Product Safety Assessment

SmartStax™

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Select a Topic:
Names
Product Overview
Manufacture of Product
Product Description
Product Uses
Exposure Potential
Health Information
Environmental Information
Regulatory Information
Additional Information
References

Names

- Bt Corn
- Bt Proteins
- Bacillus thuringiensis proteins
- Cry1A.105
- Cry1F
- Cry2Ab2
- Cry3Bb1
- Cry34Ab1
- Cry35Ab1
- TC1507
- MON 88017
- MON 89034
- DAS-59122-7
- SmartStax™
- Genuity™ SmartStax™ (see footnote)
- 5-enol-pyruvylshikimate-3-phosphate synthase (CP4 EPSPS)
- Phosphinothricin Acetyltransferase (PAT)

Product Overview

- SmartStax™ is a new transgenic corn technology developed through a research and development collaboration between Dow AgroSciences and Monsanto. It combines the benefits of both Dow AgroSciences’ Herculex® XTRA and Monsanto’s Genuity™ VT Triple PRO™ (formerly YieldGard® VT Triple Pro). SmartStax seed corn trait package is the first to provide multiple modes of action for above- and below-ground insect control traits to control root-, stalk-, and ear-feeding insects. Combining multiple modes of action reduces the likelihood of targeted insect populations developing resistance and allows corn growers to reduce the size of their non-Bt corn refuge from 20% to 5% in the Corn Belt and from 50% to 20% in cotton-growing areas. Reducing the size of the non-Bt corn refuge will enable growers to maximize yields in their SmartStax-planted acres.
- SmartStax expresses six insect-control proteins (Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, and the binary proteins Cry34Ab1 and Cry35Ab1), are all obtained from the soil bacterium Bacillus thuringiensis (Bt). The expression of the insect-control proteins provides three

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Created: January 16, 2010  The Dow Chemical Company  Page 1 of 10
modes of action for control of above-ground Lepidoptera pests and two modes of action for control of below-ground *Diabrotica* pests. For information on what insects are controlled by these traits, see Product Description.

- **SmartStax™** insect control provides season-long, whole plant protection from insect feeding damage, which allows corn plants to remain healthier throughout the growing season. Full-season protection against insect damage allows corn varieties to reach their full genetic and yield potential. For further details, see Product Uses.

- SmartStax also contains two herbicide tolerance traits—5-enol-pyruvylshikimate-3-phosphate synthase (CP4 EPSPS) obtained from the soil bacterium *Agrobacterium* sp., and phosphinothricin acetyltransferase (PAT), obtained from the soil bacterium *Streptomyces viridochromogenes*. Corn plants expressing these traits can be sprayed post emergence with glyphosate herbicides (e.g., Roundup® herbicide) and/or glufosinate-ammonium herbicides (e.g., IGNITE® herbicide), allowing for broad spectrum weed control without herbicide damage to the corn plant. Benefits to the farmer are convenient and effective weed control that ultimately enhances yield potential for the corn. For more information about these traits, see Product Description.

- On the basis of rigorous testing, regulatory agencies concluded that SmartStax corn hybrids are as safe as conventional, non-transgenic corn. Exposure to the proteins in SmartStax occurs primarily through ingestion. The *Bt* proteins and the herbicide tolerance enzymes are derived from common soil organisms. Extensive safety data were provided previously by Dow AgroSciences LLC and Monsanto Technology LLC to the U.S. Department of Agriculture (USDA), Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and the regulatory systems of other countries on the individual traits contained in SmartStax. The SmartStax product has been reviewed by the U.S. EPA and numerous importing countries. Margins of exposure previously determined for the proteins in the individual events (MON 89034, TC1507, MON 88017, and DAS-59122-7) were applicable for the risk assessment of these proteins combined in SmartStax. There is no indication of synergistic or antagonistic interactions among the components, and there has been no indication of unreasonable adverse effects to the environment. For further details, see Exposure Potential and Health Information.

** Manufacture of Product: Plant Transformation Process**

Plant biotechnology can be defined as a precise process in which scientific techniques (e.g., genetic modifications) are used to develop useful and beneficial plants with desirable “traits.” A trait refers to a characteristic that is associated with the plant, including agronomic qualities and resistance to insects, herbicides, and plant pathogens. The genes responsible for the desired traits are identified, and then the desired genes are transferred into plant cells by plant transformation. A trait may come from the plant itself (corn, in this case) or from a very specific gene of another organism, such as a bacterium.

