Monsanto Petition (10-188-01p) for Determination of Nonregulated Status of Dicamba Herbicide-resistant Soybean (*Glycine max*) MON 87708

OECD Unique Identifier: MON-877Ø8-9

Plant Pest Risk Assessment

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A. Introduction

Monsanto Company (hereafter referred to as Monsanto) has petitioned the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) for a determination that genetically engineered (GE) dicamba herbicide-resistant[1] soybean event MON 87708 and OECD Unique Identifier MON-87708-9 (hereafter referred to as MON 87708 soybean) is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under APHIS' 7 Code of Federal Regulations (CFR) part 340 (7 CFR part 340). This petition was assigned the number 10-188-01p and is hereafter referenced as (Monsanto, 2012). APHIS administers 7 CFR part 340 under the authority of the plant pest provisions of the Plant Protection Act (PPA) of 2000 (7 U.S.C. 7701 *et seq.*)[2]. This plant pest risk assessment was conducted to determine if MON 87708 soybean is unlikely to pose a plant pest risk.

APHIS regulations in 7 CFR part 340 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 PPA or to the regulatory requirements of Part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE organism is considered a regulated article under Part 340 if the donor organism, recipient organism, or vector, or vector agent used in engineering the organism belongs to any genera or taxa designated in 7 CFR 340.2 and meets the definition of plant pest, or is an unclassified organism and/or an organism whose classification is unknown, or any product which contains such an organism, or any other organism or product altered or produced through genetic engineering which the Administrator determines is a plant pest or has reason to believe is a plant pest[3]. MON 87708 soybean was produced by *Agrobacterium*-mediated transformation, and portions of the inserted genetic material were derived from plant pest organisms listed in 7 CFR part 340.2 (i.e., promoter sequence from peanut chlorotic streak caulimovirus, leader sequence from Tobacco Etch virus, and T-DNA border sequences from *Agrobacterium tumefaciens*) (Table III-1, p. 40, Monsanto, 2012). Therefore, the genetically engineered MON 87708 soybean is

[1] Monsanto has described the phenotype of MON 87708 soybean as "herbicide tolerant" and historically APHIS has also referred to GE plants with reduced herbicide sensitivity as herbicide tolerant. However, the phenotype would fall under the Weed Science Society of America (WSSA) definition of "herbicide resistance" since MON 87708 soybean has an "inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type". By the WSSA (1998) definition, "resistance (to an herbicide) may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis." Herbicide tolerance, by the WSSA definition, only applies to plant species with an "inherent ability to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

[2] Plant Protection Act in 7 U.S.C. 7702 § 403(14) defines plant pest as: "Plant Pest - The term "plant pest" means any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: (A) A protozoan. (B) A nonhuman animal. (C) A parasitic plant. (D) A bacterium. (E) A fungus. (F) A virus or viroid. (G) An infectious agent or other pathogen. (H) Any article similar to or allied with any of the articles specified in the preceding subparagraphs."

[3] Limited exclusions or exemptions apply for certain engineered microorganisms and for interstate movement of some organisms, as in 7 CFR 340.1 and 340.2.(b).

considered a regulated article under APHIS regulations at 7 CFR part 340. Monsanto has conducted field releases of MON 87708 soybean as a regulated article under APHIS-authorized permits or acknowledged notifications since 2005 (Appendix Table A-1, pp. 284-286, Monsanto, 2012), in part, to gather information to support that MON 87708 soybean is unlikely to pose a plant pest risk.

Potential impacts in this Plant Pest Risk Assessment are those that pertain to plant pest risk associated with MON 87708 soybean and its progeny and their use in the absence of confinement relative to the unmodified recipient and/or other appropriate comparators. APHIS utilizes data and information submitted by the applicant, in addition to current literature, to determine if MON 87708 soybean is unlikely to pose a plant pest risk. APHIS regulations in 7 CFR 340.6(c) specify the information needed for consideration in a petition for nonregulated status. APHIS will assess information submitted by the applicant about MON 87708 soybean related to plant pest risk characteristics; expression of the gene product, new enzymes, or changes to plant metabolism; disease and pest susceptibilities and indirect plant pest effects on other agricultural products; effects of the regulated article on nontarget organisms; weediness of the regulated article; impact on the weediness of any other plant with which it can interbreed; changes to agricultural or cultivation practices that may impact diseases and pests of plants; and transfer of genetic information to organisms with which it cannot interbreed.

APHIS may also consider information relevant to reviews conducted by other agencies that are part of the 'Coordinated Framework for the Regulation of Biotechnology' (51 FR 23302, June 26, 1986; 57 FR 22984, May 29, 1992). Under the Coordinated Framework, the oversight of biotechnology-derived plants rests with APHIS, the Food and Drug Administration (FDA), and the Office of Pesticide Programs of the U.S. Environmental Protection Agency (EPA). Depending on its characteristics, certain biotechnology-derived products are subjected to review by one or more of these agencies.

EPA regulates under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*) the distribution, sale, use and testing of pesticidal substances produced in plants and microbes, including those pesticides that are produced by an organism through techniques of modern biotechnology. 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 Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. Chapter 9). Prior to registration for a new use for a new or previously registered pesticide, EPA must determine through testing that the pesticide does 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 Data Requirements for Pesticides (40 CFR part 158). Other applicable EPA regulations include Pesticide Registration and Classification Procedures (40 CFR part 152) and Experimental Use Permits (40 CFR part 172). Dicamba was first registered in 1967 for use as a pesticide (herbicide) in the U.S. and is currently approved for preplant and preharvest labeled uses on soybean (US-EPA, 2009; BASF, 2010). However, less than 1% of soybean acreage in 2008 was estimated to be treated with dicamba (p. 28, USDA-NASS, 2007; Section VIII.G, p. 198 and Table

VIII-12, p. 199, Monsanto, 2012) due to the high sensitivity of soybean to the herbicide (BASF, 2010; Appendix C.2, Table C-7, p. 317, Monsanto, 2012). To allow for a wider window of application of dicamba on dicamba-resistant MON 87708 soybean, Monsanto has submitted an application to amend EPA Registration Number 524-582 to register a new use pattern for dicamba on MON 87708 soybean which would allow preemergence application up to the day of crop emergence and in-crop postemergence application through the early reproductive (R1/R2) growth stage (p. 35, Monsanto, 2012; 75 FR 51045, August 18, 2010). Monsanto has also requested that EPA amend 40 CFR part 180 to establish a tolerance for residues of dicamba and its relevant metabolites in or on soybean forage at 45 parts per million (ppm) and soybean hay at 70 ppm, which would allow for the feeding of dicamba-treated soybean forage and hay to livestock (p. 35, Monsanto, 2012; 75 FR 46924, August 4, 2010). EPA is currently reviewing the label changes to remove all preemergence planting restrictions (intervals and rainfall) for dicamba on MON 87708 soybean and establish tolerances for residues of dicamba on soybean forage and hay. EPA's assessment will analyze risks to non-target organisms to determine if the label is sufficient to meet EPA's standards for registration: "reasonable certainty of no harm to humans" and "no unreasonable adverse effects on the environment." If these standards are not met, EPA will apply appropriate risk mitigation strategies and propose label modifications to address the specific concerns. After EPA has completed its assessments and provided these to APHIS, APHIS will update this PPRA if needed.

The FDA under the FFDCA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those developed through modern biotechnology. To help sponsors of foods and feeds derived from genetically engineered crops comply with their obligations, the FDA encourages them to participate in its comprehensive voluntary consultation process prior to commercial distribution of food or feed (57 FR 22984, May 29, 1992). Monsanto initiated a consultation with the FDA (Biotechnology Notification File [BNF] No. 125) on the food and feed safety and compositional assessment of MON 87708 soybean (p. 34, Monsanto, 2012). Monsanto submitted a safety and nutritional assessment summary document to the FDA on November 9, 2010 and received a completed consultation letter from the FDA on October 11, 2011. A copy of the text of this letter responding to BNF 125, as well as a copy of the text of FDA's memorandum summarizing the information in BNF 125, is available via the FDA webpage "Biotechnology Consultations on Food from GE Plant Varieties" at http://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon (US-FDA, 2011).

B. Development of MON 87708 Soybean

For over 50 years, the U.S. has been the world's largest producer of soybeans (FAOSTAT, 2012). In 2011, over 3 billion bushels were produced on 74 million acres (USDA-NASS, 2012b) in over 30 states (Figure 1), generating a crop value of $35.8 billion (USDA-NASS, 2012c). To optimize yield and economic return, growers select soybean lines adapted to the local environmental and climatic conditions and grow them using appropriate cultivation practices such as seedbed preparation, planting timing and

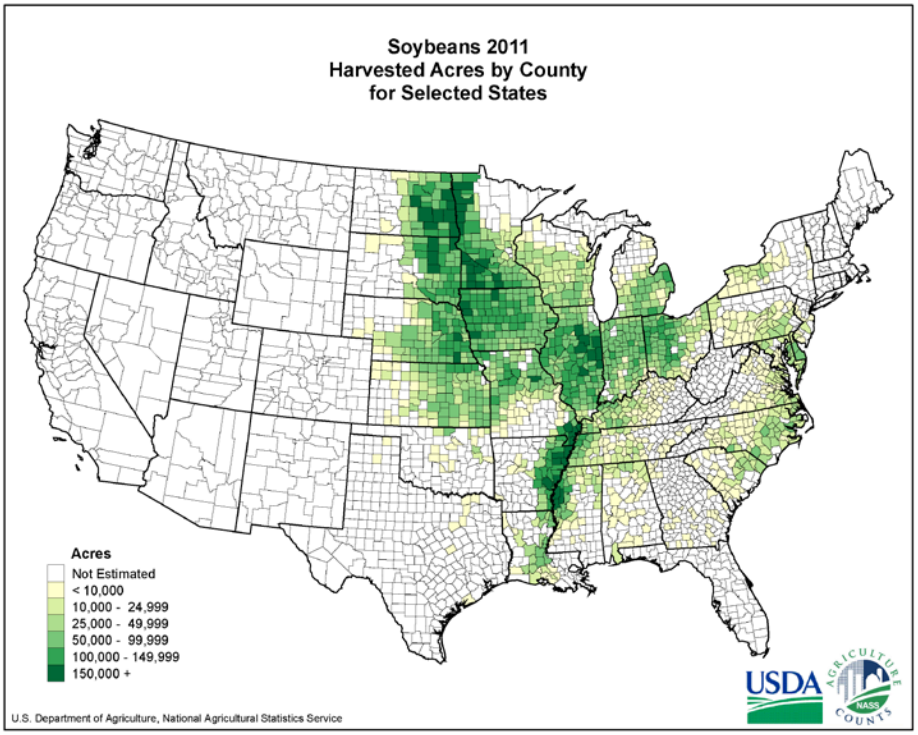

Figure 1. Soybean production areas in the U.S. (USDA-NASS, 2012d).

density, and integrated pest management to handle weed and disease pressures (Hoeft et al. 2000; OECD 2000).

The presence of weeds in soybean fields can cause greater production losses than either insects or diseases (Aref and Pike 1998; Gibson et al. 2005; Oerke 2006). Before the development of effective herbicides for the selective control of weeds in soybeans in the early 1960's, cultural practices including tillage, use of weed-free seed, row spacing and crop rotation were the only ways to control weeds (Wax 1973). By 1987, over 30 herbicides were being used on soybeans (Jordan et al. 1987). With the 1996 commercial introduction and rapid adoption of glyphosate-resistant soybeans, a major change in herbicide usage occurred; glyphosate use increased concurrent with the increase in plantings of glyphosate-resistant soybeans, and the use of other soybean herbicides decreased (Figure 2; NRC 2010; Young 2006). Consequently, the diversity of herbicides used for weed management has declined in soybean (Table 1; Young 2006) resulting in weed species shifts (Johnson et al. 2009).

Repeated and intensive use of herbicides with the same mechanisms of action can rapidly select for tolerant, difficult-to-control weeds and for herbicide-resistant weeds, especially in the absence of the concurrent use of herbicides with different mechanisms of action and/or use of different mechanical or cultural practices for weed control (Vencill et al. 2012). Currently growers are looking for more options for over-the-top herbicide

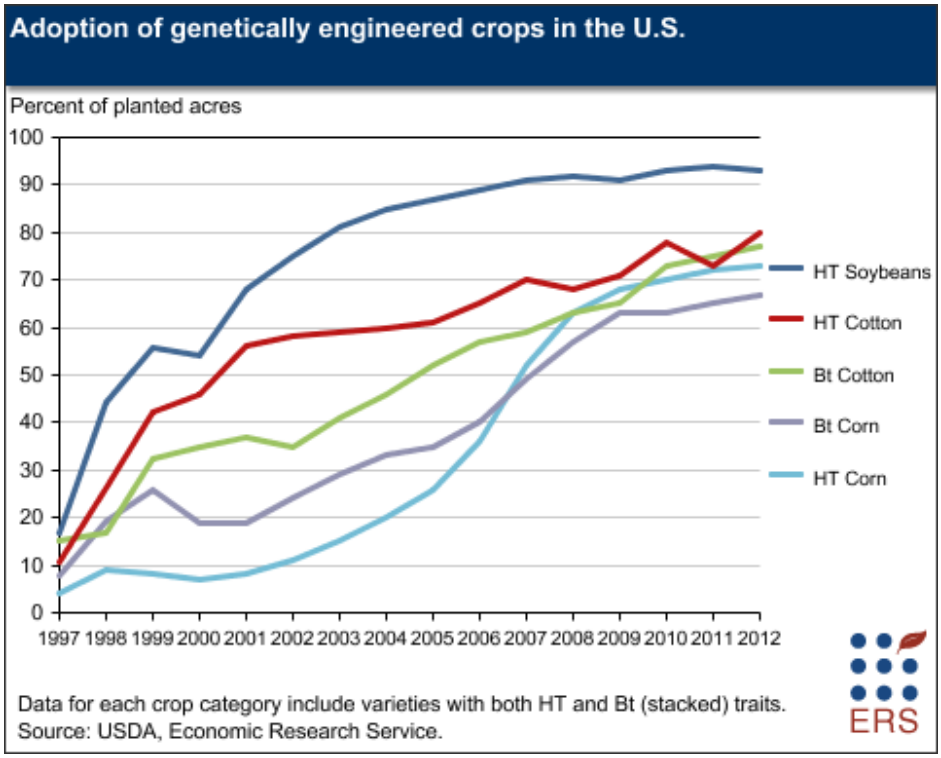

Figure 2. Percent planted acres of genetically engineered crops in the U.S. (USDA-ERS 2012).

applications for their no-till soybean crops to use in conjunction with cultural and mechanical best management practices to mitigate the evolution of herbicide-resistant weeds, especially glyphosate-resistant weeds (Service 2007).

Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a selective pre- and post-emergent herbicide used to control a wide spectrum of broadleaf weeds and woody plants. It is a synthetic auxin herbicide that acts similar to endogenous auxin (indole-3-acetic acid); low concentrations induce cellular elongation and turgor as well as cellular differentiation and division, while increased concentrations lead to abnormal cell division and growth, and higher concentrations inhibit cell division and growth to the point of plant death (Bunch et al. 2012; Kelley and Reichers 2007; WSSA 2012). Because dicamba is effective on broadleaf weeds which are hard-to-control with glyphosate or are glyphosate-resistant, Monsanto intends to commercialize GE soybeans which are resistant to both dicamba and glyphosate so that soybean growers have greater weed control options using both of these modes of herbicide action. Soybean is normally sensitive to dicamba. MON 87708 soybean was developed from A3525, a high-yielding soybean variety that was then genetically engineered to be resistant to dicamba by expressing a mono-oxygenase gene (*dmo*) from *Strenotrophomonas maltophilia* that rapidly demethylates dicamba, rendering it inactive. Although the original transformation contained a gene for resistance to glyphosate herbicide, subsequent self-pollination and selection with glyphosate herbicide was used identify the segregant which is referred to as MON 87708 soybean that lacks this glyphosate resistance gene (as

described in the next section). However, Monsanto intends to stack MON 87708 soybean with MON 89788 (glyphosate-resistant Roundup Ready 2 Yield® soybean) utilizing traditional breeding methods (p. 33, (Monsanto, 2012)). The near isogenic soybean line A3525 was used as a comparator in many of the studies submitted to support the petition.

Table 1. Average (across all states) percent of U.S. soybean acres treated with the listed herbicides in 1990, 1995, 2000 and 2006 (USDA-NASS, 2012a).

Herbicide*	1990	1995	2000	2006	Herbicide*	1990	1995	2000	2006
2,4-D	3	10	5		Glyphosate	5	21	62	92
2,4-D, Dimeth				3	Imazamox			6	
2,4-D 2-EHE				7	Imazaquin	16	15	4	1
Acifluorfen			3		Imazethapyr	11	44	12	3
Alachlor	13	4	1		Lactofen	1	5	2	
Bentazon	16	12	2		Linuron	6	2		
Chlorimuron	20	16	10	4	Metolachlor	10	7	2	
Clethodim		5	4	3	Metribuzin	19	11	4	2
Clomazone	7	4			Paraquat	2	2		1
Cloransulam			4	1	Pendimethalin	14	26	11	3
Ethalfluralin	5	1			Quizalofop	3	6		
Fenoxaprop		6	4		Sethoxydim	4	7	2	
Fluazifop	6	10	5	1	Sulfentrazone			4	1
Flumetsulam		2	2		Sulfosate			4	1
Flumioxazin				3	Thifensulfuron	4	12	6	1
Fomesafen	2	4	7	2	Trifluralin	37	20	14	2

* Herbicides used on only 1% or less acreage for all years listed are not included; 2006 was last year reported at the time the petition was submitted.

C. Description of Inserted Genetic Material, Its Inheritance and Expression, Gene Products, and Changes to Plant Metabolism

To inform the potential hazards resulting from the genetic modification and potential routes of exposure related to the inserted DNA and its expression products, APHIS assessed data and information presented in the petition related to: the transformation process; the source of the inserted genetic material and its function in both the donor organism and the GE crop event; and the integrity, stability and mode of inheritance of the inserted genetic material through sexual or asexual reproduction based on the location of the insertion (e.g. nucleus or organelle) and the number of loci inserted.

APHIS also assessed data presented in the petition on whether the genetic modification results in expression of new genes, proteins, or enzymes or changes in plant metabolism or composition in the MON 87708 soybean relative to the nontransgenic counterpart A3525 soybean or to other reference soybean varieties considered for the composition analysis. The assessment encompasses a consideration of the expressed mono-oxygenase gene (*dmo*) from *Strenotrophomonas maltophilia* and any observed or anticipated effects on plant metabolism including, e.g. any relevant changes in levels of metabolites, antinutrients, or nutrients in harvested seed or forage derived from the MON 87708 soybean compared to those in the conventional counterpart A3525 soybean or to other reference soybean varieties.

This information is used later in this risk assessment to inform whether there is any potential for plant pest vectors or sequences to cause disease or greater plant pest risks in the GE crop event; or for expression of inserted DNA, new proteins or enzymes, or changes in metabolism to affect plant pest or diseases, nontarget beneficial organisms, weediness, agricultural practices that impact pest or diseases or their management, or plant pest risks through horizontal gene flow.

Description of the genetic modification and inheritance of inserted DNA

MON 87708 soybean was developed through *Agrobacterium tumefaciens*-mediated transformation of conventional soybean variety A3525 meristem tissue without utilization of callus (Martinell et al. 2002), using plasmid vector PV-GMHT4355. The PV-GMHT4355 plasmid vector is a two-T-DNA vector and contained a Transfer DNA (T-DNA I) sequence containing the *dmo* plant expression cassette, a second Transfer DNA (T-DNA II) containing the *cp4 epsps* plant expression cassette, and plasmid backbone sequences necessary for maintenance or selection of the plasmid vector in bacteria but which are not expected to be transferred to the plant (Figure III-1, p. 39 and Table III-1, pp. 40-42, (Monsanto, 2012)), as summarized below:

T-DNA I *dmo* cassette

- Right Border Sequence: A specific DNA region from *A. tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al. 1982; Zambryski et al. 1982).
- *PC1SV* Promoter Sequence: Promoter for the Full-Length Transcript (FLt) of peanut chlorotic streak caulimovirus (Maiti and Shepherd 1998) that directs transcription in plant cells.
- TEV Leader Sequence: 5' non-translated region from the Tobacco Etch virus genome (Niepel and Gallie 1999) that is involved in regulating gene expression.
- *RbcS* Targeting Sequence: Sequences encoding the chloroplast transit peptide (CTP) and the first 24 amino acids of the mature protein of the Rubisco small subunit (*RbcS)* gene from *Pisum sativum* (pea) (Fluhr et al. 1986) that directs transport of the dicamba mono-oxygenase (DMO) precursor protein to the chloroplast.
- *dmo* Coding Sequence: Coding sequence for dicamba mono-oxygenase (DMO) from *Stenotrophomonas maltophilia* which confers resistance to the herbicide dicamba (Herman et al. 2005; Wang et al. 1997).

- RbcS2-E9 Terminator Sequence: 3′ non-translated region from the *RbcS2* gene of *Pisum sativum* (pea) encoding the Rubisco small subunit, which functions to direct polyadenylation of the mRNA (Coruzzi et al. 1984).
- Left Border Sequence: A specific DNA region from *A. tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al. 1983).

Plasmid Vector Backbone

- *oriV* Origin of Replication Sequence: Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in Agrobacterium (Stalker et al. 1981).
- *rop* Repressor of Primer Coding Sequence: Coding sequence for repressor of primer protein derived from the Co1E1 plasmid for maintenance of plasmid copy number in *Escherichia coli* (Giza and Huang 1989).
- *ori-pBR322* Origin of Replication Sequence: Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe 1979).
- *aadA* Promoter-Coding-Terminator Sequence: Bacterial promoter, coding and 3′ untranslated region sequences for an aminoglycoside-modifying enzyme, 3' (9)-*O*-nucleotidyltransferase from transposon Tn7 (Fling et al. 1985) that confers spectinomycin and streptomycin resistance.

T-DNA II *cp4 epsps* cassette

- Left Border Sequence: A specific DNA region from *A. tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al. 1983).
- RbcS2-E9 Terminator Sequence: 3′ non-translated region from the RbcS2 gene of *Pisum sativum* (pea) encoding the Rubisco small subunit, which functions to direct polyadenylation of the mRNA (Coruzzi et al. 1984).
- *cp4 epsps* Coding Sequence: Codon optimized codon sequence of the *aroA* gene from *Agrobacterium* spp. strain CP4 encoding CP4 EPSPS which confers resistance to the herbicide glyphosate (Barry et al. 1997; Padgette et al. 1996)
- CTP2 Targeting Sequence: Sequences encoding the chloroplast transit peptide region from the *shkG* gene of *Arabidopsis thaliana* encoding EPSPS (Herrmann 1995; Klee et al. 1987) that directs transport of the CP4 ESPS precursor protein to the chloroplast.
- *DnaK* Leader Sequence: 5' non-translated leader sequence from the *Petunia x hybrida Hsp70* gene (Rensing and Maier 1994) that is involved in regulating gene expression.
- *FMV* Promoter Sequence: Promoter for the 35S RNA from figwort mosaic virus (Rogers 2000) that directs transcription in plant cells.
- Right Border Sequence: A specific DNA region from *A. tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al. 1982; Zambryski et al. 1982).

In addition to the above sequences, to facilitate DNA cloning the *dmo* cassette also contained between 1 and 89 base-pair length intervening sequences, the backbone

contained between 86 and 737 base-pair length intervening sequences, and the *cp4 epsps* cassette contained between 3 and 171 base-pair length intervening sequences.

Monsanto provided evidence demonstrating the following:

- During transformation, both T-DNAs were inserted into the soybean genome, but through conventional self-pollination to segregate T-DNA I from T-DNA II, followed by a sub-lethal glyphosate selection screen, quantitative polymerase chain reaction (PCR) analysis and southern blot analysis, a homozygous segregant containing only T-DNA I was selected as MON 87708 soybean (Section III.B and Figure III-2 on pp. 43-44 and Section IV.B on pp. 58-62, (Monsanto, 2012));
- The T-DNA I inserted into the MON 87708 soybean genome is present at a single locus, and contains one functional copy of the *dmo* gene (Section IV.A, pp. 52-57, (Monsanto, 2012));
- The final product does not contain any of the backbone sequences from the plasmid PV-GMHT4355 outside of the T-DNA region or *dmo* expression cassette borders as demonstrated through Southern blot analysis (Section IV.C, pp. 63-64, (Monsanto, 2012));
- The T-DNA sequence in MON 87708 soybean is identical to the corresponding T-DNA I sequence of the original donor plasmid PV-GMHT4355 (as confirmed by DNA sequence analysis); however, as expected, both the right and left T-DNA border sequences are truncated (Table IV-2, p. 51; Section IV.D, p. 65; and Appendix Figure B-1, p. 292, (Monsanto, 2012));
- The inserted *dmo* expression cassette DNA was stably inherited across four breeding generations (Section IV.F, pp. 65-68, (Monsanto, 2012)); and
- The *dmo* expression cassette in MON 87708 soybean resides at a single locus within the soybean genome and is inherited according to Mendelian principles of inheritance (as determined by chi-square analysis for three segregating populations), consistent with the molecular characterization data that indicate MON 87708 soybean contains a single, intact copy of the *dmo* expression cassette that was inserted into the soybean genome at a single locus (Section IV.G, pp. 69-72, (Monsanto, 2012)).

Insertion of foreign genetic material tends to induce mutations at sites of insertion (generally referred to as insertional mutations) in recipient genomes (Laufs et al. 1999; Nacry et al. 1998). Monsanto examined the T-DNA insertion site in MON 87708 soybean compared to the near isogenic conventional soybean control A3525 using PCR and sequence analyses (Appendix B.10, pp. 290-293, (Monsanto, 2012)) and discovered that MON 87708 contained transformation induced mutations at the flanking site adjacent to 3' and 5' ends of the T-DNA insert (Section IV.E, p. 65, (Monsanto, 2012)). There was an 899 base pair deletion and a 128 base pair insertion just 5' of T-DNA I, and a 35 bp insertion just 3' of T-DNA I, when the conventional control sequence was compared to the genomic DNA sequence flanking the 3' and 5' ends of the T-DNA insert in MON 87708 soybean. According to Monsanto, the observed insertion and insertion-deletion mutation (indel mutation) presumably resulted from double-stranded break repair mechanisms in the plant during the *Agrobacterium*-mediated transformation process

(Salomon and Puchta 1998). As discussed later in this document, none of these mutations altered the function of the *dmo* gene or exhibited deleterious phenotypes in MON 87708 soybean.

The plant pest derived vector DNA and the plant pest vector used to insert the DNA do not pose a plant pest risk to MON 87708 soybean. The binary plasmid vector PV-GMHT4355 proved to be disarmed; the T-DNA inserted into MON 87708 soybean contained only the intended sequences as described above along with the typical insertion site mutations, and lacked sequences from Tumor-inducing (Ti) plasmids normally responsible for the formation of crown gall tumors upon *A. tumefaciens* infection (Hellens et al. 2000; Hoekema et al. 1983). The sequences derived from plant pathogens retained in MON 87708 soybean (i.e., promoter sequence from peanut chlorotic streak caulimovirus, leader sequence from Tobacco Etch virus, and the T-DNA border sequences from *Agrobacterium tumefaciens*) are non-coding sequences which do not cause plant disease. Furthermore, following transformation, the R0 plant tissue was treated with the antibiotics carbenicillin, cefotaxime, and ticarcillin/clavulanate acid mixture to inhibit the growth of excess *Agrobacterium* (Hellens et al. 2000; p. 43, (Monsanto, 2012)).

Expression of inserted DNA, changes in gene expression, new proteins or metabolism

Dicamba herbicide resistance in MON 87708 soybean is derived from a bacterial oxygenase gene (*dmo*) from *Stenotrophomonas maltophilia* isolated from a stormwater retention pond outside a dicamba manufacturing plant (Krueger et al. 1989). *S. maltophilia* is an aerobic, ubiquitous bacterium that can be found in a variety of environments, including associated with plants used as food or feed (pp. 81-82, (Monsanto, 2012)), but it is not considered a plant pathogenic bacterium (ISPP 2012). Originally classified as *Pseudomonas maltophilia*, *S. maltophilia* was also grouped in the genus *Xanthomonas* before eventually becoming the type species of the *Stenotrophomonas* genus in 1993 (Palleroni and Bradbury 1993). The bacterium can utilize dicamba as a sole carbon source through the action of a multicomponent demethylase system comprised of a reductase, a ferredoxin and an oxygenase (Chakraborty et al. 2005) which work together in a redox system similar to many other oxygenases to degrade aromatic compounds by catalyzing the incorporation of oxygen into organic substrates. In MON 87708 soybean, the reductase and ferredoxin are endogenous in the soybean chloroplast where they associate with the transgenic DMO to transport electrons from nicotinamide adenine dinucleotide (NADH) to oxygen to catalyze the demethylation of dicamba to form the non-herbicidal metabolites DCSA (3,6-dichlorosalicyclic acid) and formaldehyde (Figure 3; Behrens et al. 2007; Dumitru et al. 2009).

