we have no questions at this time regarding Dow's condusion.

Sincerely yours,

Antonia Mattia, Ph.D.
Division Director
Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety
Center for Food Safety and Applied Nutrition

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Appendix D Herbicide-Resistant Weeds

Table D-1. Common herbicide-resistant weeds.

Genus and Species (Common Name)	1st Report Country (Year)	U.S. Occurrence (Year Reported)
Glycine (G/9) Resistant (i.e., glyphosate and sulfosate)		
Amaranthus palmeri (Palmer Amaranth)[1]	U.S. (2005)	Georgia & North Carolina (2005) Arkansas & Tennessee (2006) New Mexico (2007) Mississippi & Missouri (2008) Louisiana (2010)
Amaranthus tuberculatus (syn. *rudis*) (Common Waterhemp)[2]	U.S. (2005)	Missouri (2005) Illinois & Kansas (2006) Minnesota (2007) Indiana & Iowa (2009) Mississippi (2010)
Ambrosia artemisiifolia (Common Ragweed)[2]	U.S. (2004)	Arkansas & Missouri (2004) Ohio (2006) Indiana, Kansas & North Dakota (2007) Minnesota (2008)
Ambrosia trifida (Giant Ragweed)[1]	U.S. (2004)	Ohio (2004) Arkansas & Indiana (2005) Kansas & Minnesota (2006) Tennessee (2007) Iowa & Missouri (2009) Mississippi 2010)
Chloris truncate (Australian Fingergrass)	Australia (2010)	--
Conyza bonariensis (Hairy Fleabane)	South Africa (2003)	California (2007)
Conyza canadensis (Horseweed)[2]	U.S. (2000)	Delaware (2000) Kentucky & Tennessee (2001) Indiana, Maryland, Missouri, New Jersey & Ohio (2002) Arkansas, Mississippi, North Carolina & Pennsylvania (2003) California, Illinois & Kansas (2005) Nebraska (2006) Michigan (2007) Oklahoma (2009)

Genus and Species (Common Name)	1st Report Country (Year)	U.S. Occurrence (Year Reported)
Conyza sumatrensis (Sumatran fleabane)	Spain (2009)	--
Digitaria insularis (Sourgrass)[2]	Paraguay (2006)	--
Echinochloa colona (Junglerice)[2]	Australia (2007)	--
Eleusine indica (Goosegrass)[2]	Malaysia (1997)	Mississippi (2010)
Euphorbia heterophylla (Wild Poinsettia)[1]	Brazil (2006)	--
Kochia scoparia (Kochia)[2]	U.S. (2007)	Kansas (2007)
Lolium multiflorum (Italian Ryegrass)[1, 2]	Chile (2001)	Oregon (2004) Mississippi (2005) Arkansas (2008) Oregon (2010)
Lolium perenne (Perennial Ryegrass)[1]	Argentina (2008)	--
Lolium rigidum (Rigid Ryegrass)	Australia (1996)	California (1998)
Parthenium hysterophorus (Ragweed Parthenium)	Colombia (2004)	--
Plantago lanceolata (Buckhorn Plantain)	South Africa (2003)	--
Poa annua (Annual Bluegrass)	U.S. (2010)	Missouri (2010)
Sorghum halepense (Johnsongrass)[1]	Argentina (2005)	Arkansas (2007) Louisiana (2010)
Urochloa panicoides (Liverseedgrass)	Australia (2008)	--
Glutamine Synthase Inhibitors (H/10) Resistant Weeds (i.e., glufosinate)		
Eleusine indica (Goosegrass)	Malaysia (2009)	--
Lolium multiflorum (Italian Ryegrass)[2]	U.S. (2010)	Oregon (2010)
Synthetic Auxins (O/4) Resistant Weeds (e.g., 2,4-D, 2,4-DP, dicamba, triclopyr, etc.)		
Carduus nutans (Musk Thistle)	New Zealand (1981)	--
Carduus pycnocephalus (Italian Thistle)	New Zealand (1997)	--
Centaurea solstitialis (Yellow Starthistle)	U.S. (1988)	Washington (1988)