DNA is the "genetic backbone" found in all microorganisms, plants, animals, and humans. Organisms that carry DNA introduced via genetic transformation are referred to as transgenic, and the introduced DNA is termed a transgene.

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SmartStax™ contains four independent corn transformation events; MON 89034 (Cry1A.105, Cry2Ab2), TC1507 (Cry1F, PAT), MON88017 (Cry3Bb1, CP4 EPSPS), and DAS-59122-7 (Cry34Ab1, Cry35Ab1, PAT) that are combined together through plant breeding so that all eight genes are present in the same plant.

The first step in creating the transformation events in SmartStax™ corn was the identification and isolation of DNA segments responsible for the expression of the desired insecticidal proteins (Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, and Cry35Ab1) and herbicide tolerance traits (PAT and CP4 EPSPS). The isolated DNA fragments were then chemically re-synthesized to create the final transgene, including plant-preferred DNA sequences that optimize expression of the proteins in the plant.

The transgenes were inserted into plant tissue through a process known as transformation. The four transformation events that comprise SmartStax were created through one of two means of transformation; Agrobacterium-mediated transformation or microparticle bombardment. Agrobacterium is a naturally occurring soil bacterium that can transfer and integrate selected DNA in a stable fashion into the corn plant. In Agrobacterium mediated transformation, immature corn embryos (or cells) are incubated on artificial media with special strains of Agrobacterium that transferred the desired DNA into the corn plant cells. In microparticle bombardment, the DNA is "shot" at high speed to embed the transgene DNA in the corn embryo cells. The embryos are then allowed to mature and express the inserted genes. Each unique DNA insertion or transformation is called an “event,” and many events were created and tested using a process called “event sorting” to identify the optimal protein expression.

SmartStax is produced by crossing each of the four individual transformation events, MON 89034, TC1507, MON 88017, and DAS-59122-7, using traditional plant breeding techniques to create plants that contain the Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, and CP4 EPSPS proteins.

Numerous tests and checks are in place to ensure the quality of the genetically modified seed and, ultimately, the generations of seed formed afterward. SmartStax corn was extensively tested for the stability of the traits through several growing cycles, for safety to animals, humans, and the environment, and for nutritional value.

Following this rigorous testing and approvals by regulatory agencies globally, the genetically modified material is sold to farmers to grow for human and animal consumption.

**Product Description**

The product that is purchased is seed corn with SmartStax™ traits. The protection against insects and herbicides is achieved by genetically modifying the corn to include eight genes that express proteins, making the corn plants resistant to the larval stages of multiple insect pests of corn and tolerant to certain herbicides. The expression of the proteins Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, CP4 EPSPS, and PAT by the genes cry1A.105, cry2Ab2, cry1F, cry3Bb1, cry34Ab1, cry35Ab1, cp4 epsps, and pat, respectively, allows the plants to produce the same proteins as those produced by the naturally occurring soil bacteria from which the genes...
were isolated. All of the \textit{Bt} proteins (the Cry proteins) are expressed during all growth stages of the corn plant, protecting SmartStax™ corn hybrids from insects from the time of plant emergence from the soil through ear formation, with more than one protein expressed for control of most of the insect pests. The herbicide-tolerant proteins (CP4 ESPS and PAT) expressed in SmartStax corn hybrids enable the plants to tolerate two non-selective herbicides, which provide farmers with more weed control options.

Following is background information about \textit{Bacillus thuringiensis}, and each protein and its effects.

\textbf{Bacillus thuringiensis (\textit{Bt})}—All of the insect-control proteins expressed in SmartStax corn hybrids were obtained from the common soil bacterium \textit{Bacillus thuringiensis}, often referred to as \textit{Bt}. \textit{Bt} naturally forms crystalline proteins (Cry proteins) that are toxic to some insects. Cry proteins are highly selective, with toxicity only against certain groups and/or species of insects (i.e. different Cry proteins are effective against different groups and/or species of insects).

\textit{Bt} was first discovered in 1901 by the Japanese biologist S. Ishiwatari as the source of disease that was killing large populations of silkworms. \textit{Bt} was first used as an insecticide in 1920, and spray formulations containing either \textit{Bt} bacteria or \textit{Bt} proteins have been used for more than 40 years for crop protection, including their use in organic farming operations. The development and use of EPA-approved \textit{Bt} insecticides as alternatives to synthetic chemical insecticides increased significantly during the 1980s.