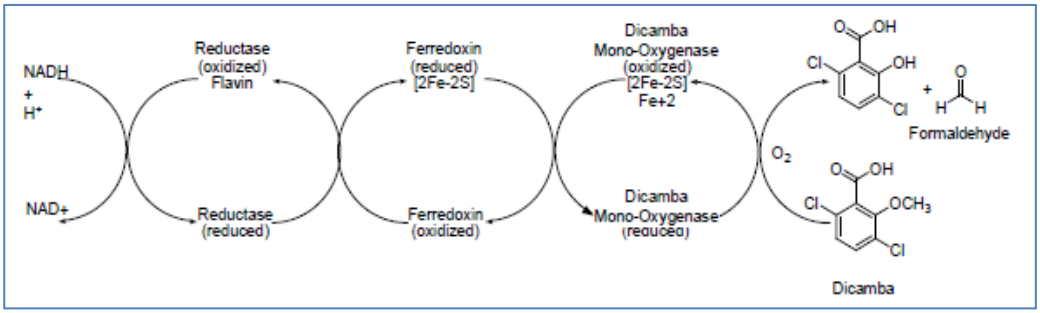

Figure 3. Demethylation of dicamba (3,6-dichloro-2-methoxybenzoic acid) catalyzed by dicamba mono-oxygenase to form the metabolites 3,6-dichlorosalicylic acid and formaldehyde (Chakraborty et al. 2005).

DCSA is a known metabolite of dicamba whose safety in soybean, soil, and livestock has been evaluated by the EPA (40 CFR part 180.227 [7-1-11 Edition]; US-EPA 2009) and whose structure is similar to salicylic acid (2-hydroxybenzoic acid), an endogenous plant benzoic acid (Frear 1976; p. 250, (Monsanto, 2012)). Formaldehyde is found naturally in plants up to several hundred ppm (Adrian-Romero et al. 1999). Because both endogenous salicylic acid compounds and formaldehyde are known to be involved in plant responses to stress, including to pests and pathogens (Szabo et al. 2003; Szende and Tyihak 2010; Wildermuth 2006), their role will be further discussed in the later section on *Plant Pest and Disease Impacts*. In addition, impacts of the application of the dicamba herbicide and/or the metabolites produced as a result of the expression of MON 87708 soybean DMO on pest and disease incidence, severity or damage will be discussed in the section on *Potential Changes to Agriculture or Cultivation Practices.*

The physicochemical characteristics and functional activity of the MON 87708 soybean DMO were determined using a panel of analytical techniques, including: 1) western blot analysis to establish identity and immunoreactivity of MON 87708 DMO using an anti-DMO antibody, 2) N-terminal amino acid sequence analysis, 3) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to generate a tryptic peptide map of the MON 87708 DMO, 4) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish the apparent molecular weight of MON 87708 DMO, 5) glycosylation status of MON 87708 DMO, and 6) MON 87708 DMO activity analysis to demonstrate functional activity (Sections V.A and V.B, pp. 74-78 and Appendix C, pp. 294 – 322, (Monsanto, 2012)).

Two forms of mature MON 87708 soybean DMO result from post-translational modification of the DMO precursor protein after transport to the chloroplast (Sections V - V.A.1 and V.B, pp. 74-75 and 77-78, and Appendix C.1, pp. 294-312, (Monsanto, 2012)):

- An expected DMO consists of 339 amino acids and lacks the 57 amino acid transit peptide and 24 amino acids of the N-terminus of the mature protein encoded by the pea *RbcS* gene, the N-terminal methionine, and three amino acids

encoded by the nine base pair intervening sequence (9521-9529) used for cloning purposes (see Table III-1, pp. 40-42 in (Monsanto, 2012)). Unexpectedly, a larger DMO consisting of 367 amino acids (referred to as DMO+27) is detected with the 57 amino acid CTP portion of the targeting sequence cleaved while the 24 amino acid N-terminal portion of the pea RbcS is retained along with the three amino acids resulting from an intervening sequence (confirmed by western blot analysis by probing with an antibody specific for both forms of the MON 87708 DMO protein, N-terminal sequencing, and peptide analysis; see V.A.1, p. 75 and Appendices C.1.11 – 1.13, pp. 304-310).

- The apparent molecular weights of DMO and DMO+27 are 39.8 and 42.0 kDa, respectively (Appendix C.1.10, pp. 301-303), and neither are glycosylated (Appendix C.1.14, p. 311).
- The specific activity of purified MON 87708 soybean DMO (naturally expressed DMO and DMO+27 mixture) was measured by quantifying the conversion of dicamba to DCSA, and it was determined to be 62.21 ± 11 nmoles DCSA/min/mg of MON 87708 DMO (Section V.B, p. 78; Appendix C.1.9, p. 301; and Appendix C.1.15, Table C-4, p. 312).

Since these studies showed that DMO purified from MON 87708 soybean seed contains a mixture of both DMO and DMO+27, and it is known that the active form of DMO necessary to demethylate dicamba and confer resistance to the herbicide is a trimer comprised of three DMO monomers (Chakraborty et al. 2005), the petitioner refers to both forms of the protein (DMO and DMO+27) and all forms of the trimer (DMO, DMO+27, or a combination of both) as MON 87708 soybean DMO (Section V, pp. 74-75). That the active binding pocket of DMO with dicamba occurs through hydrogen-bonding and steric interactions at locations not involving the N-terminus of DMO (Dumitru 2009) supports the petitioner's assertion that both forms of DMO and DMO+27 are likely involved in the formation of active trimers.

The petitioner provided data on the relative specificity of MON 87708 DMO for dicamba and also the herbicide resistance phenotype of MON 87708 soybean (Section V.A.2, pp. 75-77, and Appendix C.2, pp. 312-321, (Monsanto, 2012)):

- In addition to dicamba (formulation Clarity®; BASF 2010), a total of 19 herbicides representing eight families with distinct modes-of-action, some of which are approved for use in soybean, were tested under greenhouse conditions to compare tolerance levels of MON 87708 soybean and the near isogenic conventional soybean control A3525 at rates representative of commercial rates needed to control broadleaf weeds at the early vegetative soybean growth stage (Table V-1, p. 77). As expected, MON 87708 soybean sprayed over-the-top with dicamba exhibited negligible injury whereas the conventional soybean control A3525 sustained 100% injury; however compared to the conventional control A3525, MON 87708 showed limited but not commercially-acceptable tolerance to three other phenoxy synthetic auxin herbicides, 2,4-D (2,4-dichlorophenoxy acetic acid), MCPA (2-methyl-4-chlorophenoxy acetic acid) and 2,4-DB (2,4-dichlorophenoxy butanoic acid), but was similar to the conventional control in

level of tolerance or sensitivity to 16 other herbicides (Appendix Table C-7, pp. 317-318).

- Because 2,4-D is the most similar to dicamba of the three phenoxy herbicides tested, it was selected for further *in vitro* experimentation where it was shown that conversion of 2,4-D to the predicted, possible DMO oxidative reaction product 2,4-DCP occurred in minimal amounts in both the presence or absence of an *E. coli*-produced DMO in the reaction mix, and with a substrate only control (Figure C-9, p. 320). The degradation of a dicamba control to form DCSA was demonstrated using the *E. coli*-produced DMO, and supports Monsanto's claims that the *E. coli*-produced DMO is similar in sequence and function to MON 87708 DMO, and that therefore it is appropriate to extend specificity data generated with the *E. coli*-produced DMO to MON 87708 DMO (Section V.A.2, pp. 75-77; Appendix C.2.1, p. 312; Appendix C.2.7, p. 316; Figure C-9, p. 320); however this does not explain the slight difference in reduced sensitivity of MON 87708 soybean to the other phenoxy synthetic auxin herbicides in the greenhouse assays.
- The possibility that MON 87708 soybean DMO can metabolize plant endogenous substrates structurally similar to dicamba and abundant in soybean was also tested *in vitro*. None of the five potential substrates (o-anisic acid, vanillic acid, syringic acid, ferulic acid and sinapic acid; Figure V-2, p. 76) were metabolized by the *E. coli*-produced DMO *in vitro* (Appendix Figure C-10, p. 321).

The DMO protein is expressed in all tissues of MON 87708 soybean (Section V.C, pp. 78-80, and Appendix D, pp. 323-325, (Monsanto, 2012)):

- The levels of MON 87708 soybean DMO in various tissues collected during the 2008 growing season from replicated plots from five U.S. field test sites representative of soybean producing regions were determined based on ELISA assays (Appendix D).
- Expression analysis showed that MON 87708 soybean DMO was detected in all tissue types from MON 87708 across all five sites ranging from 3.9 – 180 µg/g dry weight (dwt). The mean levels across the five sites increased in over-season leaf collected during the later developmental stages (from the V3-V4 vegetative developmental stage at 17 µg/g dwt to the R5 (beginning seed stage) -V16 developmental stage at 69 µg/g dwt), but were lower in forage (53 µg/g dwt), seed (47 µg/g dwt), and roots (6.1 µg/g dwt) collected at later developmental stages (Table V-3, p. 80).
- MON 87708 soybean DMO makes up only approximately 0.01 to 0.02 % of the total protein in seed and forage, respectively, considering that total protein is 41.17 % and 22.7% of MON 87708 seed and forage dry weight, respectively (Table VI-5, p. 122, and Table VI-8, p. 140). As expected, MON 87708 DMO was below the limit of quantitation for all tissue types of the conventional control.

The results of the petitioner's DMO characterization studies support the conclusion that the functional activity, specificity and expression levels of the MON 87708 soybean

DMO confer the intended phenotype of dicamba resistance to MON 87708 soybean, but MON 87708 soybean also has slightly reduced sensitivity to three other phenoxy synthetic herbicides compared to the conventional control soybean A3525. The implications of the changes in sensitivity to these herbicides with respect to control of volunteer soybean will be discussed in the section of this PPRA on the *Potential for Enhanced Weediness of MON 87708 Soybean*.

The petitioner carried out a compositional assessment of forage and seed samples using the principles and analytes outlined in the OECD consensus document for soybean composition (OECD 2001) to assess whether levels of key nutrients and anti-nutrients in MON 87708 soybean were equivalent to levels in the near isogenic conventional control soybean A3525, and to the composition of twenty commercial reference varieties (Section VI, pp. 86-145, and Appendix E, pp. 326-436, (Monsanto, 2012)). The samples for compositional assessment were collected in the 2008 growing season from five replicated sites chosen to represent the typical soybean growing regions of the United States. In addition to the conventional weed control programs, MON 87708 soybean plots were either treated at the V2-V3 growth stage with a dicamba application at the target label rate of 0.5 lb acid equivalents per acre (1800 g a.e./ha) or not treated with dicamba (Section VI.A, p. 86, (Monsanto, 2012)). Four different commercial reference varieties were grown at each of the five replicated sites and their data pooled to generate a tolerance interval which the petitioner claims is expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial reference varieties (Section VI.A, p. 87; Appendix Table E-1, p. 326; and Appendix E.6, p. 333, Monsanto 2012a). The 99% tolerance interval is meant to define the natural variability of each compositional component in soybean varieties that have a history of safe consumption, and that were grown concurrently with MON 87708 soybean and the conventional control in the same trial (see p. 87, (Monsanto, 2012) for further details about data interpretations).

Nutrients assessed included proximates, fiber, amino acids, fatty acids, and vitamin E in seed, and proximates and fiber in forage. The anti-nutrients assessed in seed included raffinose, stachyose, lectin, phytic acid, trypsin inhibitor and isoflavones (daidzein, genistein, and glycitein) (see Appendix E for analytical methods, pp. 327- 333, (Monsanto, 2012)). In all, 50 components for dicamba-treated and untreated MON 87708 soybean were statistically assessed – 35 nutrients and eight anti-nutrients in seed, and seven nutrients in forage. The petitioner employed six statistical comparisons on the MON 87708 soybean compositional data. One comparison was based on compositional data combined across all five field sites (combined-site analysis) and five separate comparisons were conducted on data from each of the individual field sites.

In dicamba-treated MON 87708 soybean, the petitioner's combined site analysis of both seed and forage showed statistically significant differences ($\alpha = 0.05$) between MON 87708 and the conventional control for 29 (58%) of the 50 mean value comparisons – 24 nutrients and four anti-nutrients from the seed analysis and one nutrient from the forage analysis (Table VI-1, pp. 98-111, (Monsanto, 2012)).

- Nutrient component differences in seed were observed in mean values for ash, carbohydrates, protein and 12 amino acids, five fatty acids, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, and vitamin E. All were of relatively small magnitude with respect to the conventional control and, whether increased or decreased, ranged from a minimum difference of 1.51% (18:2 linoleic acid) to a maximum difference of 15.13% (Vitamin E). Three of the nutrient components in the combined-site analysis (decreased levels of 18:1 oleic acid and increased levels of 18:3 linolenic acid and vitamin E) were also observed to be statistically different in at least four or more of the five individual sites, whereas the other combined-site differences occurred at fewer or none of the individual sites.
- Anti-nutrient component differences in seed were observed in mean values for phytic acid, raffinose, stachyose, and daidzein. All were of small relative magnitude with respect to the conventional control, and ranged from a minimum difference of a 6.14% decrease (phytic acid) to maximum difference of an 11.51% increase (daidzein). None of the anti-nutrient components were observed to be statistically different at more than two of the five individual sites.
- The only nutrient difference in forage for the combined-site analysis was observed in ADF, and its relative magnitude of difference, with respect to the conventional control, was +10.45%. No differences between MON 87708 soybean and the conventional control ADF mean values were observed at any of the five individual sites.
- All 29 of the statistically significant differences between dicamba-treated MON 87708 soybean components compared to the conventional control were within the 99% tolerance interval established from the commercial reference varieties grown concurrently and at the same field sites, as well as within ranges in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI 2012) and the scientific literature (Table VI-9, pp. 142-143, (Monsanto, 2012)).

