Assessment of Plant Pest Risk for Pioneer 305423 Soybean

Pioneer Hi-Bred International Inc., a subsidiary company of DuPont (Pioneer), has petitioned APHIS for a determination that Pioneer 305423 soybean is unlikely to pose a plant pest risk and, therefore, is no longer a regulated article under regulations at 7 CFR part 340.

HISTORY of Development of High Oleic acid soybean

In 1997 APHIS deregulated the first transgenic high oleic acid soybean; DuPont's soybean sublines G94-1, G94-19 and G-168 (USDA-APHIS, 1997). This event involved a soybean with an inserted sense orientation soybean microsomal omega-6 desaturase gene 1 (GmFad2-1 gene). DuPont completed its food safety consultation with the U.S. Food and Drug Administration (FDA) on these high oleic acid soybean lines in 1996 (FDA, 1996b). The Australia and New Zealand Food Authority (ANZFA) also completed a Toxicological Review and Risk Assessment, and a Technical Report was published in 2001 (ANZFA, 2001). ANZFA concluded that these high oleic acid soybeans had a significantly altered fatty acid profile, but were comparable to non-genetically engineered (non-GE) soybeans in terms of their safety and nutritional adequacy. The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) concluded that DuPont's high oleic acid soybean was substantially equivalent to currently grown soybeans, in terms of their potential environmental impact and livestock feed safety and the novel traits would not have any substantial negative effect on the environment (CFIA, 2000).

Pioneer has now developed a transgenic soybean, Pioneer 305423 soybean, with increased levels of monounsaturated (oleic) fatty acids. This event contains a fragment of the same soybean microsomal omega-6 desaturase gene 1 (*gm-fad2-1* gene) as the deregulated DuPont lines. In plants, the microsomal omega-6 desaturase-catalyzed pathway is the primary route of production of polyunsaturated lipids. The *FAD2-1* gene is strongly expressed in developing seeds. The seed-specific expressed *FAD2-1* gene is likely to play a major role in controlling conversion of oleic acid (monounsaturated fatty acid) to linoleic acid (polyunsaturated fatty acid) within storage lipids during seed development (Heppard, 1996). The additional copies of the *gm-fad2-1* gene in Pioneer 305423 soybean causes a phenomenon known as "gene silencing," which results in silencing the endogenous *fad2-1* gene, thus preventing linoleic acid from being synthesized and leading to the accumulation of oleic acid in the developing soybean seed.

Non-GE soybean oil has poor oxidative stability due to naturally occurring high levels (~54%) of polyunsaturated fatty acids. Polyunsaturated fatty acids can quicken the rancidity of the soybean oil compared with saturated and monounsaturated fatty acids, especially after prolonged exposure to oxygen, light and/or heat. This characteristic reduces product stability and shelf life. Hydrogenation is a chemical process that is used to stabilize oils high in polyunsaturated fatty acids against the potential negative effects of oxidation by reducing the polyunsaturated fat content. But partial hydrogenation produces *trans* fatty acids which adversely affect serum cholesterol levels in humans, as has been consistently demonstrated in multiple research studies conducted in last 10 years

(Mazaffarian et al., 2006; Meister, 2006). *Trans* fatty acids also have been linked to heart disease (Mozaffarian et al., 2006; Katan et al., 1995; Nestel et al., 1994). Both the American Heart Association and the World Health Organization have recommended limiting intake of *trans* fatty acids. In July 2003, FDA issued a regulation requiring manufacturers to list *trans* fatty acids on the Nutrition Facts panel of foods and some dietary supplements. Food manufacturers had until January 1, 2006, to list the amount of *trans* fat in their products (FDA, 2003). With this rule, consumers have more information to make healthier food choices that could lower their consumption of *trans* fat as part of a heart-healthy diet.

The intentional increase in oleic acid content in Pioneer 305423 soybean is aimed at improving stability of the vegetable oils. The commercialization of Pioneer 305423 soybean could be beneficial for the consumers. From a nutritional standpoint, the consumption of *trans* fatty acids results in considerable potential harm, but no apparent known benefit to human health (Modaffarian et al., 2006). Increased intake of oils high in monounsaturated fatty acids, such as oleic acid have been shown to have positive effects on total cholesterol levels when compared to equal intakes of hydrogenated oils (Lichtenstein et al. 2006). Likewise, increased intake of oils high in oleic acid can decrease LDL-cholesterol levels compared to equal intakes of saturated oils (Mensink et al., 1989) and increased HDL-cholesterol levels compared to an equal intake of polyunsaturated oil (Mata et al., 1992). Moderate consumption of oil high in oleic acid has also demonstrated decreases in systolic blood pressure (Bondia-Pons et al., 2007).