Beginning in the 1980s, the genes responsible for making \textit{Bt} proteins were isolated and transferred into corn plants. \textit{Bt} proteins were commercially approved as plant-incorporated protectants in transgenic corn and cotton seed in the mid-1990s. Compared with conventional \textit{Bt} spray formulations, transgenic plants expressing \textit{Bt} proteins provide much more effective insect protection throughout the growing season. Several different \textit{Bt} genes have been used to genetically modify corn, cotton, and potatoes, as well as other transgenic crops currently in development.

\textit{Bt} proteins must be ingested to kill the insect. A susceptible immature insect (larva) eats the protein, which then binds to a specific receptor in the larva’s gut. Binding initiates a cascade of effects in the larva that ultimately leads to death.

Each type of \textit{Bt} protein expressed in a plant binds to a specific receptor in the insect’s gut. Different \textit{Bt} proteins targeting the same insect species will bind in different places in the insect’s gut. If a target insect population were to develop resistance to one \textit{Bt} protein, it is likely that the other \textit{Bt} protein(s) targeting the same insect species would remain effective.

\textbf{Cry1A.105, Cry2Ab2}—These two \textit{Bt} proteins, which are expressed in Genuity™ VT Triple PRO corn hybrids, protect the corn from lepidopteran insects that feed on above-ground portions of the plant—leaves, stalks, ears. The insects targeted by these proteins are the corn earworm (\textit{Helicoverpa zea}), European corn borer (\textit{Ostrinia nubilalis}), fall armyworm (\textit{Spodoptera frugiperda}), and southwestern corn borer (\textit{Diatraea grandiosella}). Corn borers are considered to be the second most important complex of insect pests in North America, second only to the corn rootworm complex. The fall armyworm causes injury to corn leaves (particularly on late-planted corn) and corn ears. The fall armyworm is the most important insect pest of corn in South America.
Cry1F—This Bt protein, which is expressed in corn hybrids with Herculex® I Insect Protection and Herculex XTRA Insect Protection, protects the corn from lepidopteran insects that feed on above-ground portions of the plant—leaves, stalks, ears—all season long. The insect targeted by these proteins are the black cutworm (Agrotis ipsilon), European corn borer, fall armyworm, lesser cornstalk borer (Elasmopalpus lignosellus), southern corn stalk borer (Diatraea crambidoides), southwestern corn borer, and western bean cutworm (Striacosta albicosta). The protein also provides suppression of corn earworm injury. The black cutworm is an occasionally destructive pest that feeds on corn seedlings shortly after emergence, severely reducing corn populations when infestations are significant. The western bean cutworm is an increasingly threatening insect pest whose range has expanded rapidly eastward in the United States from the Great Plains to New York (for the first time in 2009) within the past decade. It feeds on the ears, reducing yields because of its destruction of kernels and because damaged ears are open for infection ear rot organisms. The Cry1F protein provides excellent control of major lepidopteran pests from early in the season (black cutworm) to late in the season (ear-feeding insects such as fall armyworm and western bean cutworm).

Cry3Bb1—This Bt protein, which is expressed in YieldGard™ Plus, YieldGard Rootworm, YieldGard VT Triple, and Genuity™ VT Triple Pro corn hybrids, protects corn roots from the larvae of Mexican corn rootworm (Diabrotica virgifera zeae), northern corn rootworm (Diabrotica barberi), and western corn rootworm (Diabrotica virgifera virgifera). Collectively, corn rootworm species are considered to be the most destructive and important insect pests of corn in North America. Since 1992, the western corn rootworm also has become established in Europe. Over the past several years, the western corn rootworm has become established in much of eastern Europe (from its initial discovery near Belgrade), has spread through northern Italy, and has been found occasionally as far west as Germany, France, and the United Kingdom.

Cry34Ab1, Cry35Ab1—These binary proteins, which are expressed in Herculex RW and Herculex XTRA corn hybrids, protect corn roots from larvae of the Mexican, northern, and western corn rootworms. Injury caused by rootworm larvae decreases a corn plant’s ability to take up water and nutrients, which can result in significant yield loss, particularly when plants are under environmental stress. If damage to the root system is extensive, the plants “lodge,” or fall over, further compromising yield. It has been estimated that at least 30 million acres of corn in the United States (of 80 million acres grown) are infested regularly with corn rootworms. The number of acres infested is expected to expand over the next 20 years, especially if the variant western corn rootworm (a variant of the species that lays its eggs in soybeans) expands its range further east and west from Illinois, Indiana, southern Michigan, western Ohio, and southern Wisconsin. The United States Department of Agriculture estimates that corn rootworms causes more than $1 billion in lost revenue each year, which includes $800 million in yield loss and $200 million in cost of treatment for corn growers.