In dicamba-untreated MON 87708 soybean, the petitioner's combined site analysis of both seed and forage showed statistically significant differences ($\alpha = 0.05$) between MON 87708 and the conventional control for 20 (40%) of the 50 mean value comparisons – 17 nutrients and three anti-nutrients from the seed analysis (Table VI-5, pp. 122-131, (Monsanto, 2012)).

- Nutrient component differences in seed were observed in mean values for protein and eight amino acids, five fatty acids, ADF, NDF and vitamin E. All were of relatively small magnitude with respect to the conventional control and, whether increased or decreased, ranged from a minimum difference of 1.45% (18:2 linoleic acid) to a maximum difference of 18.16% (vitamin E). As in the dicamba-treated trials, three of the nutrient components in the combined-site analysis (decreased levels of 18:1 oleic acid and increased levels of 18:3 linolenic acid and vitamin E) were also observed to be statistically different in at least four of the five individual sites, whereas the other combined-site differences occurred at fewer or none of the individual sites. The small relative magnitudes of the differences in 18:3 linolenic acid, 18:1 oleic acid and vitamin E compared

to the conventional control, as well as the even broader range of these fatty acids and vitamin E present in commercial soybean varieties, suggest that the differences are not meaningful to nutritional quality in MON 87708 soybean.

- Anti-nutrient component differences in seed were observed in mean values for trypsin inhibitor, daidzein and genistein. All were of small relative magnitude with respect to the conventional control, and ranged from increases of 11.59% for genistein, 15.37% for trypsin inhibitor and 17.24% for daidzein. None of the anti-nutrient components were observed to be statistically different at more than one of the five individual sites.
- No nutrient component differences in forage for the combined-site analysis were observed.
- All 20 of the statistically significant differences between dicamba-untreated MON 87708 soybean components compared to the conventional control were within the 99% tolerance interval established from the commercial reference varieties grown concurrently and at the same field sites, as well as within ranges in the ILSI Crop Composition Database and the scientific literature (Table VI-9, pp. 142-143, (Monsanto, 2012)).

Based on the data presented by the petitioner on the composition of key nutrients and anti-nutrients in seed and key nutrients in forage for both dicamba-treated and untreated MON 87708 soybean, it is reasonable to conclude that neither the dicamba tolerance trait nor the dicamba herbicide treatment have a meaningful impact on the composition of seed or forage derived from MON 87708 soybean compared to other commercial soybean varieties.

Based on all the above noted considerations, APHIS concludes that MON 87708 soybean poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional soybean varieties.

D. Potential Plant Pest and Disease Impacts

APHIS assessed whether potential plant pest or disease impacts are likely to result from the transformation process, from DNA sequences from plant pests, or from any other expression products, new enzymes, proteins or changes in plant metabolism or composition in MON 88708 soybean that are known or anticipated to cause disease symptoms, or to affect plant pests or diseases or plant defense responses (as identified from the previous section). APHIS also assessed or whether MON 88708 soybean is likely to have significantly increased disease and pest susceptibility based on data and observations from field trials on specific pest and disease damage or incidence and any agronomic data that might relate to such damage. Impacts or changes are assessed to determine if they would (1) affect the new GE crop and/or result in significant introduction or spread of a damaging pest or disease to other plants; (2) result in the introduction, spread, and/or creation of a new disease; and/or (3) result in a significant exacerbation of a pest or disease for which APHIS has a control program. Any increase in pest or disease susceptibility is evaluated with respect to the context of currently

cultivated varieties, the ability to manage the pest or disease, and the potential impact on agriculture.

Plant Protection and Quarantine (PPQ) is an APHIS program that safeguards agriculture and natural resources from the entry, establishment, and spread of animal and plant pests and noxious weeds into the United States of America; and supports trade and exports of U.S. agricultural products. PPQ responds to many new introductions of plant pests to eradicate, suppress, or contain them through various programs in cooperation with state departments of agriculture and other government agencies. These may be emergency or longer term domestic programs that target a specific pest. A variety of insect, plant disease, mollusk, nematode or weed programs exist (USDA-APHIS 2014a). For instance, a PPQ program was initiated for soybean rust prior to its November 2004 discovery in the continental US, in order to train diagnosticians to identify the causal organisms and to prepare U.S. soybean growers for the arrival of this rapidly spreading disease which causes serious crop losses in many parts of the world. Soybean rust is caused by two fungal species, *Phakopsora pachyrhizi* and *Phakopsora meibomiae*, with *P. pachyrhizi* being the more virulent strain in the U.S. for which all currently available commercial varieties of soybeans are susceptible (ACES 2012).

Soybean is not a plant pest in the U.S. (7 CFR part 340.2; USDA-NRCS 2012) and the *Agrobacterium tumefaciens* transformed plants used in the generation of MON 87708 soybean were treated with antibiotics to kill *A. tumefaciens* cells. The description of the introduced genetic elements, expression of the gene products and their functions in MON 87708 soybean has been summarized above. DNA sequences derived from plant pests that were incorporated in MON 87708 soybean do not result in the production of infectious agents or disease symptoms in plants, and so it is unlikely that MON 87708 soybean could pose a plant pest risk.

MON 87708 soybean was released within confined field trials in the U.S. from 2005 through 2009 in at least 75 locations across 24 states and territories covering a diverse range of environmental conditions representative of where soybeans are currently grown and bred, and where MON 87708 is expected to be grown (Appendix A, Table A-1, pp. 283-286, (Monsanto, 2012)). In addition to the observational data that Monsanto annually reported to USDA-APHIS from these product development trials, which would have included reports of unusual pest and/or disease incidence, Monsanto also carried out a comparative assessment of both dicamba-treated and untreated MON 87708 soybean with the near isogenic conventional soybean control A3525 to evaluate environmental interactions (i.e., interactions between the crop plants and their receiving environment, including responses to abiotic stress, disease damage, arthropod-related damage, and pest and beneficial arthropod abundance) (Section VII, pp. 146-169, and Appendix G, pp. 443-485, (Monsanto, 2012)). The petitioner assessed both treated and untreated MON 87708 soybean to allow for not only the assessment of the effect of the inserted *dmo* gene on environmental interactions, but also the assessment of the effect of the dicamba herbicide treatment which is important for this plant pest risk assessment since the reaction products formed in MON 87708 soybean in the presence of dicamba are analogs

of endogenous salicylic acid compounds and formaldehyde which are known to be involved in plant responses to stress, including to pests and pathogens.

Environmental interaction data were collected in 2008 on untreated MON 87708 soybean at 18 field sites and in 2009 on treated MON 87708 soybean at 8 field sites (Tables VII-3 and VII-4, pp. 157-158, (Monsanto, 2012)).

- These 26 locations provided a diverse range of environmental and agronomic conditions representative of commercial soybean production areas in North America (Appendix G.1, p. 443, and Tables G-3 and G-4 for 2008 and 2009 trials respectively, pp. 448-450, (Monsanto, 2012)). Multiple commercial reference varieties (including some Roundup Ready - glyphosate resistant soybeans) were grown concurrently with MON 87708 soybean and the conventional A3525 control at each site to establish a range of natural variability for the assessed stressors (Tables G-1 and G-2 for 2008 and 2009 field trials, respectively, pp. 445-447, (Monsanto, 2012)).
- The researchers at each field site were expected to be familiar with the growth, production, and evaluation of soybean characteristics, and to use well-established qualitative and/or quantitative techniques to observe and evaluate environmental interactions. They chose abiotic stressors, diseases and arthropod pests that were either actively causing injury or were likely to occur in soybean during the given observation period. The assessed stressors were present at natural levels, as no artificial infestation or imposed abiotic stress was used.
- For plant responses to abiotic stress, disease damage and arthropod-related damage, at least three abiotic stressors, three diseases and three arthropod pests were evaluated up to four times during the growing season at all 26 sites. The researcher at each field site chose abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in soybean during the given observation period. Therefore, the stressors typically varied between observations at a site or among sites (Appendix G.7, pp. 451-452, (Monsanto, 2012)).
- Arthropod abundance was measured only in 2008, and only at four of the 18 sites which were designed to contain plots suited for the purpose of collecting robust arthropod abundance data (Section VII.C.2.4, pp. 162-163; Appendix G.3, pp. 443-444; and Appendix G.8, p. 453, (Monsanto, 2012)).
- Qualitative categorical data on observations of plant response to abiotic stress and disease damage were not statistically analyzed; they were considered different on a particular observation date at a site if the range of injury severity to MON 87708 soybean did not overlap with the range of injury severity to the control across all three replications. Qualitative numerical data for arthropod damage and quantitative data for arthropod abundance underwent statistical analysis ($\alpha = 0.05$) (Section VII.C.2.4, pp. 162-163, (Monsanto, 2012)).
- The results of the 2009 study which included only dicamba-treated MON 87708 soybean (not untreated MON 87708) will also be discussed later in the section on *Potential Changes to Agriculture or Cultivation Practices.*

Neither the dicamba tolerance trait nor the dicamba herbicide treatment altered the

assessed environmental interactions of MON 87708 soybean compared to the conventional A3525 control (Section VII.C.2.4, pp. 162-166; Appendices G.6-G.11, pp. 451-454; and Tables G-9 through G-16, pp. 469-484; (Monsanto, 2012)).

Abiotic stressors:

- In 2008, 193 out of 194 observations between untreated MON 87708 soybean and the conventional control showed no meaningful differences in damage from 12 abiotic stressors (cold, compaction, crusting, drought, excess moisture, flooding, frost, hail, heat damage, mineral toxicity, nutrient deficiency and wind) (Table G-9, p. 469). Slight damage due to wind was observed on untreated MON 87708 soybean compared to the control (no wind damage) for one of the four observations at one of the sites, but since the difference was slight and no differences were observed in any of the other wind evaluations, the difference is not considered biologically meaningful.
- In 2009, no differences were observed between treated MON 87708 soybean and the conventional control for all 89 observations of damage from eight abiotic stressors (cold, compaction, drought, flood, frost damage, hail damage, nutrient deficiency and wind damage) (Table G-14, p. 481).

Disease damage:

- In 2008, no differences were observed between untreated MON 87708 soybean and the conventional control for all 215 observations for damage from all 22 diseases evaluated (*Alternaria* leaf spot, anthracnose, Asian rust, bacterial blight, brown stem rot, *Cercospora*, charcoal rot, downy mildew, frogeye leaf spot, *Phytophthora*, pod and stem blight, powdery mildew, *Pythium*, *Rhizoctonia*, *Septoria* brown spot, soybean cyst nematode, soybean mosaic virus, soybean rust, stem canker, sudden death syndrome, white mold and yellow mosaic virus) (Table G-10, p. 470).
- In 2009, 92 of 93 observations between treated MON 87708 soybean and the conventional control showed no differences in damage for all 18 diseases evaluated (anthracnose, bacterial blight, bacterial leaf spot, brown stem rot, *Cercospora*, charcoal rot, downy mildew, frogeye leaf spot, leaf spot, *Phytophthora* root rot, powdery mildew, *Pythium*, *Rhizoctonia*, soybean mosaic virus, soybean rust, stem canker, sudden death and white mold) (Table G-15, p. 482). One difference was observed for white mold during a single observation (slight on MON 87708 soybean vs. none on the control) which was outside the reference range (no white mold was observed in the references), but since the difference was slight and no differences were observed in any of the other white mold evaluations, the difference is not considered biologically meaningful.

Arthropod damage:

- In 2008, 89 out of 95 comparisons between untreated MON 87708 soybean and the conventional control showed no significant damage from all 19 arthropods assessed (aphid, armyworm, bean leaf beetle, blister beetle, cabbage looper, corn rootworm beetle, cutworm, fall armyworm, grasshopper, green cloverworm, Japanese beetle, leafhopper, seedcorn maggot, soybean looper, spider mite, stink

bug, thistle caterpillar, thrips and yellow woollybear) (Table G-11, pp. 471-472). The six differences in arthropod damage were from four taxa (aphid, blister beetle, potato leafhopper and Japanese beetle) and were all small in magnitude and not consistent across observations or sites.

- In 2009, 56 out of 59 comparisons between treated MON 87708 soybean and the conventional control showed no significant damage from all 14 arthropods assessed (aphids, bean leaf beetle, blister beetle, cabbage looper, corn earworm, fall armyworm, grasshopper, green cloverworm, Japanese beetle, potato leafhopper, soybean looper, stink bugs, three cornered alfalfa hopper and velvet leaf caterpillar) (Table G-16, pp. 483-484). The three differences in arthropod damage were from two taxa (leaf beetle and grasshopper) and were small in magnitude and/or not consistent across observations or sites.

Arthropod abundance (pests and beneficials):

- In 2008, 142 out of 151 comparisons between untreated MON 87708 soybean and the conventional control showed no significant differences in abundances of all eleven pest and seven beneficial arthropod taxa assessed (Tables G-12 and G-13, pp. 473-480). Pest arthropods included aphid, bean leaf beetle, grape colaspis, garden flea-hopper, green clover-worm, Japanese beetle, potato leafhopper, stink bug, tarnished plant bug, velvet-bean caterpillar and woolly-bear caterpillar. Beneficial arthopods included *Araneae* (spiders), big eyed bug, *Carabidae,* lacewings, ladybird beetles, micro-parasitic wasps, *Nabis* spp., *Opilones*, *Orius* spp. and syrphid larvae. The seven differences detected for abundance for three pest taxa (green cloverworm, Japanese beetles and stink bugs) and the two differences detected for abundance of beneficial arthropod taxa (*Aranaea* and *Nabis* spp.) were all small in magnitude and were not detected or were not consistent in other collections or sites.
- No arthropod abundance data were collected from the 2009 field trials between treated MON 87708 soybean and the conventional control, so no assessment can be made on whether the dicamba herbicide treatment alters arthropod abundance, however, impacts of herbicide treatment on nontargets are assessed by the EPA. Impacts of the herbicide on nontarget organisms are not considered a plant pest risk.

The results of the petitioner's field studies on the assessed environmental interactions between MON 87708 soybean and its receiving environment indicate that neither the dicamba resistance trait nor the dicamba herbicide treatment alter the response of MON 87708 to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, and nor were pest arthropods more abundant around MON 87708 plots.

