Product Safety Assessment

Herculex® RW Rootworm Protection

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Names

- Bt Proteins
- \textit{Bacillus thuringiensis} proteins
- Cry34Ab1
- Cry35Ab1
- Herculex® RW Rootworm Protection
- DAS-59122-7 (event number)

Product Overview

- Herculex® RW Rootworm Protection is a new transgenic corn trait developed through a collaborative agreement between Dow AgroSciences and Pioneer Hi-Bred International. Herculex RW is the first insect protection trait to co-express unique binary proteins (Cry34Ab1 and Cry35Ab1) from a new strain of the natural soil microorganism \textit{Bacillus thuringiensis} (\textit{Bt}). For more information on \textit{Bt}, Cry34Ab1 and Cry35Ab1, see Product Description. Herculex RW provides high levels of protection against larval stages of major corn rootworm pests, including western corn rootworm (\textit{Diabrotica virgifera virgifera}), northern corn rootworm (\textit{Diabrotica barberi}), and Mexican corn rootworm (\textit{Diabrotica virgifera zeae}).
- Herculex RW provides season-long root protection from larval corn rootworm feeding damage, which allows corn plants to remain healthier and better fight off environmental stresses throughout the growing season. Full-season protection against corn rootworms and other stress factors allows corn hybrids to reach their full genetic growing potential. Thus, Herculex RW protects and enhances yield potential for the corn hybrid. Herculex RW can also increase farmer’s productivity by reducing various inputs (e.g., labor, fuel, equipment, pesticides) typically required for conventional corn rootworm control programs.
- In addition to corn rootworm protection, corn hybrids with the Herculex® RW trait contain LibertyLink® technology which provides tolerance to glufosinate-ammonium herbicides by the expression of a protein generally referred to as PAT. (For more information see Product Description.) Corn plants possessing this tolerance can be directly sprayed after emergence with glufosinate-ammonium herbicides (e.g., LIBERTY® 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.
- On the basis of rigorous testing, regulatory agencies concluded that corn with Herculex RW Rootworm Protection is as safe as conventional, non-transgenic corn. Exposure occurs primarily by ingestion. The Cry34Ab1, Cry35Ab1 and PAT proteins are present in common soil organisms, so exposure can also occur naturally but at much lower levels (see Exposure Potential and Health Information). Extensive safety data was provided by Dow AgroSciences LLC and Pioneer Hi-Bred International, Inc., to the U.S. Department of Agriculture (USDA), Environmental Protection Agency (EPA), Food and Drug Administration (FDA) and the regulatory systems of other countries before corn with Herculex RW Rootworm Protection was approved for sale (see Environmental Information 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 characteristics that are associated with the plant, including agronomic qualities and resistance to insects, herbicides, and disease. Plant biotechnology first identifies the genes responsible for the desired trait and then transfers them into plant cells by plant transformation. The trait may come from the plant itself (in this case corn) 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 modification are referred to as transgenic, and the introduced DNA is termed a transgene. The first step in creating Herculex® RW corn was the isolation and replication of DNA segments from *Bacillus thuringiensis* (strain PS149B1) that were responsible for the expression of the desired insecticidal proteins (Cry34Ab1 and Cry35Ab1). The isolated DNA fragments were then chemically re-synthesized to create the final transgene, including plant-preferred DNA that optimizes expression of the insecticidal protein in the plant.

As shown in Figure 1, the transgene for Herculex RW Rootworm Protection is comprised of a protein coding region (expressing Cry34Ab1 and Cry35Ab1), a preceding promoter element (PRO) for each cry gene and a trailing regulatory element (TERM) for each cry gene. The promoter element determines the strength at which a trait will be expressed in the plant. The promoter also determines in which plant tissues the trait will be expressed. The trailing regulatory element defines the length of the DNA to be expressed.

A “selectable marker gene” is normally included with a gene of interest during plant transformation. Selectable marker genes can be used in both the laboratory and the field to quickly determine if plants contain the desired genes and are expressing the desired proteins. For Herculex RW Rootworm Protection, the pat gene is used as the selectable marker. (For more information on PAT, see Product Description.) The pat gene, which is located next to the cry35Ab1 gene, is controlled by a different promoter and trailing regulator. The pat gene provides tolerance to glufosinate-ammonium herbicides and facilitates the selection of plants containing Cry34Ab1 and Cry35Ab1 in the laboratory and field.

Fig. 1: Transgene used to produce corn with the Herculex RW trait.