Description of the modification

Pioneer 305423 soybean has been genetically engineered to contain two transgene fragments:

(1) PHP 19340A fragment contains three genetic elements: KTi3 promoter, *Gm-fad2-1* gene and Kti3 terminator.

- KTi3 promoter is the promoter from Kunitz trypsin inhibitor 3 of soybean (,Jofuku, 1989). This is a seed specific promoter that allows high level gene expression during seed development.

- *Gm-fad2-1* gene fragment codes for the middle region of the 597 base pair (bp) fragment of soybean microsomal omega-6 desaturase gene 1, which is responsible for the synthesis of the polyunsaturated fatty acids found in the oil fraction. The presence of the inserted copy of the *Gm-fad-2-1* gene causes “gene silencing” which results in both copies of *fad2-1* gene (the inserted copy as well as the original endogenous soybean copy) being switched off, thus preventing linoleic acid from being synthesized and leading to the accumulation of oleic acid in the soybean seed.

- KTi3 terminator is the 3' terminator region from Kunitz trypsin inhibitor 3 of soybean (Jofuku, 1989). This terminator contains signals for termination of transcription and directs polyadenylation.

(2) PHP 17752A fragment contains four genetic elements: S-adenosyl-L-methionine synthetase (SAMS) promoter region, *Gm-hra* gene, *als* terminator and *Flp* recombinase recombination sites.

- SAMS promoter region from soybean that consists of a constitutive SAMS promoter and an intron that interrupts the SAMS 5' untranslated region.

- *Gm-hra* gene encoding the GM-HRA protein was generated by site-specific mutagenesis of the endogenous herbicide sensitive soybean acetolactate synthase *als1* gene.

- *als* terminator is the endogenous soybean acetolactate synthase terminator. (Falco, 2003)

- Flp recombinase target sequences (FRT1 and FRT6) – there are two FRT1 and one FRT6 sites. In the development of the Pioneer 305423 soybean, these were not used. A specific Flp recombinase enzyme was needed in order to make these sites function.

Plant Pest Risk Assessment

This plant pest risk assessment is to determine whether Pioneer 305423 soybean is unlikely to pose a plant pest risk. If APHIS determines that a GE organism is unlikely to pose a plant pest risk, APHIS then has no regulatory authority over that organism under 7CFR part 340.

APHIS administers the regulations 7 CFR part 340 under the authority of the Plant Protection Act of 2000 (PPA). The PPA states that:

> "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.

Assessment of Plant Pest Risk for Pioneer 305423 Soybean

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS) has prepared a Plant Pest Risk Assessment in response to a petition (APHIS Number 06-354-01p) from Pioneer. APHIS regulation 7 CFR 340.6(c) stipulates the information needed for consideration in a petition for nonregulated status. APHIS will evaluate information submitted by the applicant related to plant pest risk characteristics, disease and pest susceptibilities, expression of the gene product, new enzymes, or changes to plant metabolism, weediness of the regulated article, any impacts on the weediness of any other plant with which it can interbreed for Pioneer 305423 soybean. Issues related to agricultural or cultivation practices and the effects of the regulated article on nontarget organisms will be considered in the Environmental Assessment for Pioneer 305423 soybean.

Based on information on the biology of soybean (OECD, 2000), data presented by Pioneer (APHIS Number 06-354-01p) and scientific data relevant to a discussion of plant pest risk, APHIS concluded the following regarding Pioneer 305423 soybean:

Potential impacts of altered disease and pest susceptibilities

USDA-APHIS assessed whether Pioneer 305423 soybean is likely to have significantly increased disease and pest susceptibility. The assessment encompasses a thorough consideration of introduced traits and interactions with pest and disease.