Monsanto claims that the DCSA metabolite produced from applications of dicamba on DMO-expressing crops such as MON 87708 soybean may actually confer beneficial health effects on the crop plants, such as increased resistance against biotic (e.g., insects, fungi, viruses, nematodes, and other pathogens) and abiotic stresses (e.g., drought, cold, ozone, soil nutrient deficiencies), with resulting increases in yields and improved quality of crops. This reported discovery was made by Monsanto during research on a variety of

dicamba-tolerant crops currently under development (corn, cotton as well as soybean) and is currently claimed under a pending patent (Bhatti et al. 2010). Endogenous salicylic acid is known to be a common and widespread mediator of plant responses to biotic and abiotic stress (Vlot et al. 2008; Vlot et al. 2009; Wildermuth 2006). Since DCSA is a synthetic analog of salicylic acid, it is not surprising it could play a similar role as salicylic acid in plants, but it is also plausible it could interfere with plant defense signaling such as other salicylic analogs have been shown to do (Park et al. 2009; Silverman et al. 2005). Monsanto's patent claim indicates they have evidence supporting the former; however, as shown above, the beneficial plant health effects described in the patent claim are not evident in the data provided in the petition for the determination of nonregulated status of MON 87708 soybean.

Formaldehyde, the other breakdown product of dicamba by MON 87708 soybean DMO, is found naturally in plants up to several hundred ppm (Adrian-Romero et al. 1999) and is incorporated into the one-carbon pool reactions which are essential to all organisms (Hanson and Roje 2001). In plants, these reactions supply the C1 units needed to synthesize proteins, nucleic acids, pantothenate, and a great variety of methylated molecules (Kalasz 2003). C1 pathways are particularly active in tissues that produce methylated compounds such as lignin, alkaloids, and betaines because the C1 demands for these physiologically and economically important secondary metabolites can dwarf those of primary metabolism. C1 transfers are also central to the massive photorespiratory fluxes that occur in C3 plants (Hanson and Roje 2001). Formaldehyde production in plants has been shown to change in response to abiotic and biotic stress (Sardi et al. 1996; Szabo et al. 2003; Szende and Tyihak 2010) although the mechanism and function is unclear. In MON 87708 soybean, the dicamba-derived formaldehyde is expected to be produced in small amounts; the maximum theoretical amount is estimated to be 16.7-37.5 mg/kg based on an assumption that the entire 0.56 kg/ha (0.5 lb/acre) a.e. of dicamba application at the V3 to R1 growth stage of MON 87708 soybean is intercepted and instantaneously metabolized by the MON 87708 DMO (pp. 251-252, (Monsanto, 2012)). This is well within the range of formaldehyde measured in a variety of dicot plants (up to several hundred mg/kg) (Adrian-Romero et al. 1999) and agricultural commodities (WHO-IPCS 1989), so it is likely that dicamba-produced formaldehyde will play a similar role as naturally produced endogenous formaldehyde. This is supported by the compositional analysis of seed and forage from dicamba-treated MON 87708 soybean vs. dicamba-untreated conventional controls which revealed no biologically meaningful differences in nutrients or anti-nutrients; e.g., differences in means from the combined site analysis were relatively small, were within the 99% tolerance interval established from the commercial reference varieties, and often were not consistent across sites. Similarly, the environmental interactions study indicated that the dicamba herbicide treatment did not alter the response of MON 87708 soybean to abiotic stress, diseases, or arthropod pests under natural levels of these stressors.

In conclusion, for MON 87708 soybean, no plant pest or disease impacts are expected from the plant pest transformation vector or plant pest sequences used in the transformation, nor the expression of the new enzyme for dicamba resistance and minor changes in composition, nor from the herbicide metabolites produced as a result of

treating MON 87708 soybean with dicamba. Field study observations on the assessed environmental interactions between MON 87708 soybean and its receiving environment indicate that neither the dicamba resistance trait nor the dicamba herbicide treatment (including any metabolites produced as a result) significantly alter the response of MON 87708 to diseases, or arthropod pests under natural levels of these stressors, and nor were pest arthropods more abundant around MON 87708 soybean plots compared to the control line. As discussed earlier, there were no significant changes in MON 87708 soybean compositions that would render MON 87708 more susceptible to pests and diseases over its control or reference soybean varieties. As presented later in this document, the observed agronomic traits also did not reveal any significant changes that would indirectly indicate that MON 87708 soybean is or could be relatively more susceptible to pests and diseases over control or reference soybean varieties. Thus MON 87708 is unlikely to be more susceptible to plant pathogens and insect pests than conventional soybean. For this reason, MON 87708 soybean is unlikely to differ from conventional soybean in its ability to harbor or transmit plant pathogens or pests and cause indirect plant pest effects on other agricultural products.

E. Potential Impacts on Nontarget Organisms Beneficial to Agriculture

MON 87708 soybean is not engineered for pest resistance, thus there are no 'target' species, and thus no 'nontarget' species either. APHIS assessed whether exposure or consumption of MON 87708 soybean would have a direct or indirect adverse impact on species beneficial to agriculture**.** Organisms considered were representatives of the species associated with production of the regulated crop in the agricultural environment. The assessment includes an analysis of data and information on MON 87708 soybean compared to the non-GE counterpart A3525 soybean (or other comparators) for any biologically relevant changes in the phenotype or substances produced (e.g. the MON 87708 DMO, nutrients, antinutrients, metabolites, etc.) which may be novel or expressed at significantly altered amounts that are associated with impacts on organisms beneficial to agriculture, and/or any observations of beneficial organisms associated with the plants.

APHIS reviewed information Monsanto provided justifying the safety of MON 87708 soybean (Sections V.D-V.F, pp. 81-85, (Monsanto, 2012)) as well additional literature:

- The donor organism for the *dmo* gene, *S. maltophilia,* has been previously reviewed as part of a safety and nutritional assessment of MON 87708 soybean that Monsanto completed with the FDA (US-FDA 2011). *S. maltophilia* is an aerobic, ubiquitous, environmental, gram-negative bacterium, and although its close genetic relatives are plant pathogens, it is not classified as such. APHIS examined a recent review of *S. maltophilia* by Brooke (2012) that indicates that it has been isolated from soil, water, animals, invertebrates, plant matter including food, and hospital equipment, and can cause infections in humans, particularly immunocompromised and debilitated individuals; but there is no indication that the *dmo* from *S. maltophilia* plays a role in pathogenicity,

virulence, antibiotic resistance, adhesion or other interactions with human, animals or invertebrates.

- The DMO enzyme present in MON 87708 soybean shares sequence identity and many catalytic and domain structural similarities with a wide variety of oxygenases found in numerous species of microorganisms widely distributed and prevalent in the environment (Chakraborty et al. 2012), and with oxygenases such as pheophorbide A oxygenase also found in plants such as rice, maize, canola and pea (Rodoni et al. 1997; Yang et al. 2004) that are consumed in a variety of food and feed sources which have a history of safe human consumption, establishing that plants, animals and humans are extensively exposed to these types of enzymes (Section V.E.2, pp. 82-83, (Monsanto, 2012)).
- Bioinformatics analyses demonstrated that MON 87708 soybean DMO does not share amino acid sequence similarities with known allergens, gliadins, glutenins or protein toxins which could have adverse effects to human or animal health (Section V.D, p. 81, (Monsanto, 2012); US-FDA 2011).
- MON 87708 soybean DMO is readily digestible in simulated gastric and intestinal fluids, making it highly unlikely that it would be absorbed in the small intestine and have any adverse effects on human or animal health (Section V.E.5, p. 84, (Monsanto, 2012)).
- An acute oral toxicity study indicated no adverse effects in mice at the highest dose tested (140 mg/kg body weight), and by extrapolation using the Margin of Error approach, no meaningful risk to human or animal health from dietary exposure to MON 87708 soybean DMO (Section V.E.6, p. 84, (Monsanto, 2012)).

As indicated earlier in this plant pest risk assessment, the petitioner's characterization of MON 87708 soybean showed nutrient and anti-nutrient levels in seed and forage were within the 99% tolerance intervals for commercial reference varieties, and that the MON 87708 soybean DMO protein makes up only approximately 0.01 to 0.02 % of the total protein in seed or forage, respectively, so there is unlikely to be nontarget effects resulting from changes in composition or from consumption of MON 87708 DMO. Also the study on environmental interactions found that there were no changes in beneficial arthropod abundance in field plots of MON 87708 soybean.

Honeybees were not among the arthropods sampled in the beneficial arthropod study and are not essential for soybean pollination, with natural outcrossing rates in cultivated soybean often lower than 1% (Table IX-1, p. 259, (Monsanto, 2012)). Monsanto examined MON 87708 soybean pollen and found there was no difference in pollen viability, size or visual morphology due to the dicamba resistance trait (Section VII.C.3, pp.166-167). MON 87708 soybean DMO is targeted to the chloroplast and is not expected to be found in nectar. Soybean does not produce a lot of pollen and so would not be a significant source of protein for honeybees, however soybean flour can be used to provide supplemental protein to bee colonies to support low levels of brood rearing during winter months in the South, Southwest, and Southeast when pollen sources are low (Standifer et al. 1977). Since the protein has no known toxicity and is present at low levels in soybean seed, no adverse effect on honeybees would be expected from such use.

Monsanto also found that the nitrogen-fixing plant-microbe symbiotic relationship between MON 87708 soybean and *Bradyrhizobiaceae japonicum* was unaltered due to the introduction of the dicamba resistance trait, as there was no significant difference detected in nodule number, nodule dry weight, root dry weight, shoot dry weight and shoot total nitrogen (percent and mass) between MON 87708 and the conventional control when grown in nitrogen-deficient potting medium from seeds inoculated with a solution of the symbiont (Section VII.C.4 and Table VII-8, pp.168-169, (Monsanto, 2012)).

Therefore, based on the above analysis of Monsanto's studies on MON 87708 soybean food and feed safety, nutrient and anti-nutrient composition, levels of DMO in tissues, environmental interactions with beneficial arthropods, pollen characteristics and association with symbionts, APHIS concludes exposure to and/or consumption of MON 87708 soybean are unlikely to have any adverse impacts on organisms beneficial to agriculture.

F. Potential for Enhanced Weediness of MON 87708 Soybean

APHIS assessed whether the MON 87708 soybean is likely to become more weedy (i.e. more prevalent, competitive, damaging or difficult-to-control in situations where it is not wanted) than the nontransgenic progenitor from which it was derived, or other varieties of the crop currently under cultivation. The assessment considers the basic biology of the crop, the situations in which crop volunteers or feral populations are considered weeds, and an evaluation of the MON 87708 soybean compared to its nontransgenic progenitor A3525 soybean evaluated under field conditions characteristic for the regions of the U.S. where soybean is grown (and/or under laboratory or greenhouse conditions) for characteristics related to establishment, competiveness, reproduction, survival, persistence and/or spread that could influence weediness and the ability to manage the crop as a weed. For soybean, such characteristics include in particular, hard seededness, vegetative vigor, harvest seed loss and lodging. The assessment also considers whether the engineered trait affects methods of control for the crop in situations where it is managed as a weed or volunteer in subsequent crops or in feral populations.

In the U.S., soybean is not listed as a weed in the major weed references (Crockett 1977; Holm et al. 1979; Holm et al. 1997), nor is it designated as a noxious weed by the federal government (USDA-APHIS 2012), although it has been mentioned as an agricultural weed by the Southern Weed Science Society (USDA-NRCS 2012). Soybean does not possess any of the attributes commonly associated with weeds (Baker, 1965), such as long persistence of seed in the soil, the ability to disperse, invade, and become a dominant species in new or diverse landscapes, or the ability to compete well with native vegetation. Furthermore, mature soybean seeds have no innate dormancy; germinating seedlings and plants are sensitive to cold, are not expected to survive in freezing winter conditions and do not vegetatively reproduce (OECD 2000; Raper Jr. and Kramer 1987). Soybeans that volunteer from the previous year's crop are rarely a management issue, as

crop losses attributable to interference from soybean volunteers are minimal (Owen and Zelaya 2005). However, volunteers can be a problem particularly in the South where winters are milder, and particularly if weather events lead to soybean seed loss prior to harvest (York et al. 2005).

To test the expectation that MON 87708 soybean has not obtained characteristics that would increase its weediness, Monsanto conducted a combination of replicated laboratory, greenhouse and multi-site field experiments in 2008 and 2009, similar to the design of the compositional and environmental interaction assessments previously discussed which compared dicamba-treated or untreated MON 87708 with the conventional soybean control A3525, to evaluate phenotypic characteristics that may impact weediness (e.g., viable hard seed as an indication of seed dormancy, vegetative vigor as an indication of competitiveness, pre-harvest seed loss and lodging as indications of the potential for seed to occur on the soil following harvest and potentially volunteer as a weed in the subsequent crop) (Section VII, pp. 146-169, and Table VII-1, pp. 148-149, (Monsanto, 2012)). Multiple commercial reference varieties were included in the assessment of weediness characteristics to provide a range of comparative values that are representative of existing commercial soybean varieties.

For seed dormancy and germination characteristics, the seed lots for MON 87708 soybean, the conventional control and eight commercial reference varieties were produced in replicated field trials during 2008 in Iowa, Illinois and Missouri, which represent environmentally relevant conditions for soybean production. In addition to the Association of Official Seed Analysts (AOSA 2007) recommended temperature range of 20/30°C, seed was tested at five additional temperature regimes of 10, 20, 30, 10/20, and 10/30°C to assess seed germination properties, following the methods presented in Appendix F (pp. 437-442, (Monsanto, 2012)). In a combined site analysis, in which the data were pooled among the three seed production sites, none of the significant differences detected ($\alpha = 0.05$) between MON 87708 soybean and the conventional control were outside the range of the eight commercial reference varieties nor were they in the direction that would increase potential weediness; all were small in magnitude (Table VII-2, p. 155, (Monsanto, 2012)):

- MON 87708 soybean had lower percent germinated seed at 10°C (98.9% vs. 99.7%) and at 10/30°C (98.6% vs. 99.7%). Concurrently, MON 87708 soybean had higher percent dead seed at 10°C (0.8% vs. 0.2%) and 10/30°C (1.4% vs. 0.3%). These differences are small and would not increase plant weediness; on the contrary, there would be lower stand count and less viable seed available for seed dispersal or soil seed banking under these temperature regimes, all other factors being equal.
- There were no differences for percentages of germinated or dead seed in the temperature regimes 20, 30, 10/20, and at the optimal temperature range of 20/30°C.
- There were also no detectable differences for percentages of viable hard seed or viable firm-swollen seed in any temperature regime.