Once the transgenes were constructed, they were then inserted into plant tissue through a process known as transformation. Herculex RW Rootworm Protection was created through Agrobacterium-mediated transformation of corn. Agrobacterium is a naturally occurring soil bacterium that can transfer and integrate selected DNA in a stable fashion into the corn plant. Immature corn embryos (or cells) were incubated on artificial media with special strains of Agrobacterium that transferred the desired DNA into the corn plant cells. Then the embryos were allowed to mature and express the inserted genes. Each plant transformation experiment produced an “event” and many events were created and tested to identify the optimal protein expression. For Herculex RW Rootworm Protection, the final event chosen during the “event sorting” process is known by the event code name DAS-59122-7.

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

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Now that this process has proven successful on all counts, and has been approved by the USDA, FDA and EPA as well as other 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 Herculex® RW Rootworm Protection. The protection is achieved by genetically modifying the corn to include three genes which express proteins, making it resistant to larval corn rootworm pests and tolerant to certain herbicides. The expression of the new proteins (Cry34Ab1, Cry35Ab1 and PAT) by the new genes (cry34Ab1, cry35Ab1 and pat) allows the plants to produce the same proteins as those produced by the naturally occurring soil bacteria from which the genes were isolated. Following is background information about each protein and their effects.

**Cry34Ab1, Cry35Ab1 and Bt** – The cry34Ab1 and cry35Ab1 genes, isolated from the common soil bacterium Bacillus thuringiensis strain PS149B1 (often referred to as Bt), produces the insect control protein. Bt was first discovered in 1901 by the Japanese biologist S. Ishiwatari as the source of disease that was killing large populations of silkworms. Bt was first used as an insecticide in 1920, and spray formulations containing either Bt bacteria or Bt proteins have been used for more than 40 years for crop protection, including organic farming operations. EPA-approved Bt insecticides saw expanded use and development in the 1980s as an alternative to synthetic chemical insecticides.

Beginning in the 1980’s, the genes responsible for making Bt proteins were isolated and transferred into corn plants. Bt was commercially approved in transgenic corn seed in the mid-1990s. Compared to conventional Bt spray formulations, transgenic plants with the Bt protein provide much more effective insect protection throughout the growing season. Other Bt proteins besides Cry34Ab1 and Cry35Ab1 have been used to genetically modify potatoes, cotton, and other types of commercial corn.

Bt must be ingested to kill the insect. A susceptible immature insect (larva) eats the protein, which then binds to specific receptors in the larva's gut. Binding initiates a cascade of effects in the larva that ultimately leads to death.

Bt proteins are highly selective on only certain categories and species of insects. The Cry34Ab1 and Cry35Ab1 proteins affect the larvae of damaging corn rootworm (beetle) species including western corn rootworm (Diabrotica virgifera virgifera), northern corn rootworm (Diabrotica barberi), and Mexican corn rootworm (Diabrotica virgifera zeae).

Corn rootworm larvae can destroy significant percentages of corn if left untreated. In the United States, current estimates show that 30 million acres of corn (out of 80 million grown) are infested with corn rootworms, and the number of infested acres is expected to grow over the next 20 years. Corn rootworms can cause significant reductions in yield and revenue for growers. The United States Department of Agriculture estimates that corn rootworms cause $1 billion in lost revenue each year, which includes $800 million in yield loss and $200 million in cost of treatment for corn growers.

The Cry34Ab1 and Cry35Ab1 proteins in Herculex RW Rootworm Protection are expressed at efficacious concentrations throughout the growth cycle of the corn plant. The targeted corn rootworm larvae are exposed to efficacious levels of insecticidal protein in the corn root tissues at all stages in their life cycle. Thus, Herculex RW corn provides season-long protection against larval corn rootworms.

Cry34Ab1 and Cry35Ab1 have been found to not adversely affect beneficial insects or other organisms, including honeybees, earthworms, lady beetles, springtails, green lacewings, monarch butterfly caterpillars or endangered species. For more information, see Environmental Information.

**PAT** – Phosphinothricin-N-acetyltransferase (PAT) is an enzyme isolated from the common soil bacterium Streptomyces viridochromogenes. Used as the “marker gene”, PAT imparts transgenic plants with tolerance to glufosinate-ammonium, the active ingredient in herbicide products such as Liberty® herbicide. Corn with Herculex RW Rootworm Protection can be sprayed with this herbicide to control weeds with no crop loss.
Glufosinate-ammonium was developed from the same bacteria as PAT and causes ammonia to build up in the plant tissues. Excess ammonia disrupts cell membranes and stops photosynthesis. 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. When PAT is produced by the crop, in this case corn, the herbicide targets only the weeds without detriment to the crop.

**Product Uses**

Corn seed with Herculex® RW Rootworm Protection is used to produce high quality corn with greater efficiency for higher yields, and less loss due to feeding damage by larval corn rootworms. It reduces the need for pesticide applications.