Soybean (*Glycine max*) is not a plant pest in United States (USDA-APHIS, 2000), and the introduced DNA in Pioneer 305423 soybean is unlikely to pose a plant pest risk because there are no pathogenic DNA sequences present. The description of the genetic modifications, including genetic elements, expression of the gene product and their functions for Pioneer 305423 soybean has been summarized above. The data submitted by Pioneer indicated no significant differences between Pioneer 305423 soybean and the non-transgenic counterparts for disease (as measured by *Alternaria*, bacterial blight, bacterial pustule, brown spot, Downey mildew, frogeye leaf spot and powdery mildew) and pest susceptibility (as measured by damages cause by bean leaf beetle, grasshopper, corn leaf aphid, rose beetle, soybean aphid, soybean leaf miner, stink bug and whitefly) (Pavely, 2007, Table 11 and 12 of petition).

The introduced gene (*Gm-fad2-1* gene) that impacts lipid biosynthesis comes from soybean and only increases the amount of oleic acid in the seed, a constituent which is present in the parent cultivar. Such genes have been used in field trials previously and are not known to cause plant disease. There is no indication that inserting the *Gm-fad2-1* gene will result in increased likelihood of introduction or dissemination of a plant pest. Oleic acid is the primary ingredient of olive oil. The higher concentration of oleic acid is not known to pose an environmental hazard in unconfined releases. APHIS has not identified any plant pest risk following the introduction of the high oleic acid trait in the deregulated DuPont product (USDA-APHIS, 1997). The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA, 2000) also concluded that this intended effect of this novel trait (high oleic acid) is not related to altered plant pest potential.

The gene (*Gm-hra)* that is used as the selectable marker is minimally modified from the endogenous soybean *als* gene and results in production of a slightly modified acetolactate synthase enzyme (ALS). ALS enzymes are widely distributed in nature. ALS genes have been isolated from bacteria, fungi, algae and plants (Friden et al., 1985; Falco et al., 1985; Reith et al., 1995; Mazur et al., 1987). Several commercialized non-GE crops (Clearfield®) with a similar herbicide tolerant *als* gene are available. A plant pest risk assessment of the GM-HRA protein has been evaluated by APHIS, USDA as part of the Petition of Pioneer 356043 herbicide tolerant soybean (USDA-APHIS, 2006a).

Since the transcription of the *gm-fad2-1* gene in Pioneer 305423 soybean is driven by the promoter from soybean Kunitz trypsin inhibitor gene 3 (KTi3) (Jofuku, 1989), the insertion of the promoter fragment effectively silenced its endogenous KTi3 gene expression (Pavely, 2007). It has been noted in the literature that these classes of inhibitors slow the growth of insect pests and fungi which attack many of our important food crops (Ryan, 1990). Johnson et al (1989) studies had firmly established that the expression of different families of trypsin protease inhibitor genes in different host plants can provide resistance against insect pests. This protective effect is generally attributed to the ability of these protease inhibitors to inhibit the digestive enzymes present in the gut of these insects. Several studies have also shown that protease inhibitors are associated with resistance against plant pathogens. Peng et al. (1976) found that levels of trypsin protease inhibitor increased more in leaves in varieties of tomato that were resistant to *Phytophthora infestans* than in susceptible varieties. In some cases, trypsin protease inhibitors have pesticidal properties toward target pests such as bean weevil, corn borer, tomato fruit worm, beet army worm, *Fusarium*, cricket, codling moth (EPA SAP Report, 2000). Expression analysis of the endogenous KTi3 gene supports the observation from the compositional assessment of Pioneer 305423 soybean (Pavely, 2007, page 88) showing that the amount of trypsin protease inhibitor was significantly lower in Pioneer 305423 soybean as compared to the control lines. The reported trypsin inhibitor concentration levels for Pioneer 305423 soybean is still well within the ranges of trypsin inhibitor found in published literature for soybean (OECD, 2001; ILSI, 2004). The reduced trypsin protease inhibitor levels could potentially make the Pioneer 205435 soybean more susceptible to insect damage or fungal disease than the non-GE control lines. However, Pioneer conducted field trials to evaluate the severity of insect and disease stress at total of 13 field trial locations in soybean growing regions of the United States and Canada in 2005 and 2006. Table 11, Table 12, and Appendix 4 (Pavely, 2007, page 74, 75 and 158-160) identified that there were no statistically significant differences between Pioneer 305423 soybean and various other control lines for any of the disease incidence and insect damage. Based on this analysis, it is unlikely that the inserted KTi3 promoter would cause Pioneer 305423 soybean to pose a plant pest risk.