For the phenotypic and agronomic characteristics, APHIS evaluated 14 characteristics assessed in the field by Monsanto to determine if MON 87708 soybean was likely to be more weedy than the conventional control (Section VII, pp. 146-169, and Appendix G, pp. 443-468, (Monsanto, 2012)):

- Eleven quantitative characteristics (early stand count, seedling vigor, days to 50% flowering, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, test weight and yield) underwent statistical analysis ($\alpha = 0.05$), whereas three qualitative characteristics (flower color, plant pubescence and plant growth stage) were assessed for categorical differences (Table VII-1, pp. 148-149).
- MON 87708 soybean and the conventional control were grown in the same replicated field trials previously described for the environmental interaction study described in the section of this PPRA on the *Potential Plant Pest and Disease Impacts,* following the methods presented in Section V.II (V.II.A-VII.B, pp. 146-152 and V.II.C.2, pp. 156-158) and Appendix G.1-G.11 (pp. 443-454).
- Data were collected in 2008 on untreated MON 87708 soybean at 18 field sites and in 2009 on dicamba-treated MON 87708 soybean at 8 field sites (Tables VII-3 and VII-4, pp. 157-158).

An evaluation of the data shows that neither the dicamba tolerance trait nor the dicamba herbicide treatment altered the weediness potential of MON 87708 soybean compared to the conventional control based on the assessed phenotypic and agronomic traits (Section VII.C.2.3, pp. 158-162; Appendix G.12, pp. 454-456; Tables G-5 through G-8, pp. 457-468, (Monsanto, 2012)):

2008 Analysis with Untreated MON 87708 Soybean

- In the combined-site analysis for 2008, no significant differences were detected between untreated MON 87708 soybean and the control for 9 of 11 quantitative characteristics (e.g., early stand count, seedling vigor, days to 50% flowering, lodging, pod shattering, final stand count, seed moisture, test weight and yield). For the other two characteristics, untreated MON 87708 soybean had significantly taller plants (6% taller, with a plant mean height of 33.5 inches vs. 31.6 inches) and significantly lighter seeds (3.3% smaller, weighing 15.0 vs. 15.5 grams per 100 seed weight) (Table VII-5, p. 159).
- While the differences in plant height and seed weight were small in magnitude, an examination of the corresponding individual-site data revealed that the differences were consistent; untreated MON 87708 soybean had taller plants at 16 of the 18 sites with 8 of those significantly taller, and had lighter seeds at 15 of the 18 sites with 5 of those significantly lighter (Table G-5, pp. 457-459). None of these differences were outside the reference ranges (Table VII-5, p. 159) calculated from the 18 commercial reference varieties (Table G-1, p. 445) grown across the 18 sites. Nevertheless the differences are significant, although increased plant height and lower seed weight are not characteristics associated with plant weediness for soybean.
- There were 13 other significant differences detected out of 179 comparisons of

quantitative traits at individual sites, but these are not indicative of a consistent or biologically meaningful response since no significant differences were detected in the corresponding combined-site analyses.

- Untreated MON 87708 soybean developed similar to the conventional control, as indicated by no categorical differences in plant growth stages for 131 out of 132 observations among all sites (Table G-7, pp. 463-466), and no differences in flower color and plant pubescence (Table G-5, p. 457).

2009 Analysis with Dicamba-Treated MON 87708 Soybean

- In the combined-site analysis for 2009, no significant differences were detected between treated MON 87708 soybean and the control for 9 of 10 quantitative characteristics (e.g., early stand count, seedling vigor, days to 50% flowering, plant height, lodging, pod shattering, final stand count, seed moisture and yield). The only significant difference was that treated MON 87708 soybean had significantly lighter seeds (6.4% smaller, weighing 14.6 vs. 15.6 grams per 100 seed weight) (Table VII-6, p. 161).
- While the difference in seed weight was small in magnitude, an examination of the corresponding individual-site data revealed that the differences were very consistent, with treated MON 87708 soybean having lighter seeds at all 8 individual sites, with 4 of these significantly lighter (Table G-6, pp. 460-462), similar to the 2008 untreated study. Furthermore, the mean 100 seed weight of treated MON 87708 soybean (14.6 grams) was slightly below the reference range (15.0 – 17.7 grams, Table VII-6, p. 161) calculated from the 14 commercial reference varieties (Table G-2, p. 446) grown across the 8 sites, although reported ranges of commercial soybean seed weight are as broad as 13 – 20 grams per 100 seed (Fasoula et al. 2004). While the reduced 100 seed weight did not manifest into a significant reduction in yield, nevertheless the reduction is significant and so far appears durable across years, sites and herbicide treatment. However, while the seed weight reduction has interesting implications for how the inserted dmo gene may be ultimately affecting seed weight, for the purposes of this plant pest risk assessment, lower seed weight is not a characteristic associated with plant weediness for soybean and therefore is not a cause for concern by APHIS.
- There were 10 other significant differences detected out of 71 comparisons of quantitative traits at individual sites, but these are not indicative of a consistent or biologically meaningful response since the 10 differences were distributed widely among eight of the 11 characteristics and no significant differences were detected in the corresponding combined-site analyses.
- Treated MON 87708 soybean developed the same as the conventional control, as indicated by no categorical differences in plant growth stages for 44 out of 44 observations among all sites (Table G-8, pp. 467-468) and no differences in flower color (Table G-6, p. 460).

APHIS evaluated information provided in the petition regarding the dicamba herbicide use in the U.S. and control of volunteers of MON 87708 soybean in other crops (Sections VIII.G.1 – VIII.G.2, pp. 197 – 206, and Section VIII.J, pp. 238-240, (Monsanto, 2012)).

Resistance of MON 87708 soybean volunteers to dicamba would increase its survival compared to its conventional control in situations where it is treated with dicamba, e.g., in subsequent rotation with dicamba-resistant soybean or in fallow land or another crop with a labeled application of dicamba. In crops that are normally treated with dicamba to control broadleaf weeds, should volunteers of MON 87708 soybean appear, they could be controlled with other effective herbicides or cultural control methods. Since Monsanto intends to commercialize MON 87708 soybean as a stack with glyphosate herbicide-resistant MON 89788 soybean (Roundup Ready 2 Yield), glyphosate would also not be an effective herbicide for volunteer control. As indicated earlier, MON 87708 soybean is also reduced in sensitivity to at least three other phenoxy synthetic auxin herbicides: 2,4-D, MCPA and 2,4-DB.

An analysis of crop rotation practices in soybean and the use of dicamba in crops following soybean in rotation by region and by state are summarized in Section VIII.I of the petition (pp. 224-237 (Monsanto, 2012), including revised Tables VIII-24 through VIII-27 received from Monsanto on October 10, 2012). Crops that follow soybean in rotation and soybean volunteer pressure differ depending on the geographical growing region. Volunteer soybeans are less of a concern in the Midwest region where the majority of soybean in the U.S. is grown, since their lack of innate seed dormancy and sensitivity to cold causes soybean seeds or seedlings to be killed by the cold temperatures over the winter or in the early spring (Andersson and de Vicente 2010; Carpenter et al. 2002; OECD 2000); however volunteer soybeans may be more problematic in the southern growing regions. According to analysis in the petition, based on 2008 planting data compiled from all the regions (Midwest, Southeast, and East Coastal), U.S. soybean acreage is most frequently rotated the following year to corn (68.6%), soybean (14.5%), wheat (11.2%), cotton (2.1%), rice (1.4%) or sorghum (1.1%) with other minor crops making up less than one percent of the estimated rotated acreage (Table VIII-24). Of the total soybean acreage rotated, the acreage of the rotational crops (other than soybean) treated with dicamba is expected to be a relatively small percentage due to the generally low percentage of dicamba use for the other crops typically rotated with soybean, and there are expected to be regional and state differences as shown below in Table 2.

Preplant tillage and/or herbicides can be used to control emerging volunteers of soybean in most rotation crops, and additionally flooding can be used as a control measure in rice (Carpenter et al. 2002; York et al. 2005). Furthermore, as summarized in Table VIII-28 (p. 240, (Monsanto, 2012)), excellent ratings are obtained for postemergence control of volunteer soybeans in the rotational crops corn, sorghum, wheat, barley, oats, cotton and rice for one or more labeled herbicides with different modes of action than the synthetic auxins to which MON 87708 soybean has acquired complete or partial resistance (Appendix Table C-7, pp. 317-318, (Monsanto, 2012)). Depending on the herbicide resistance trait of the rotational crop, additional herbicide options may be possible.

Table 2. The acreage of the rotational crop following soybean that is treated with dicamba (Dicamba Acreage) and the percent of the total acreage rotated to the crop that is treated with dicamba (% Dicamba Usage), for the U.S. and each region (where available or applicable) (summarized from columns H and I, respectively, from revised Tables VIII-24 through VIII-27 received from Monsanto on October 10, 2012).

Major Crop Following Soybean In Rotation[1]	Dicamba Acreage / (% Dicamba Usage)[2]			
	United States	Midwest Region	Southeast Region	East Coastal Region
Corn	5053 / (9.8)	4591 / (9.7)	72 / (3.2)	390 / (22.4)
Soybean	4350 / (40.0)	1954 / (40.0)	2299 / (40.0)	97 / (40.0)
Wheat	448 / (5.3)	448 / (5.5)		
Cotton	153 / (9.7)	14 / (18.0)	139 / (9.4)	

[1] Those rotation crops that follow soybean for which planted acreage is estimated to exceed 2.0% of the total soybean acres planted in the U.S. as compiled in Table VIII-24 Column G.
[2] Acreage expressed as 1000s of acres based on 2008 planting data, during which 75,037,000 acres of soybean were planted in the US; percent dicamba usage for soybean was based on a future market estimate of 40% ((Monsanto, 2012)) and revised Tables VIII-24 through VIII-27 received from Monsanto on October 10, 2012.

APHIS concludes, based on the agronomic field data and literature survey concerning weediness potential of the crop, the there is no indication that MON 87708 soybean possesses a selective advantage that would result in increased weediness either in cultivated or unmanaged fallow fields. MON 87708 soybean is no more likely to establish troublesome volunteer populations than either existing transgenic or nontransgenic herbicide-resistant or nontransgenic herbicide sensitive soybean varieties. Volunteer populations of MON 87708 soybean, although resistant to dicamba and less sensitive to other phenoxy synthetic auxin herbicides, could still be controlled using other currently available weed control methods.

G. Potential Impacts on the Weediness of Any Other Plants with which MON 87708 Soybean Can Interbreed

Gene flow is a natural biological process with significant evolutionary importance. A number of angiosperm taxa are believed to be derived from hybridization or introgression between closely related taxa (Grant, 1981; Rieseberg and Wendel, 1993; Soltis *et al.*, 1993; Grant, 1994; Hegde *et al.*, 2006) and even in the existing floras, the occurrence of hybridization or introgression is reported to be widespread (Stace, 1987; Rieseberg and Wendel, 1993; Rieseberg 1997; Peterson et al., 2002). It has been a common practice by plant breeders to artificially introgress traits from wild relatives into crop plants to develop new cultivars (Khoury *et al.*, 2013). However, gene flow from crops to wild relatives is also thought of as having a potential to enhance the weediness of wild

relatives, as observed in rice, sorghum, sunflower and a few other crops (see Table 1 in Ellstrand *et al*. (1999). By providing fitness-related traits such as resistance to insects, diseases, herbicides or harsh growing conditions, gene flow from crops to their wild relatives could allow the hybrids to compete better, produce more seeds, and become more abundant (Snow 2002). Besides weediness, other concerns are the loss of herbicide resistance as a tool to protect crops from closely related weeds (Gepts and Papa 2003). This topic is covered in two sections: 1) the potential for gene flow, hybridization and introgression from MON 87708 soybean to sexually compatible relatives, including wild, weedy, feral or cultivated species in the United States and its territories, and 2) if so, the risk potential with respect to weediness of those taxa following introgression, based on the phenotypic changes that have been observed in the engineered plants.

Potential for gene flow, hybridization and gene introgression

Gene flow is unlikely to occur between MON 87708 soybean and other soybean crops due to its highly self-pollinating nature. Natural outcrossing rates in soybean are usually lower than 1% (Andersson and de Vicente 2010; Table IX-1, p. 259, (Monsanto, 2012)). This is predominantly due to soybean flower physiology and anatomy, where the anthers mature in the flower buds and directly shed their pollen onto the stigma of the same flower before flower opening (Andersson and de Vicente 2010; 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 flower orientation which reduces its exposure to wind and the clumping and stickiness of soybean pollen decreases the dispersion ability of pollen (Yoshimura 2011). The limited potential for cross-pollination in soybean is evident in the Federal Seed Act Rules of Practice used by growers of Foundation, Registered and Certified soybean seed, which permit an isolation distance of zero between fields of different varieties of soybean, with the caveat that there also be adequate distance around the field to prevent mechanical mixing with farm equipment (7 CFR part 201.76, 2014 Edition).

Gene flow potential of MON 87708 soybean was evaluated thoroughly. The introduced *dmo* gene in MON 87708 soybean is not expected to change the ability of the plant to interbreed with other plant species. Furthermore, the APHIS evaluation of data provided by Monsanto (2012) of agronomic and phenotypic properties of MON 87708 soybean, including those characteristics associated with reproductive biology such as days to 50% flowering, plant growth stage, pollen morphology and viability, seed dormancy and germination and pod shattering, indicated no unintended changes likely to affect the potential for gene flow from MON 87708 soybean to sexually compatible species. The potential for gene flow to occur specifically between herbicide-resistant crop varieties and their sexually compatible relatives has been previously addressed (Mallory-Smith and Olguin 2011). Gene flow does not differ whether the herbicide resistance trait is introduced via genetic engineering or via conventional breeding techniques, and gene

flow has been occurring between non-GE soybean and GE soybean varieties, albeit at very low levels in accordance with soybean's highly self-pollinating nature. Therefore, the potential for gene flow and introgression of the dicamba-resistant trait from MON 87708 soybean to other soybean varieties and its consequences are anticipated to be similar to those as for existing commercial soybean varieties.