**Exposure Potential**

The Cry34Ab1, Cry35Ab1 and PAT proteins are present in common, non-pathogenic soil bacteria, so exposure can occur naturally and without concern. Greatest external exposure potential to genetically modified corn seed and plants will involve farmers involved in planting the seed and growing the plants. Because harvested corn with Herculex RW Rootworm Protection is as safe as corn without it, standard farm workplace procedures and precautions should be followed. See Health Information.

Internal exposure to Herculex RW Rootworm Protection, however, occurs mainly through ingestion of corn-derived foods. Consumers and animals eat processed corn products that may contain the Cry34Ab1, Cry35Ab1 and PAT proteins. Several studies have demonstrated the safety of these proteins for human and animal consumption. Neither protein is associated with toxicity or allergenicity. See Health Information.

**Health Information**

On the basis of rigorous testing, regulatory agencies concluded that corn with Herculex RW Rootworm Protection is as safe as non-transgenic corn. The Herculex RW trait has received full food, feed and environmental approval by the United States and Canada. Herculex RW also has received regulatory approvals for import into Japan, Mexico, Taiwan, Korea, Australia and New Zealand. Approvals are currently pending in other countries such as those in the European Union, Russia and Switzerland.

The EPA found that Herculex RW does not pose risks to human health. All of the proteins expressed in Herculex® RW (Cry34Ab1, Cry35Ab1 and PAT) are present in soil bacteria and are not considered as pathogens for humans or animals. Moreover, none of the proteins expressed in Herculex RW have biochemical characteristics or homology (relevant similarities) with known food allergens or toxins, indicating that Herculex RW Rootworm Protection is highly unlikely to pose any risk of toxic or allergic reaction.

Healthy mice demonstrated lack of acute toxicity after ingesting a dose of Cry34Ab1, Cry35Ab1 or PAT protein many thousand times the estimated dietary intake of humans.

Studies conducted on the nutrient composition profiles of grain and whole plant forage confirmed that Herculex RW corn hybrids and corn hybrids not containing the trait are compositionally equivalent, including nutrients and antinutrients. A chicken broiler study has been conducted, using grain harvested from Herculex RW corn hybrids. No differences in nutritional quality or growth of the broilers were observed between those animals that consumed the Herculex RW corn and those that were fed conventional corn.

**Seed Treatments.** Agricultural seeds, including corn with the Herculex RW trait, may be treated with an insecticide and/or a fungicide and these seed treatments can present certain health risks. These risks are associated with the seed treatments and not the Herculex® RW trait. Consult the appropriate Safety Data Sheet (SDS) and/or label or tag for seed treatment hazard information, and wear all recommended personal protective equipment.

**Environmental Information**

Before a biotech 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 Herculex RW trait, extensive safety data was provided to the EPA as part of the registration application, to the USDA in a petition for

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deregulation, to the FDA as part of a pre-market consultation process and to the regulatory agencies of other countries.

The corn with the Herculex® RW trait produces minute quantities of the Cry34Ab1 and Cry35Ab1 proteins, contained in the plant and plant parts such as pollen, roots, and leaves. Both proteins degrade rapidly in the soil, minimizing the potential for run-off or exposure to soil-dwelling non-target organisms.

A number of non-target organisms were tested as part of the registration process for Herculex RW Rootworm Protection and no adverse effects were observed. Some of the organisms tested include honeybees, earthworms, lady beetles, springtails, green lacewings, and monarch butterfly caterpillars. No adverse effects were observed in birds or fish. In addition, the EPA found that Herculex RW has no effects on endangered species.

Another important environmental consideration with a biotech product is how cross-pollination will affect the environment. Gene exchange between corn with Herculex RW trait and other cultivated corn varieties can occur. The exchange will be similar to that which occurs naturally between cultivated corn varieties at the present time. In the U.S. and Canada (the current two countries approved for Herculex RW cultivation) there is no plant species closely related to corn in the wild, so gene flow to other species is unlikely. There is no selective advantage for corn hybrids with Herculex RW Rootworm Protection in the natural environment.

There is a potential long-term risk of target pest adaptation to the Cry34Ab1 and Cry35Ab1 proteins leading to the possibility of reduced efficacy. An insect resistance management plan is in place with growers to mitigate this risk.

Regulatory Information

Regulations may exist that govern the manufacture, sale, transportation, use, and/or disposal of corn with Herculex RW trait, 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 MSDS and product label for the product you are using.

Additional Information

- Dow AgroSciences Herculex web site (http://www.dowagro.com/herculex)
- Expanded technical summary and additional references that support the information summarized here on Herculex RW (DAS-59122-7) can be found at Agbios web site at: http://www.agbios.com/dbase.php?action=ShowProd&data=DAS-59122-7&frmat=LONG
- www.cropcomposition.org
- www.biotradestatus.com

For more business information about Herculex® RW Rootworm Protection, visit Dow AgroSciences.
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