Potential impacts from new gene products, changes to plant metabolism or composition

There is no new protein expressed with the insertion of the *Gm-fad2-1* gene fragment. The only novel protein expressed in Pioneer 305423 soybean is GM-HRA, as the selectable marker. The GM-HRA protein is minimally modified compared to the

endogenous soybean ALS (acetolactate synthase). The expression of the GM-HRA protein confers tolerance to acetolactate synthase inhibiting herbicides. The scientific basis for the ALS tolerance trait in GM flax (USDA-APHIS, 1998; FDA, 1998), cotton (APHIS-APHIS, 1995; FDA, 1996a), and soybean (USDA-APHIS, 2006a; FDA, 2007) is very similar. A nearly identical ALS protein is found in a number of non-GE Clearfield® and STS® varieties that have been produced by chemical-induced mutagenesis. Those non-GE products have been grown widely across the US since 1980's and have a history of safe use. Although ALS proteins are ubiquitous in nature, there is no evidence of unexpected plant pest risk from these proteins.

Southern blots were used to determine the copy number of each of the genetic elements and to examine the integrity of each fragment inserted into the soybean genome. APHIS review of Pioneer's submission indicates that there is one intact and seven truncated copies of the PHP 19340A fragment (Pavely, 2007, Appendix 2; p. 134-148) and a single intact copy of the PHP 17752A fragment inserted into Pioneer 305423 soybean (Pavely, 2007, Appendix 2; p.127-129). All of the fragments were stably inserted into the soybean genome. Some studies (El-Shemy et al., 2004) indicate that multiple copies of a transgene are needed for transgene silencing and the associated co-suppression of homologous endogenous genes. Pioneer indicated that multiple copies of the *gm-fad2-1* fragment appear to be necessary for effective co-suppression of the endogenous gene.

A 495 base pair fragment of the plasmid backbone DNA (derived either from plasmid PHP19340 or plasmid PHP17752 (Pavely, 2007, pp. 25-31) was also present (Pavely, 2007, Appendix2; p 130-133). Southern blot analysis and sequence data confirm the absence of all functional elements (i.e. the hygromycin resistance gene and the plasmid origin of replication) from the plasmid backbone. Flanking sequence analyses indicate that there is no evidence of a fusion protein. There is no new protein coded and no safety concerns can be identified.

The compositional assessment data supplied in the Petition and reviewed by APHIS support the conclusion that Pioneer 305423 soybean contains introduced transgene *Gm-fad2-1* fragments and an intact *Gm-hra* gene. These data support the conclusion that Pioneer 305423 soybean produces soybean seeds with increased levels of monounsaturated (oleic) fatty acids and decreased levels of polyunsaturated fatty acids (linoleic and linolenic), and to a lesser extent, palmitic acid.

Compositional and nutritional data was collected on Pioneer305423 soybean and comparisons were made to a conventional control line and a set of reference soybean varieties. Results of these comparisons indicate that Pioneer 305423 soybean is compositionally and nutritionally equivalent to conventional soybean varieties currently in commerce.

Based on all the noted considerations, APHIS concludes that Pioneer 305423 soybean poses no more of a plant pest risk from new gene products, changes to plant metabolism or composition than conventional soybean.

Assessment of Plant Pest Risk for Pioneer 305423 Soybean

Potential impacts from outcrossing of the Pioneer 305423 soybean to wild relatives

Soybean is a self-pollinated species propagated by seed (OECD, 2000). In its papilionaceous flower, the anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self fertilization. Natural or artificial cross-pollination can only take place during the short time when the pollen is viable. The cross pollination rate (with and without pollinators) is less than 1.5% beyond one meter from the pollen source (Garber, et al., 1926; Carviness, 1966; Ahrent et al., 1994). At greater distances from the pollen source, cross pollination rates decrease rapidly. Based upon these factors, it is highly unlikely for Pioneer 305423 soybean to naturally outcross or hybridize with other soybean varieties in agricultural settings.

In assessing the risk of gene introgression from Pioneer 305423 soybeans into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression and, 2) the potential impact of introgression.

Cross pollination with wild species
The genus *Glycine* is divided into two subgenera, *glycine* and *soja*.

The subgenus ***soja*** consists of three annual species: *G. soja* Sieb. and Zucc., the wild form of soybean, *G. gracilis* Skvortz., the weedy form of soybean and *G. max,* which is the cultivated soybean. They grow wild or semi-wild in Asia. Fertile hybrids between *G. max* and *G. soja* (Broich, 1978), and between *G. max* and *G. gracilis* (Karasawa, 1952) occur. *G. soja* and *G. gracilis* grow naturally only in Asia and Australia, not in the United States (Skvortzow, 1972).