Many conditions have been identified that are required for gene flow and introgression to occur between a crop and its wild relatives (Carpenter et al. 2002; Jenczewski et al. 2003; Lu 2005; Stewart et al. 2003), including flowering synchrony, abundance and method of pollen spread, distance of pollen movement, genetic compatibility, and environmental conditions pertinent to cross-pollination, but the foremost condition is the presence of wild relatives within pollen or seed dispersal range from the crop. In the U. S., the lack of sexually compatible wild relatives of *Glycine max* precludes any opportunity for gene flow to occur between cultivated soybean and its wild relatives. The genus *Glycine* is divided into two distinct subgenera, the annual subgenus *Soja* which contains three species including *G. max*, and the perennial subgenus *Glycine* which contains multiple species distantly related and not known to be found in the American continents or to produce fertile hybrids with *G. max*. Within the annual subgenus *Soja*, all three species (*G. max*, *G. soja*, and *G. gracilis*) can hybridize to some extent, but the latter two are not found in America (Hymowitz 2004; Lu 2005; Section IX.D.3, p. 256, (Monsanto, 2012); OECD 2000). Even if free-living wild relatives of cultivated soybean were to somehow be introduced into the soybean growing regions in the United States, the same reproductive characteristics previously discussed which limit cross-pollination among cultivated soybean varieties would limit gene flow to wild relatives.

Potential for enhanced weediness of recipients after hybridization and/or introgression

As described earlier, there is no indication that MON 87708 soybean possesses a selective advantage that would result in increased weediness either in cultivated or unmanaged fallow fields. In the extremely unlikely event successful hybrids of cultivated soybean and wild relatives were to occur in the U.S., the herbicide resistance trait would only provide selective advantage in situations in which the hybrid was in contact with the herbicide (i.e., in an agricultural or fallowed field or field edge). Any herbicide-resistant hybrid-derived populations are likely to be controlled using other available chemical or mechanical means. Many broadleaf and/or broad spectrum herbicides that are effective for control of dicamba-resistant soybean as volunteers (see above section of this PPRA on the *Potential for Enhanced Weediness of MON 87708 Soybean*; and Table VIII-28, p. 240, (Monsanto, 2012)) would likely be effective for control of hybrids formed with other conventional soybeans or related species.

APHIS concludes, based on the information presented in the petition and in relevant literature, that MON 87708 soybean is not expected to increase the weed risk potential of other soybeans, nor of other species with which it can interbreed in the U.S. or its territories as other sexually compatible species do not occur there. The genetic modification in MON 87708 soybean is not expected to increase the potential for gene flow, hybridization and/or introgression to occur to sexually compatible taxa compared to the nontransgenic recipient or other varieties of the crop commonly grown. It is highly

unlikely that soybean plants will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between MON 87708 soybean plants and sexually compatible relatives in a natural environment, since sexually compatible relatives do not occur in the U.S. Herbicides and other methods are available to control volunteer dicamba-resistant soybeans and other soybeans and *Glycine* species with which it might cross.

H. Potential Changes to Agriculture or Cultivation Practices

APHIS assessed whether there are likely to be significant changes to agricultural or cultivation practices associated with adoption of MON 87708 soybean, and if so, are they likely to impact plant diseases or pests or their management, including any APHIS control programs. This includes consideration of any changes in pesticide applications, tillage, irrigation, harvesting, etc. as they relate to plant pests and diseases.

The only agricultural or cultivation practices that are currently employed for soybean production that are expected to change if MON 87708 is determined to be no longer subject to the regulatory requirements of 7 CFR part 340 are those related to weed management: in particular, choice of herbicide(s) or herbicide combinations, times of application, and potential crop choices or buffer zones for adjacent lands to avoid spray drift or volatilization to sensitive plants. Although dicamba spray drift and volatilization can potentially injury susceptible crops in proximity to MON 88708 soybean, such impacts are not considered plant pest risks, and such impacts are assessed by the U.S. EPA.

The current and proposed uses of dicamba in soybean are described in the petition (Section VIII.H, pp. 208- 224, (Monsanto, 2012). Dicamba is currently labeled only for preplant and late postemergence (preharvest) applications in soybean. Due to insufficient crop tolerance of soybean to applications of dicamba, preplant restrictions regarding preplant treatment are required to avoid soybean injury: i.e. a maximum application rate of 0.5 lbs a.e. per acre, a minimum of one inch of rainfall, and a 28-day interval between preplant application and planting of soybean. Due to insufficient crop tolerance, dicamba currently also cannot be used for in-crop postemergence applications. If EPA approves Monsanto's submitted application to amend Registration Number 524-582 for a DGA salt formulation of dicamba to remove all pre-emergence planting restrictions and to allow in-crop postemergence dicamba applications to MON 87708 soybean through the reproductive R1/R2 (beginning and full bloom) growth stage of soybean, growers would be authorized to apply dicamba alone or in mixtures with glyphosate (when MON 87708 soybean is stacked with MON 89788 Roundup Ready 2 Yield soybean) or other herbicides for preplant or in-crop postemergence applications on MON 87708 soybean. Non-aerial applications of dicamba would be authorized preemergence up to crop emergence as a single application or split applications up to a total of 1.0 lb a.e. per acre, and up to two postemergence applications up to 0.5 lb a.e. per acre, each through the R1/R2 growth stage of soybean, with a total maximum annual application rate of 2.0 lb dicamba a.e. per acre.

As with soybean, the timing of application of dicamba differs for labeled crops depending on their tolerance level (Section VIII.G.1, pp. 200 - 201, (Monsanto, 2012). Since the requested change in the use of dicamba in MON 87708 soybean would allow in-crop postemergence applications at times not previously allowed or practiced, and later than many other postemergence applications applied to other crops grown in the regions where soybeans are grown, there is an increased opportunity for exposure to sensitive plants (both in terms of timing and total amount) from offsite drift or volatilization. Analysis in the petition shows that application of dicamba at or following the V4 vegetative growth stage is expected to be later in the growing season than the current latest in-crop application timings to corn, sorghum, and wheat; and dicamba is not currently applied in-crop to cotton (Section VIII.H.1, pp. 217- 218, (Monsanto, 2012), although a separate petition for nonregulated status for dicamba and glufosinate herbicide-resistant cotton is also pending with APHIS (petition 12-085-01pp) (Monsanto, 2012). When Monsanto submitted the petition for deregulation of MON 87708 soybean, corn had the largest in-crop application use of dicamba (Table VIII-14, p. 201, (Monsanto, 2012). Based on the planting times and growth stages of corn and soybean and environmental conditions, the most likely application timing for dicamba to MON 87708 soybean was projected to be approximately 20 days later than the current latest application timing for corn in central Illinois (representative of Midwest soybean regions) and approximately 37 days later than the current latest application timing for corn in western Tennessee (representative of southern soybean growing regions). As noted in the *Introduction* in this PPRA, EPA is currently reviewing the proposed label changes described above and a request to establish tolerances for residues of dicamba on soybean forage and hay. EPA's assessment will analyze risks to off-target plants as well as other non-target organisms to determine if the label is sufficient to meet EPA's standards for registration: "reasonable certainty of no harm to humans" and "no unreasonable adverse effects on the environment." If these standards are not met, EPA will apply appropriate risk mitigation strategies and propose label modifications to address the specific concerns. After EPA has completed its assessments and provided these to APHIS, APHIS will update this PPRA if necessary. In addition, issues related to herbicide drift and volatilization will be addressed in the NEPA document for this petition.

Upon integration of MON 87708 soybean into the Roundup Ready soybean system, aside from the anticipated label changes requested, Monsanto expects that growers will have the ability to continue to use established soybean production practices including crop rotation, tillage systems, labeled herbicides, row spacing, and planting and harvesting machinery currently being utilized (Section VIII.H, pp. 208 - 216, (Monsanto, 2012). The anticipated label change would facilitate a wider window of application for dicamba and is expected to provide a tool for improved control of broadleaf weeds (including some with resistance to other herbicides such as glyphosate, ALS, and PPO chemistries) that can be integrated into weed management programs using no-till or reduced tillage or conventional tillage. Their anticipated weed management recommendations for MON 87708 soybean combined with Roundup Ready 2 Yield soybean also include a preemergence (burndown at planting) application of a residual herbicide alone with conventional tillage, or combined with glyphosate and dicamba in conservation tillage

(Table VIII-16, p. 210, (Monsanto, 2012). The impacts of this system for reducing or managing weeds and the evolution of herbicide-resistant weeds are being addressed in the NEPA document for this petition. Greater weed control could potentially reduce disease and pest pressure in soybean if the diseases and pests of the weeds also use soybean as a host.

Crop rotation practices are not expected to be impacted by the use of dicamba on fields planted to MON 87708 soybean. Crop rotation practices in soybean were analyzed in the petition (Section VIII.I, pp. 224-237, (Monsanto, 2012), and Tables VIII-24 - VIII-27 revised by Monsanto received on October 10, 2012). Soybeans are grown in rotation for many reasons, including to break or mitigate disease, insect, nematode and weed cycles or damage (Al-Kaisi et al. 2003; Hoeft et al. 2000). In comparison to alternative herbicides to dicamba for use in soybean, Monsanto has indicated that dicamba has either an improved or neutral risk profile with respect to long rotational crop restrictions (e.g., through a moderate potential for residual activity) (Table VIII-19, p. 215, (Monsanto, 2012). Dicamba can be absorbed through leaves and roots and translocated, but is considered only moderately persistent in soil, with a half-life of six days for dicamba acid under aerobic soil conditions with formation of the non-persistent degradate DCSA, and a half-life of 141 days under anaerobic soil conditions (US-EPA 2009). Crop rotation restrictions range from 30 to approximately180 days, depending on the rate applied, inches of rainfall and the following crop, according to the Clarity® label (BASF 2010), and these should be adequate for rotation to other crops the spring following harvest of soybeans.

The field studies on MON 87708 soybean conducted in 2008 and 2009 in the absence or inclusion of dicamba treatment, respectively, described in the section *Potential Plant Pest and Disease Impacts*, demonstrate that neither the dicamba resistance trait nor the dicamba herbicide treatment nor the metabolites produced as a result of the breakdown of dicamba by MON 87708 soybean DMO appear to alter the response of MON 87708 soybean to abiotic stress, diseases, or arthropod pests under natural levels of these stressors, and nor were pest arthropods more abundant around MON 87708 soybean plots. Agronomic practices used to prepare and maintain each study site were characteristic of those used in each respective geographic region. All maintenance operations were performed uniformly over the entire trial area (p. 444, (Monsanto, 2012). Therefore, no changes are expected for insect and disease control practices with MON 87708 soybean. The agronomic characteristics and cultivation practices employed when growing MON 87708 soybean are essentially indistinguishable from practices used to grow other soybean varieties, including other herbicide-resistant varieties (Section VIII, pp. 170-243, (Monsanto, 2012) with the exception of the changes in herbicide choices and timing discussed previously.

Although pest and disease susceptibility data were not presented for MON 87708 soybean stacked with the glyphosate-resistant trait, an evaluation of current literature does not suggest that the intended stacking will increase plant pest and diseases. In a recent review regarding claims made that glyphosate-resistant (GR) crops sometimes have mineral deficiencies and increases in plant disease, an evaluation of the literature

showed that: "1) although there is conflicting literature on the effects of glyphosate on mineral nutrition on GR crops, most of the literature indicates that mineral nutrition in GR crops is not affected by either the GR trait or by application of glyphosate; 2) most of the available data support the view that neither the GR transgenes nor glyphosate use in GR crops increases crop disease; and 3) yield data on GR crops do not support the hypotheses that there are substantive mineral nutrition or disease problems that are specific to GR crops" (Duke et al. 2012). This review included an evaluation of literature relevant to disease development and severity of many of the major pathogens of soybean, comparing effects of the GR trait and glyphosate treatment. When differences in cultivar sensitivities to pathogens were taken into account, there was no consistent effect correlating the GR trait or glyphosate treatment on GR soybeans with an increase in *Sclerotinia* stem rot or white mold disease caused by *Sclerotinia sclerotiorum* (Lee et al. 2000; Lee et al. 2005; Nelson et al. 2002), sudden death syndrome caused by *Fusarium virguliforme* (Njiti et al. 2003; Sanogo et al. 2000; Sanogo et al. 2001), or infections caused by *Rhizoctonia solani* (Harikrishnan and Yang 2002) or soybean cyst nematode (Noel and Wax 2009; Yang et al. 2002). Some efficacy of glyphosate against *Phakopsora pachyrhizi*, the cause of Asian soybean rust, was reported in both greenhouse (Feng et al. 2005) and field studies on GR soybeans (Feng et al. 2008).

Changes in agricultural practices related to weed control are unlikely to adversely impact pest and disease management practices in soybean and may provide some benefit by providing another tool for in-crop control of broadleaf weeds that may serve as alternative hosts for pests and diseases. For example, soybean cyst nematode (SCN) is the most important soybean 'pathogen' in the United States, and in addition to soybean, several other crops and broadleaf weeds are also hosts for SCN. Some weed hosts include chickweed, common mullein, henbit, hop clover, purslane, Rocky Mountain Bee plant, and toothed medic. Proper crop rotation and control of weeds can help to reduce levels of SCN in the field (Nelson and Bradley 2003). Several broadleaf weeds (including burdock, chickweed, lambsquarters, purslane, ragweed, vetch, curly dock, dandelion, redroot pigweed, shepard's-purse, velvetleaf, wild mustard and wild parsnip) are also hosts of white mold (*Sclerotinia* stem rot) (Fyksen 2012).

Most seedling diseases are controlled with fungicides included in seed treatments. For soybean rust, no resistant varieties are available, but several fungicides are available for soybean rust and recommendations for these are that the first spray should not be applied prior to bloom. Other major pod and stem diseases are controlled by using disease-free seed, rotation with non-leguminous crops, and foliar fungicides where the first application to soybeans are in mid-bloom to early pod-set stages (ACES 2012). Most of these fungicides are expected to be applied later than most of the additional application windows for dicamba based on Monsanto recommendations for dicamba use on MON 87708 soybeans (stacked with glyphosate resistance) since their recommendation for postemergence application of dicamba (plus glyphosate) at the V4-R2 stage is only for more aggressive glyphosate-resistant weed species, such as *Ambrosia* or *Amaranthus* species (Table VIII-16, p. 210, (Monsanto, 2012).