CP4 EPSPS—5-Enol-pyruvylshikimate-3-phosphate synthase (CP4 EPSPS) is the protein expressed in Roundup® Ready® Corn 2 hybrids, imparting tolerance to glyphosate-based herbicides. Glyphosate-based herbicides, such as Durango® and Roundup®, are non-selective, providing control of a broad spectrum of weeds. Glyphosate-based herbicides kill non-tolerant plants (e.g., weeds) by inhibiting the enzyme EPSPS. EPSPS interferes with the development of aromatic amino acids in plants, resulting in reduced plant growth. SmartStax corn can be sprayed with glyphosate-based herbicides to control weeds with no resulting crop loss.
**Product Safety Assessment: SmartStax™**

**PAT**—Phosphinothricin acetyltransferase (PAT) is the protein expressed in Herculex® corn hybrids with LibertyLink® technology, making them tolerant to over-the-top applications of glufosinate herbicides, such as IGNITE®. PAT is an enzyme isolated from the common soil bacterium *Streptomyces viridochromogenes*. SmartStax™ corn can be sprayed with glufosinate-based herbicides to control weeds with no resulting crop loss.

Glufosinate-ammonium, developed from the same bacterium as PAT, lowers glutamine levels of non-tolerant corn plants. The reduction of glutamine results in increased concentrations of ammonia, disrupting the cell membrane, halting photosynthesis, and resulting in necrosis. Eventually the plant, preferably a weed, dies. PAT is essentially the antidote to the herbicide, allowing the plant to detoxify the active ingredient in this particular herbicide.

**Product Uses**

SmartStax™ corn hybrids are used to produce high-quality corn with greater efficiency for higher yields and less loss due to feeding damage caused by multiple above- and below-ground insect pests. Growing SmartStax corn hybrids reduces the need for insecticide applications.

**Exposure Potential**

The Bt and herbicide tolerance proteins are derived from common, non-pathogenic soil bacteria, so environmental exposure is natural and without concern. The potential for external exposure to genetically modified corn seed and plants is greatest for farmers who plant the seed, grow the plants, and harvest the crop. Because harvested corn with SmartStax traits is as safe as corn without them, standard farm workplace procedures and precautions should be followed. See Health Information.

Internal exposure to the proteins expressed in SmartStax occurs mainly through ingestion of corn-derived foods. Consumers and animals are already consuming processed corn products that contain the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, and CP4 EPSPS proteins. None of the proteins is associated with toxicity or allergenicity and numerous studies have demonstrated the safety of these proteins for human and animal consumption. See Health Information.

**Health Information**

On the basis of rigorous testing, regulatory agencies concluded that corn with SmartStax™ traits is as safe as non-transgenic corn. The SmartStax traits have full food, feed, and environmental approval by the United States and Canada. SmartStax also has received regulatory approvals for import into Japan and Mexico. Approvals are currently pending in other countries that regulate combined event traits like SmartStax.

SmartStax™ does not pose risks to human health. All of the proteins expressed in SmartStax are derived from naturally-occurring soil bacteria and are not considered as pathogens for humans or

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Created: January 16, 2010   The Dow Chemical Company   Page 6 of 10
animals. Moreover, none of the proteins expressed in SmartStax™ has biochemical characteristics or homology (relevant similarities) with known food allergens or toxins, indicating that SmartStax is highly unlikely to pose any risk of toxic or allergic reaction.

Studies conducted on the nutrient composition profiles of grain and whole plant forage confirmed that SmartStax corn hybrids and conventional non-transgenic corn hybrids are compositionally equivalent, including nutrients and anti-nutrients.

The four individual transformation events that comprise the SmartStax product have all previously passed regulatory safety assessments globally and are available commercially as individual traits. In combining the four individual events into SmartStax™, no further modifications were made to the DNA or transgenes. Studies demonstrated that there are no synergistic or antagonistic interactions among the events, and protein expression levels in SmartStax are similar to those in the individual events. Therefore the individual safety assessments of the proteins and traits in SmartStax also apply to the SmartStax product. Those assessments included investigating toxicity in mice; where healthy mice demonstrated lack of acute toxicity after ingesting a dose of protein many thousand times the estimated dietary intake of humans. Broiler chicken feeding studies were also conducted with grain harvested from each of the individual transformation event corn hybrids. No differences in nutritional quality or growth of the broilers were observed between those animals that consumed the transgenic corn and those that were fed conventional corn.