The subgenus ***glycine*** consists of twelve wild perennial species. These species grow wild in Australia, South pacific Islands and Asia (Newell et al., 1978), and do not exist naturally in the U.S. Hybrids between perennial *Glycine* species are fertile.

G. max is the only *Glycine* species located in the United States, thus there are no other plant species with which *G. max* can interbreed. *G. max* has never been found in the wild (Hymowitz and Singh, 1987) without human intervention. Therefore, it is highly unlikely that soybean plants in the United States will be found outside of an agricultural setting. It is also highly unlikely that gene flow and introgression will occur between Pioneer 305423 soybean and soybean plants in a natural environment. USDA has determined that any adverse consequences of gene flow from Pioneer 305423 soybean to wild or weedy species in the United States are highly unlikely.

Potential impacts based on the relative weediness of Pioneer 305423 soybean.

APHIS assessed whether Pioneer 305423 soybean is any more likely to become a weed than the nontransgenic recipient soybean line, or other soybean currently cultivated. The

assessment encompasses a thorough consideration of the basic biology of soybean and an evaluation of unique characteristics of Pioneer 305423 soybean.

In the U.S., soybean is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980) nor is it listed as a noxious weed species by the U.S. Federal Government (USDA-APHIS, 2006b). Soybeans are not frost tolerant, do not survive freezing winter conditions (OECD, 2000), and do not reproduce vegetatively. After crop harvest, soybean may germinate as a volunteer weed in the succeeding crop due to lack of dormancy (Padgette et al., 1996). If any seeds remain viable and germinate the following year, mechanical methods or herbicides can be used to control volunteers. Soybean is one of the largest acreage crops in the world. Soybean was never reported as a serious weed. In the U.S., soybean is grown on over 70 millions acres (USDA-NASS, 2008). Based on the familiarity of soybean as the parent plant, there has been no report of soybean escaping cultivation and becoming established as a weed in United States (Holm et al., 1979).

Weediness for the purposes of this part of the plant pest risk assessment is an attribute, which causes a crop to act as a weed due to the addition of genes, in comparison to the non-GE comparator. If the fitness of Pioneer 305423 soybean improves in natural or agricultural ecosystems due to the inserted DNA, the potential for weediness could increase. The following analysis of the inserted DNA is intended to document that Pioneer 305423 soybean has a negligible likelihood of increased weediness.

Pioneer 305423 soybean differs from conventional soybeans only in the expression of the ALS protein (acetolactate synthase) and silencing of the endogenous *fad2-1* gene, that encodes omega-6-desaturase. The insertion of the *fad2-1* gene fragment which is driven by the promoter for the KTi3 results in decreased conversion of oleic acid to linoleic acid. Scientific literature (Kodama et al., 1994 and Kodama et al.,1995) has reported that the increases in levels of trienoic fatty acids such as hexadecatrienoic acid and linolenic acid can enhance cold tolerance in model plants such as *Arabidopsis* and tobacco. In Pioneer 305423 soybean, the levels of linolenic acid are significantly decreased. Therefore, Pioneer 305423 soybean will not be expected to have enhanced cold tolerance. If the seed overwintering capacity improved, the potential for a successful weed could increase. There is no indication that Pioneer 305423 soybean possesses a selective advantage that would result in increased weediness potential. Therefore, the chances for Pioneer 305423 soybean to behave as a weed are negligible.

The ALS protein is a modified soybean acetolactate synthase with two site-specific point mutations known to confer tolerance to sulfonylurea herbicides. Pioneer indicated that the *als* gene is only used as selectable marker, and would not confer commercial levels of herbicide tolerance. Pioneer does not plan to promote 305423 soybean commercially as a sulfonylurea tolerant variety. Therefore normal agronomic practices and weed control measures for soybean can be used.

Assessment of Plant Pest Risk for Pioneer 305423 Soybean

Potential impacts from transferring genetic information from Pioneer 305423 soybean to organisms with which it cannot interbreed.