In conclusion, MON 87708 soybean is similar to conventional soybean in its agronomic, phenotypic, environmental response, and compositional characteristics and has levels of pests and diseases or their damage levels comparable to conventional soybean. The only changes in agricultural or cultivation practices that are anticipated with adoption of MON 87708 soybean (including the anticipated stack with MON 89788 Roundup Ready 2 Yield soybean) are related to weed management practices. Anticipated changes in herbicide use patterns in MON 87708 soybean alone or stacked with MON 89788 Roundup Ready 2 Yield soybean are unlikely to increase pests or diseases or adversely impact their management, nor will they impact APHIS pest control programs.

I. Potential Impacts from Transfer of Genetic Information to Organisms with which MON 87708 Soybean Cannot Interbreed

APHIS examined the potential for the new genetic material inserted into MON 87708 soybean to be horizontally transferred to other organisms without sexual reproduction and whether such an event could lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

The horizontal gene transfer between unrelated organisms is one of the most intensively studied fields in the biosciences since 1940, and the issue gained extra attention with the release of transgenic plants into the environment (Dröge et al. 1998). Potential risks from stable horizontal gene transfer (HGT) from genetically engineered organisms to another organism without reproduction or human intervention were recently reviewed (Keese 2008). Mechanisms of HGT include conjugation, transformation and transduction, and other diverse mechanisms of DNA and RNA uptake and recombination and rearrangement, most notably through viruses and mobile genetic elements. HGT has been a major contributor to the spread of antibiotic resistance amongst pathogenic bacteria; emergence of increased virulence in bacteria, eukaryotes and viruses; and, in the long run, to major transitions in evolution (Brown, 2003; Keeling and Palmer, 2008; Keese, 2008).

MON 87708 soybean contains a protein coding region derived from the *dmo* gene from the bacterium *Stenotrophomonas maltophilia* and a transit peptide protein coding region from pea (*Pisum sativum*) for chloroplast targeting of the DMO protein in MON 87708 soybean. It also contains non-protein-coding regions from *Agrobacterium*, plant viruses, and pea. One example of HGT involves a class of enzymes similar to DMO. Chakraborty et al. (2012) propose that HGT contributed to the distribution of ring-hydroxylating oxygenase (*rho*) genes among prokaryotic phyla (proteobacteria, actinobacteria, cyanobacteria, and archaea), and note that homologues of *rho* genes are found in plants (in strains of *Arabidopsis, Zea mays, Oryza sativa, Physcomitrella patens and Amaranthus tricolor*). Ring-hydroxylating oxygenases (RHO) catalyze the addition of hydroxyl groups to aromatic ring compounds, initiating one of the major pathways for oxidative degradation of both natural and synthetic aromatic compounds in the environment (Peng et al. 2010). Dicamba mono-oxygenase is a unique type of RHO that

initiates the degradation of dicamba by oxygenating the exocyclic methyl group, rather than the more conventional oxygenation of the aromatic ring of the substrate seen in most other RHOs (Dumitru et al. 2009). Chakraborty et al. (2012) suggest that distribution and diversification of *rho* genes can be explained by the mechanisms of gene duplication, transposition events and DNA rearrangements in most cases, but that HGT is assumed to be the primary mechanism in cases where occurrence of the genes was found to be limited to just one or two organisms within phyla (such as *rho* genes in some cyanobacteria, firmicutes and crenarchaeota), since the possibility of being remnants of a partially deleted *rho* operon is ruled out due to the absence of similar genes in any other member of these genera. Although it is widely accepted that HGT has generated novel degradation capabilities and increased metabolic diversity among bacterial communities exposed to an ever-evolving array of polycyclic aromatic compounds, such degradative capabilities are mostly indicative of divergent evolution from a common ancestor, not HGT (Peng et al. 2010).

Potential for horizontal gene transfer to bacteria, fungi, or invertebrates

Horizontal gene transfer and expression of DNA from a plant species to bacterial, fungal or invertebrate species is unlikely to occur based on the following observations. Although there are many opportunities for plants to directly interact with fungi and bacteria (e.g. as commensals, symbionts, parasites, pathogens, decomposers, or in the guts of herbivores) and with invertebrates as plant pests, there are almost no evolutionary examples of HGT from eukaryotes to bacteria or from plants to fungi or invertebrates (Keese 2008). Examples of HGT between eukaryotes and fungi primarily involve gene acquisition or transfer by fungi to or from other distantly related fungi or bacteria (Keeling and Palmer 2008; Keese 2008) and HGT between plants and fungi is extremely rare (Richards et al 2009). Examples of HGT between plants and invertebrates are also extremely rare, and most examples of HGT in insects involve acquisition of genes from their pathogens or endosymbionts (Keese 2008; Zhu et al, 2011; Acuna et al 2012).

Horizontal transfer from and expression in bacteria of the foreign DNA inserted into the nuclear genome of the GE plant is unlikely to occur. First, many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Wood et al. 2001; Kaneko et al. 2002). There is no evidence that these organisms contain genes derived from plants. HGT from plants to bacteria is a very low frequency event, primarily because functional and selective barriers to HGT increase with genetic distance (Keese 2008). Second, 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; EFSA 2009; Koonin et al. 2001). Third, transgene DNA promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. Thus even if horizontal gene transfer occurred, proteins corresponding to the transgenes are not likely to be produced. Fourth, both the FDA (US-FDA 1998) and the European Food Safety Authority (EFSA 2009) have 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 very rare or remote.

Potential for horizontal gene transfer to viruses

APHIS also considered whether horizontal transfer of DNA from the GE plant to plant viruses was likely to occur and would lead to the creation or selection of plant viruses that are more virulent or have a broader host range. This issue has been considered before by other science review panels and government regulatory bodies (EPA-FIFRA-SAP 2006; Keese 2008). HGT is not unusual among plant viruses; however this is generally limited to exchange between viruses present in the same host organism in mixed infections, and most commonly involves homologous recombination, relying on sequence similarity at the point of crossover (Keese 2008). HGT from virus sequences engineered into plants has been demonstrated with infecting or challenge viruses, including both DNA viruses (e.g. geminiviruses which replicate in the nucleus) (Frischmuth and Stanley 1998) and RNA viruses (which typically replicate in the cytoplasm); however most have been under conditions that favor recombination to restore a defective virus (Fuchs and Gonsalves 2007; Keese 2008; Thompson and Tepfer 2010). Populations of recombinants between virus transgenes expressed in transgenic plants infected with related viruses are similar to recombinants found in mixed infections of the same viruses in nontransgenic plants, indicating that there was no novel recombination mechanism in the transgenic plants and no increased risk is expected over what is expected from mixed infections (Keese 2008; Turturo et al 2008). Nonhomologous recombination in HGT among viruses or between virus transgenes and infecting viruses can occur, but frequently results in gene deletions which can result in nonviable viruses (Morroni et al 2013). Depending on the particular virus and sequences involved, various hot-spots for recombination have been found in both coding and noncoding regions, and strategies implemented in design of transgenes to avoid recombination have been suggested. No recombinant or undesirable viruses with new properties have been detected for over at least 8-10 years in field tests or during commercial growth of deregulated virus resistant plum, squash, or papaya engineered with genes from viruses that have been deregulated in the U.S. (Fuchs and Gonsalves 2007).

The only virus sequences inserted in MON 87708 soybean are the promoter for the peanut chlorotic streak virus (PCSV) and the 5' non-translated region from the tobacco etch virus (TEV) involved in regulating gene expression. Soybean is considered susceptible to PCSV, but not to TEV (Brunt et al. 1996). PCSV belongs to the Caulimovirus family of pararetroviruses, double-stranded DNA viruses in which replication occurs in the cytoplasm via reverse transcription of an RNA intermediate. Caulimoviruses generally have a narrow host range (Hansen and Heslop-Harrison 2004). The only other caulimovirus that soybean is susceptible to is a soybean chlorotic mottle caulimovirus (SbCMV) (Brunt et al. 1996). Neither of these viruses are considered widely prevalent in the United States (University of Georgia 2012), therefore exposure of either of these two viruses to the PCSV sequences in MON 87708 soybean is expected to be low or unlikely. Moreover, recombination in Caulimoviruses occurs predominantly, if not exclusively, in the cytoplasm by template switching between RNA transcripts during

the replication process, although a low level of recombination involving viral DNA may occur in the nucleus (Froissart et al. 2005). Since the Caulimovirus promoter sequences are not transcribed in transgenic plants, there is little or no opportunity for them to recombine with any related Caulimoviruses that may infect soybean. Although TEV occurs in the United States and is considered widely prevalent (University of Georgia 2012), since soybean is not susceptible to this virus, it is unlikely that TEV would be exposed to sequences from TEV in MON 87708. Since the TEV sequence in MON 87708 is a 5' non-translated region, even if recombination were to occur with another related potyvirus that infects soybean, it is unlikely to encode a peptide. Based on the foregoing, horizontal transfer of DNA from MON 87708 soybean to plant viruses is unlikely to occur or is unlikely to lead to the creation or selection of plant viruses that are more virulent or have a broader host range.

Potential for horizontal gene transfer to parasitic plants

Evidence for HGT from plants to other plants is limited to two specific scenarios: (1) exchange of genes between a parasitic plant and its host; and (2) exchange of genes between cells of two plants living in close proximity, such as in a graft junction. In both cases, this type of HGT requires physical contacts between the two plants. Most cases of HGT in plants involve transfer of mitochondrial genomes, which are primarily maternally inherited in plants (Barr et al 2005), to other mitochondria genomes, and mostly involve parasitic plants and their hosts (Richardson and Palmer 2007). Recently, Yoshida et al (2010) through a comparative genomics analysis implicated HGT for the incorporation of a specific genetic sequence in the parasitic plant purple witchweed (*Striga hermonthica*) from its monocot host plant. According to this study, the incorporation of the specific genetic sequence (with an unknown function) occurred between sorghum and purple witchweed. However, this HGT occurred before speciation of purple witchweed and related cowpea witchweed (*S. gesnerioides*) from their common ancestor. More recent studies demonstrated that in a few parasitic species of the Rafflesiaceae family, out of several genetic sequences examined, about 2.1% of nuclear (Xi et al 2012) and 24 –41% of mitochondrial (Xi et al 2013) gene transcripts appeared to be acquired from their obligate host species. However, all the above-mentioned instances of HGT between parasitic plants and their hosts were reported to be of ancient origins, on an evolutionary time scale spanning thousands to millions of years ago. Furthermore in the GE crop, the DNA sequences were inserted into the nuclear genome, not the mitochondrial genome. genome.

If the GE plant becomes infected by a parasitic plant or is naturally grafted to another plant, there is a very low probability that HGT could result in the other plant acquiring DNA from the GE plant. However, in both scenarios this newly introduced DNA would likely reside in somatic cells, and with little chance of reaching the germ cells, this introduced DNA could not persist in subsequent generations unless the recipient plant reproduced asexually from the affected cells.

Based on the above analysis, APHIS therefore concludes that HGT of the new genetic material inserted into MON 87708 soybean to a variety of other organisms with which it cannot interbreed is highly unlikely, and is not expected to lead directly or indirectly to

disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

J. Conclusion

APHIS has reviewed the information submitted in the petition, supporting documents, public comments in response to the Federal Register notice concerning this petition and other relevant information to assess the plant pest risk of MON 87708 soybean compared to the unmodified variety from which it was derived and other commercial reference soybean varieites. APHIS concludes that the MON 87708 soybean is unlikely to pose a plant pest risk based on the following findings.

- No plant pest risk was identified from the transformation process or the insertion of new genetic material in MON 87708: the *Agrobacterium* transformation vector was disarmed, transformed material was treated to kill the bacterium, and the plant pest sequences inserted do not cause disease or create an infectious agent.
- No increase in plant pest risk was identified from expression of the inserted genetic material, the new MON 87708 DMO protein, or changes in metabolism or composition. The composition of MON 87708 grain and forage were determined to be substantially equivalent to other soybeans commercially grown and the mode of action and specificity of MON 87708 DMO raises no plant pest concerns.
- Disease and pest incidence and/or damage were not observed to be significantly increased or atypical in MON 87708 compared to the nontransgenic counterpart in field trials conducted in growing regions representative of where this soybean is expected to be grown**.** Neither the dicamba resistance trait nor the dicamba herbicide treatment (including any metabolites produced as a result) significantly alter the response of MON 87708 to diseases, or arthropod pests under natural levels of these stressors, and nor were pest arthropods more abundant around MON 87708 plots compared to the control line. Observed agronomic traits also did not reveal any significant differences that would indirectly indicate that MON 87708 is more susceptible to pests or diseases**.** Therefore no plant pest effects are expected on these or other agricultural products and no impacts are expected to APHIS pest control programs.
- Exposure to and/or consumption of MON 87708 soybean are unlikely to have any adverse impacts on organisms beneficial to agriculture based on APHIS analysis of studies on MON 87708 soybean food and feed safety, nutrient and anti-nutrient composition, levels of DMO in tissues, environmental interactions with beneficial arthropods, pollen characteristics and association with nitrogen fixing symbionts.
- MON 87708 soybean is unlikely to become more of a weed or volunteer problem than other conventional or commercial soybean varieties based on its observed agronomic characteristics, the low weediness potential of soybean and current management practices available to control MON 87708 as a weed. MON 87708 volunteers, although resistant to dicamba and less sensitive to some other phenoxy synthetic auxin herbicides, can still be controlled with other currently available weed control methods.

- MON 87708 is not expected to increase the weed risk potential of other soybeans, and other species with which it can interbreed do not naturally occur in the U.S. or its territories. The genetic modification in MON 87708 soybean is not expected to increase its potential for gene flow, hybridization and/or introgression to sexually compatible taxa, nor is it likely to increase their weediness potential in the event that such species were to be introduced. Introgression of the dicamba resistant trait into other soybeans or related species will likely make them resistant to dicamba and less sensitive to other phenoxy synthetic auxin herbicides, but other currently available weed control methods could be used for their control.
- Changes in agricultural or cultivation practices anticipated with adoption of MON 87708 soybean (including the anticipated stack with MON 89788 Roundup Ready 2 Yield soybean) are only related to weed management practices and herbicide use patterns, and these are unlikely to increase pests or diseases or adversely impact their management, nor will they impact APHIS pest control programs.
- Horizontal gene transfer of the new genetic material inserted into MON 87708 soybean to other organisms is highly unlikely and is not expected to lead directly or indirectly to disease, damage, injury or harm to plants, including the creation of new or more virulent pests, pathogens, or parasitic plants.

K. References

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