Genus and Species (Common Name)	**1st Report Country (Year)**	**U.S. Occurrence (Year Reported)**
Chenopodium album (Lambsquarters)[1]	New Zealand (2005)	--
Cirsium arvense (Canada thistle)[1]	Sweden (1979)	--
Commelina diffusa (Spreading Dayflower)	U.S. (1957)	Hawaii (1957)
Convolvulus arvensis (Field Bindweed)[1]	U.S. (1964)	Kansas (1964)
Daucus carota (Wild Carrot)[1]	Canada (1957)	Michigan (1993) Ohio (1994)
Digitaria ischaemum (Smooth Crabgrass)	U.S. (2002)	California (2002)
Echinochloa colona (Junglerice)	Colombia (2000)	--
Echinochloa crus-galli (Barnyardgrass)	U.S. (1998)	Louisiana (1998) Arkansas (1999)
Echinochloa crus-pavonis (Gulf Cockspur)	Brazil (1999)	--
Fimbristylis miliacea (Globe Fringerush)	Malaysia (1989)	--
Galeopsis tetrahit (Common Hempnettle)	Canada (1998)	--
Galium spurium (False Cleavers)	Canada (1996)	--
Kochia scoparia (Kochia)[1]	U.S. (1995)	Montana (1995) North Dakota (1995) Idaho (1997)
Lactuca serriola (Prickly Lettuce)	U.S. (2007)	Washington (2007)
Limnocharis flava (Yellow bur-head)	Indonesia (1995)	--
Limnophila erecta (Marshweed)	Malaysia (2002)	--
Matricaria perforate (Scentless Chamomile)	France (1975)	--
Papaver rhoeas (Corn Poppy)	Spain (1993)	--
Ranunculus acris (Tall Buttercup)	New Zealand (1988)	--
Raphanus raphanistrum (Wild Radish)	Australia (1999)	--
Sinapis arvensis (Wild Mustard)[1]	Canada (1990)	--
Sisymbrium orientale (Indian Hedge Mustard)	Australia (2005)	--

Genus and Species (Common Name)	1st Report Country (Year)	U.S. Occurrence (Year Reported)
Soliva sessilis (Carpet Burweed)	New Zealand (1999)	--
Sphenoclea zeylanica (Gooseweed)	Philippines (1983)	--
Stellaria media (Common Chickweed)	United Kingdom (1985)	--

Source: Heap (2011).
[1]Weeds commonly found in genetically engineered crops
[2] Having multiple mode of action resistance (G/9 and H/10)

Table D-2. 2,4-D activity on glyphosate- and ALS-resistant weeds.

Weed Species - Scientific Name (Common Name)	Glyphosate	ALS Herbicides	2,4-D
Chenopodium album (Common lambsquarters)	Difficult: Suspected Resistant (2004)	Resistant (2001)	Susceptible
Ambrosia artemisiifolia (Common ragweed)	Confirmed Resistant (2004)	Resistant (1998)	Susceptible
Solanum ptycanthum (Eastern black nightshade)	Difficult (2004)	Resistant (1999)	Susceptible
Ambrosia trifida (Giant ragweed)	Confirmed Resistant (2004)	Resistant (1998)	Susceptible
Conyza canadensis (Marestail [horseweed])	Confirmed Resistant (2000)	Resistant (2000)	Susceptible
Amaranthus palmeri (Palmer amaranth)	Confirmed Resistant (2005)	Resistant (1991)	Susceptible
Amaranthus spp. (Waterhemp spp.)	Confirmed Resistant (2005)	Resistant (1993)	Susceptible
Sida spinosa (Prickly sida)	Difficult (2004)	Resistant (1993)	Susceptible

Source: (DAS, 2010; Heap, 2011)

Appendix E APHIS Threatened and Endangered Species Decision Tree for FWS Consultations

DECISION TREE ON WHETHER SECTION 7 CONSULTATION WITH FWS IS TRIGGERED FOR PETITIONS OF TRANSGENIC PLANTS

This decision tree document is based on the phenotypes (traits) that have been permitted for environmental releases under APHIS oversight (for a list of approved notifications and environmental releases, visit Information Systems for Biotechnology, at http://isb.vt.edu.) APHIS will re-evaluate and update this decision document as it receives new applications for environmental releases of new traits that are genetically engineered into plants.