Horizontal gene transfer and expression of DNA from a plant species to bacteria 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* (Kaneko et al., 2000; Wood et al., 2001; Kaneko et al., 2002). There is no evidence that these organisms contain genes derived from plants. 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 (Koonin et al., 2001; Brown, 2003). 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, the FDA has evaluated horizontal gene transfer from the use of antibiotic resistance marker genes, and concluded that the likelihood of transfer of antibiotic resistance genes from plant genomes to microorganisms in the gastrointestinal tract of humans or animals, or in the environment, is remote (http://vm.cfsan.fda.gov/~dms/opa-armg.html). Therefore APHIS concludes that horizontal gene transfer is unlikely to occur and thus poses no significant environmental or plant pest risk.

Conclusion

APHIS has reviewed and conducted a plant pest risk assessment on Pioneer 305423 soybean. Due to the lack of plant pest risk from the inserted genetic material, the lack of weediness characteristics of Pioneer 305423 soybean, the lack of atypical responses to disease or plant pests in the field, the lack of deleterious effects on non-targets or beneficial organisms in the agro-ecosystem, and the lack of horizontal gene transfer, APHIS concludes that Pioneer 305423 soybean is unlikely to pose a plant pest risk.

Reference:

Ahrent, D.K., Caviness, C.E. (1994) Natural cross-pollination of twelve soybean cultivars in Arkansas. Crop Sci. 34:376-378.

ANZFA (Australia New Zealand food Authority) (2001) Draft risk analysis report for application A387 – food derived from high oleic acid soybean lines G-94-1, G94-19 and G168. http://www.foodstandards.gov.au/_srcfiles/A387_Far.pdf. Accessed 11/09/2008

Bondia-Pons, I., chroder, H., Covas, M., Castellote, A.I., Kaikkonen, J., Poulsen, H.E., gaddi, A.V., Machowetz, A., Kiesewetter, H., Lopez-Sabater, C. (2007) Moderate consumption of olive oil by healthy European men reduces systolic blood pressure in non-Mediterranean participants. The Journal of Nutrition (137):84-87.

Broich, S.L. (1978) The systematic relationships within the genus *Glycine* Willd. Subgenus *soja* (Moench) F.J. Hermann. M.S. thesis. Iowa State University, Ames.

Brown, J. R. (2003) Ancient horizontal gene transfer. Genetics. 4:121-132.

CFIA (Canadian Food Inspection Agency) (2000) Decision Document 2001-34: Determination of the Safety of Optimum Quality Grains L.L.C.'s High Oleic Soybean (*Glycine max*) lines G94-1, G94-19, and G-168 Derived from Transformation Event Line 260-05 http://www.inspection.gc.ca/english/plaveg/bio/dd/dd0134e.shtml#a13. Accessed on 11/03/2008

Crockett, L. (1977) Wildly Successful Plants: North American Weeds. University of Hawaii Press, Honolulu, Hawaii. 609 pp.

El-Shemy, H.A., Teraishi, M., Khalafalla, M.M., Katsube-Tanaka, T., Utsumi, S., Ishimoto, M. (2004) Isolation of soybean plants with stable transgene expression by visual selection based on green fluorescent protein. Molecular Breeding 14:227-238

EPA SAP Report (2000) FIFRA Scientific Advisory Panel Meeting, Sets of scientific issues being considered by the Environmental Protection Agency regarding: Mammalian Toxicity Assessment Guidelines for protein plant pesticides. SAP Report No. 2000-03B

Falco, C.S. and Li, Z. (2003) S-adenosyl-L-methionine synthetase promoter and its use in expression of transgenic genes in plants. United States Patent Application No. 2003/0226166.

Falco, S.C., Dumas, K.D. (1985). Genetic analysis of mutants of *Saccharomyces cerevisiae* resistant to the herbicide sulfometuron methyl. Genetics 109:21-35.

FDA (1996a) Biotechnology Consultation Note to the file on sulfonylurea tolerant cotton line 19-51a (BNF No. 000030). http://www.cfsan.fda.gov/~rdb/bnfm030.html. Accessed 11/11/2008

FDA (1996b) Biotechnology Consultation Note to the file on High Oleic Acid transgenic soybean (BNF No. 000039). http://www.cfsan.fda.gov/~rdb/bnfm039.html. Accessed 11/11/2008

FDA (1998) Biotechnology Consultation Note to the file on sulfonylurea tolerant linseed flax (BNF No. 000050). http://www.cfsan.fda.gov/~rdb/bnfm050.html. Accessed 11/11/2008

FDA (2003) Food Labeling: *Trans* Fatty Acids in Nutrition Labeling. 21 CFR Part 101. Government Publishing Office. Accessed 11/10/2008

FDA (2007) Biotechnology Consultation Note to the file on herbicide-tolerant soybean event 356043 (BNF No. 000108). http://www.cfsan.fda.gov/~rdb/bnfm108.html. Accessed 11/11/2008

Friden, P., Donegan, J., Mullen, J., Freundlich, M., Eoyang, L., Weber R., Siverman, P.M. (1985). The *ilv*B locus of *Escherichia coli* K-12 is an operon encoding both subunits of acetohydroxyacid synthase I. Nucleic Acids Research 13:3979-3993.