**Seed Treatments**

Agricultural seeds, including corn with SmartStax™ traits, may be treated with an insecticide and/or a fungicide. These seed treatments can present certain health risks, which are associated with the seed treatments and not the SmartStax traits.

For more information, farmers should consult the appropriate Safety Data Sheet and/or label or tag for seed treatment hazard information, and wear all recommended personal protective equipment.

**Environmental Information**

Before a biotechnology product can be introduced to the market, approval by appropriate governmental agencies is required. Using the criteria established by these agencies, Dow AgroSciences conducts extensive, validated tests for its biotechnology products. For the approval of corn with the SmartStax traits, extensive safety data were provided to the EPA as part of the registration application and to the regulatory agencies of other countries.

SmartStax corn produces minute quantities of the Cry1A.105, Cry1F, Cry2Ab2, Cry3Bb1, Cry34Ab1, Cry35Ab, CP4 EPSPS, and PAT proteins, which are contained in the plant and plant parts such as pollen, roots, and leaves. These proteins, collectively, comprise only 0.7 to 1.6% of the total proteins produced by corn plants. All of the transgenic proteins degrade rapidly in the soil, minimizing the potential for run-off or exposure to soil-dwelling non-target organisms.

Each of the events (MON 89034, TC1507, MON 88017, and DAS-59122-7) that comprise SmartStax has been previously tested extensively, as required by the EPA and USDA as
Herculex® and YieldGard™ products. A number of non-target organisms were tested as part of the registration process for these products, and no adverse effects were observed. Some of the organisms tested include honeybees, earthworms, lady beetles, springtails, green lacewings, and monarch butterfly caterpillars. Additionally, no adverse effects were identified for birds or fish, and the product does not pose a threat to endangered species.

The protein expression levels in SmartStax are comparable to the protein levels in the individual events that comprise it. Consequently, margins of exposure previously determined for the proteins in the individual events (MON 89034, TC1507, MON 88017, and DAS-59122-7) were applicable for the risk assessment of these proteins combined in SmartStax. There is no indication of synergistic effects, and no indication of adverse effects to the environment.

Another important environmental consideration with a biotechnology product is how cross-pollination will affect the environment. Gene exchange between corn with SmartStax traits and other cultivated corn varieties can occur. The exchange will be similar to that which occurs naturally between cultivated corn varieties (both transgenic and non-transgenic) at the present time. In the U.S. and Canada (the current two countries that have approved SmartStax cultivation), there is no plant species closely related to corn in the wild, so gene flow to other species does not occur. There is no selective advantage for corn hybrids with SmartStax™ traits in the natural environment.

There is a potential long-term risk of target pest adaptation to the insect-control proteins in SmartStax, which could lead to the possibility of reduced efficacy. However, SmartStax hybrids produce multiple Bt proteins to control key pests (i.e., gene pyramiding). SmartStax can thereby control insects that may be resistant to one or other Bt protein. Consequently, the target insects are much less likely to develop resistance to the trait combination than to individual traits. The EPA has recognized this dramatically reduced likelihood for development of insect resistance by approving reduction of the non-Bt corn refuge from 20% to 5% in the Corn Belt, and from 50% to 20% in cotton-growing regions in the United States where the corn earworm/bollworm (Helicoverpa zea) is a major pest of both corn and cotton. An insect resistance management plan with a reduced refuge is in place with growers to mitigate the risk of target insect pests developing resistance to the insect-control proteins.

For more information, farmers should consult the appropriate Safety Data Sheet and/or label or tag for seed treatment hazard information, and wear all recommended personal protective equipment.

Regulatory Information

Regulations may exist that govern the manufacture, sale, transportation, use, and/or disposal of corn with SmartStax™ traits, especially if the seed is treated with an insecticide or fungicide. These regulations may vary by city, state, country, or geographic region. Please review the Product Use Guide for SmartStax. For more information, farmers should consult the appropriate Safety Data Sheet and/or label or tag for seed treatment hazard information, and wear all recommended personal protective equipment.

Back to top

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Additional Information

- Dow AgroSciences website (http://www.dowagro.com)
- Dow AgroSciences SmartStax™ Seed Brands website (www.dowagro.com/na/usa/en/traitstwd/seeds.htm)
- Agbios technical summary for SmartStax™ (http://agbios.com/dbase.php?action=Submit&evidcode=MON89034+x+TC1507+x+MON88017+x+DAS-59122-7)
- Biotechnology Industry Organization “Commercial Status of Certain Agricultural Biotechnology Products” (www.biotradestatus.com)

References

NOTICES:

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