BACKGROUND

For each transgene(s)/transgenic plant the following information, data, and questions will be addressed by APHIS, and the EAs on each petition will be publicly available. APHIS review will encompass:

- 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 threatened or endangered plant species (TES) or a host of any TES.

FDA published a policy in 1992 on foods derived from new plant varieties, including those derived from transgenic plants (http://vm.cfsan.fda.gov/~lrd/fr92529b.html and http://vm.cfsan.fda.gov/~lrd/consulpr.html). The FDA's policy requires that genetically engineered foods meet the same rigorous safety standards as is required of all other foods. Many of the food crops currently being developed using biotechnology do not contain substances that are significantly different from those already consumed by human and thus do not require pre-market approval. Consistent with its 1992 policy, FDA expects developers to consult with the agency on safety and regulatory questions. A list of consultations is available at http://vm.cfsan.fda.gov/~lrd/biocon.html. APHIS considers the status and conclusion of the FDA consultations in its EAs.

Below is a description of our review process to whether a consultation with U.S. Fish and Wildlife Service is necessary.

If the answer to any of the questions 1-4 below is yes, APHIS will contact FWS to determine if a consultation is required:

Is the transgenic plant sexually compatible with a TE plant[9] without human intervention?

[9] APHIS will provide FWS a draft EA that will address the impacts, if any, of gene movement to the TES plant

1. Are naturally occurring plant toxins (toxicants) or allelochemicals increased over the normal concentration range in parental plant species?
2. Does the transgene product or its metabolites have any significant similarities to known toxins[10]?
3. Will the new phenotype(s) imparted to the transgenic plant allow the plant to be grown or employed in new habitats (e.g., outside agro-ecosystem)[11].
4. Does the pest resistance[12] gene act by one of the mechanisms listed below? If the answer is YES then a consultation with U.S. Fish and Wildlife Service is NOT necessary.

A. The transgene acts only in one or more of the following ways:

i. As a structural barrier to either the attachment of the pest to the host, to penetration of the host by the pest, to the spread of the pest in the host plant (e.g., the production of lignin, callose, thickened cuticles);
ii. In the plant by inactivating or resisting toxins or other disease causing substances produced by the pest;
iii. By creating a deficiency in the host of a component required for growth of the pest (such as with fungi and bacteria);
iv. By initiating, enhancing, or potentiating the endogenous host hypersensitive disease resistance response found in the plant;
v. In an indirect manner that does not result in killing or interfering with normal growth, development, or behavior of the pest;

B. A pest derived transgene is expressed in the plant to confer resistance to that pest (such as with coat protein, replicase, and pathogen virulence genes).

For the biotechnologist:
Depending on the outcome of the decision tree, initial the appropriate decision below and incorporate its language into the EA. Retain a hard copy of this decision document in the petition's file.

________ BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that the release following a determination of nonregulated status would have no effects on listed threatened or endangered species and consequently, a written concurrence or formal consultation with the Fish and Wildlife Service is not required for this EA.

________ BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS reached a determination that the release

[10] Via a comparison of the amino acid sequence of the transgene's protein with those found in the protein databases like PIR, Swiss-Prot and HIV amino acid data bases.

[11] Such phenotypes might include tolerance to environmental stresses such as drought, salt, frost, aluminum or heavy metals.

[12] Pest resistance would include any toxin or allelochemical that prevents, destroys, repels or mitigates a pest or effects any vertebrate or invertebrate animal, plant, or microorganism.

following a determination of non-regulated status is not likely to adversely affect any listed threatened or endangered species and consequently obtained written concurrence from the Fish and Wildlife Service.

________ BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS reached a determination that the release following a determination of non-regulated status is likely to affect adversely one or more listed threatened or endangered species and has initiated a formal consultation with the Fish and Wildlife Service.