Heppard, E.P., Kineey, A.J., Stecca, K.L., Miao, G.-H. (1996) Developmental and growth temperature regulation of two different microsomal omeg-6 desaturase genes in soybeans. Plant Physiology 110:311-319

Holm, L., Pancho, J. V., Herbarger, J. P., and Plucknett, D. L. (1979) A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Hymowitz, T., Singh, R.J. (1987) Taxonomy and Speciation in Soybean: Improvement, Production, and Uses-Second Edition. American Society of Agronomy.

ILSI (International Life Science Institute) (2004) ILSI Crop Composition Database Version 3.0. www.cropcomposition.org/ Accessed on 11/10/2008

Jofuku, K.D. and Goldberg, R.B. (1989) Kunitz trypsin inhibitor genes are differentially expressed during the soybean life cycle and in transformed tobacco plants. Plant Cell 1:1079-1093.

Johnson, R., Narvaez, J., AN, G., Ryan, C.A. (1989) Expression of proteinase inhibitors I and II in transgenic tobacco plants: Effects on natural defense against *Manduca sexta* larvae. Proc. Natl. Acad. Sci. USA 86:9871-75

Kaneko, T., Y. Nakamura, S. Sato, E. Asamizu, T. Kato, and S. Sasamoto. (2000) Complete genome structure of the nitrogen-fixing xymbiotic bacterium Mesorhizobium loti. DNA Research. 7:331-338.

Karasawa, K. (1952) Crossing Experiments with *Glycine soja* and *G. gracilis.* Genetica 26:357-358.

Kodama, H., Hamada, T., Horiguchi, G., Nishimura, M., and Iba, K. (1994) Genetic enhancement of cold tolerance by expression of a gene for chloroplast ω-3 fatty acid desaturase in transgenic tobacco. Plant Physiology 105:601-605.

Kodama, H., Horiguchi, G., Nishiuchi, M., Nishimura, M., and Iba, K. (1995) Fatty acid desaturation during chilling acclimation is one of the factors involved in

conferring low temperature tolerance to young tobacco leaves. Plant Pathology 107:1177-1185.

Lichtenstein, A., Mathan, N.R., Jalbert, S.M., Resteghini, N.A., Schaefer, E.j., Ausman, L.M. (2006) Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. Am. J. Clin. Nutr. 84:497-504

Mata, P., Alvarez-Sala, L.A., Rubio, M.J., Oya, M.D. (1992) effects of long-term monounsaturated- vs polyunsaturated-enriched diets on lipoproteins in healthy men and women. Am J clin Nutr 55:846-850

Mazur, B., Chiu, C-F., Smith, J.e. (1987) Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. Plant Physiology 85:1110-1117.

Meister, K. (2006) Trans fatty acids and heart disease. The American Council on Science and Health. http://www.acsh.org/publications/pubID.1415/pub_detail.asp, accessed 3/23/2009

Mensink, R.P., Katan, M.B. (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high density lipoprotein cholesterol in healthy women and men. New England Journal of Medicine Vol. 321 No 7.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. (2006) Trans fatty acids and cardiovascular disease. *N Engl J Med.* 13;354(15):1601-13.

Muenscher, W. C. (1980) Weeds. Second Edition. Cornell University Press, New York and London. 586 pp.

Nestel, P., Clifton, P., Noakes, M. (1994) Effects of increasing dietary palmitoleic acid compared with palmitic and oleic acids on plasma lipids of hypercholesterolemic men. J. Lipid Res. 35:656-662

Newell, C.A., Hymowitz, T. (1978) A reappraisal of the subgenus. Am. J. Bot. 65(2):168-179

OECD (Organization of Economic Co-operatin and Development). (2000) Consensus document on the biology of *Glycine max* (soybean). OECD, ENV/JM/MONO(2000)15.

OECD (Organization of Economic Co-operatin and Development) (2001) Consensus Document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. Organisation for Economic Co-operation

and Development ENV/JM/MONO (2001)15, http://www/olis.oecd.org/olis/2001doc.nsf/LinkTo/env-jm-mono(2001)15.

Padgette, S.R., Re, D.B., Barry, G.F., Eichholtz, D.E., Delannay, X., Fuchs, R.L., Kishore, G.M., Fraley, R.T. (1996) New weed control opportunities: development of soybeans with a Roundup Ready gene. Pp53-84. In S.O. Duke (ed.). Herbicide Resistant Crops-Agricultural, Environmental, Economic, Regulatory, and Technical Aspects. CRC Press, Boca Raton, Florida.

Pavely, C. (2007) Pioneer Hi-Bred International Petitions 06-354-01p: Revised Petition for Determination of Nonregulated Status for transgenic High Oleic 305423 soybean. http://www.aphis.usda.gov/brs/not_reg.html. Accessed 11/10/2008

Peng, J. H., black, L.L. (1976) Increased proteinase inhibitor activity in response to infection of resistant tomato plants by *Phytophthora infestans*. Phytopathology 66:958-63.

Reith, M., Munholland, J. (1995) Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome. Plant Molecular Biology Reporter 7:333-335.

Ryan, C.A. (1990) Protease inhibitors in plants: Genes for improving defenses against insects and pathogens. Annu. Rev. Phytopathology 28:425-49

Skvortzow, B.V. (1972). The soybean-wild and cultivated in Eastern Asia. Proc Manchurian Res. Soc Publ Ser A. Natural History Sect. No. 22:1-8

USDA-APHIS (1995) DuPont Petitions 95-256-01p for determination of Nonregulated Status for transgenic sulfonylurea tolerant cotton line 19-51A: Environmental Assessment. http://www.aphis.usda.gov/brs/not_reg.html. Accessed 11/11/08

USDA-APHIS (1997) DuPont Petition 97-008-01p for determination of nonregulated status for transgenic High Oleic Acid soybean sublines G94-1, G94-19, and G-168: Environmental Assessment and Finding of No Significant Impact. http://www.aphis.usda.gov/brs/aphisdocs2/97_00801p_com.pdf. Accessed 11/07/2008

USDA-APHIS (1998) University of Saskatchewan Petitions 98-335-01p for determination of Nonregulated Status for transgenic Sulfonylurea tolerant flax: Environmental Assessment. http://www.aphis.usda.gov/brs/not_reg.html. Accessed 11/11/2007

USDA-APHIS (2000) U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Regulated Plant Pest List. http://www.invasivespecies.org/RegulatedPestList.pdf. Accessed 10/14/2008

USDA-APHIS (2006a) Pioneer Hi-Bred International Petitions 06-271-01p for determination of Nonregulated Status for transgenic herbicide tolerant 356043 soybean: Environmental Assessment. http://www.aphis.usda.gov/brs/not_reg.html. Accessed 11/10/2008

USDA-APHIS (2006b) Federal Noxious Weed List as of June 30, 2006 http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/index.shtml. Accessed on 11/11/2008

USDA-NASS (2008) Acreage (Publication No. Cr.Pr 2-5 (6-08)). http://usda.mannlib.cornell.edu/usda/current/acre/acre-06-30-2008.pdf. Accessed 12/17/2008

Wood, D. W., J. C. Setubal, R. Kaul, D. E. Monks, J. P. Kitajima, V. K. Okura, Y. Zhou, L. Chen, G. E. Wood, N. F. Almeida Jr., L. Woo, Y. Chen, I. T. Paulsen, J. A. Eisen, P. D. Karp, D. Bovee Sr., P. Chapman, J. Clendenning, G. Deatherage, W. Gillet, C. Grant, T. Kutyavin, R. Levy, M.-J. Li, E. McClelland, A. Palmieri, C. Raymond, G. Rouse, C. Saenphimmachak, Z. Wu, P. Romero, D. Gordon, S. Zhang, H. Yoo, Y. Tao, P. Biddle, M. Jung, W. Krespan, M. Perry, B. Gordon-Kamm, L. Liao, S. Kim, C. Hendrick, Z.-Y. Zhao, M. Dolan, f. Chumley, S. V. Tingey, J.-F. Tomb, M. P. Gordon, M. V. Olson, and E. W. Nester. (2001) The genome of the natural genetic engineer Agrobacterium tumefaciens C58. Science. 294:2317-2323.