MON 89034 x 1507 x NK603 maize

Genuity® Powercore®

Insect protection and herbicide tolerance

Key Facts

Monsanto EMEA
and Dow AgroSciences
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Maize, a key crop

Today, maize is the largest cultivated crop in the world followed by wheat (*Triticum* sp.) and rice (*Oryza sativa* L.) in total global metric ton production. Following European discovery of the Americas where this crop is indigenous, maize was rapidly adopted in Europe, Africa and Asia. Today, it is one of the few intensively cultivated crops in European agriculture. Significant areas of production include the Danube basin from southwest Germany to the Black Sea and southern France through to the Po Valley of northern Italy.

As in other world areas, maize use in Europe is dominated by the demand for animal feed. Maize is also processed into valuable industrial and food products such as ethyl alcohol, maize meal, starch and sweeteners.

In 2011/12, the estimated area of maize harvested in the European Union (EU) was approximately 9.1 million hectares, with a production of around 68.09 million tons[1]. The EU is a large importer of maize, having imported in 2010/11 about 7.53 million tonnes of maize grain per year (the majority from Brazil, Ukraine and Serbia)[2].

What is MON 89034 x 1507 x NK603?

MON 89034 x 1507 x NK603 is a traditionally bred maize, produced by the crossing of three genetically modified (GM) maize lines: MON 89034, 1507 and NK603.

MON 89034 x 1507 x NK603 combines the traits of agronomic interest from the three parental lines, *i.e.* protection against certain lepidopteran insect pests and tolerance to the glufosinate–ammonium and the glyphosate herbicides.

Insect protection

MON 89034 is a GM maize developed through *Agrobacterium*-mediated transformation to express two proteins: Cry1A.105 and Cry2Ab2 modified from the common soil bacteria *Bacillus thuringiensis*. These proteins protect the plants from feeding damage caused by the European corn borer (*Ostrinia nubilalis*) and other lepidopteran (moths and butterflies) insect pests.

1507 is a GM maize developed through the particle acceleration method and carries a gene coding for the Cry1F protein which provides protection against a broad spectrum of lepidopteran insect pests.

Herbicide tolerance

Together with the insecticidal protein Cry1F, 1507 also expresses the phosphinothricin acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, a commonly occurring soil organism. PAT provides tolerance to the glufosinate-ammonium herbicide. Glufosinate inhibits glutamine synthase, which synthesizes glutamine from glutamic acid and ammonia. When this enzyme is inhibited, ammonia accumulates in the plant body and causes plant death. The PAT protein acetylates glufosinate to N-acetylglufosinate, which does not inhibit glutamine synthase and is thus not herbicidally active.

On the other hand, NK603 generated by particle acceleration method, expresses the 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) enzymes derived from the CP4 strain (CP4 EPSPS) of the common soil bacterium *Agrobacterium* sp. CP4 EPSPS provides tolerance to the glyphosate herbicide. Glyphosate (N-phosphonomethyl glycine) inhibits EPSPS (5-enolpruvylshikimate-3-phosphate synthase), an enzyme in the shikimate pathway involved in the production of aromatic amino acids. Inhibition of the production of aromatic amino acids, which are necessary for plant growth and development, causes the plant to die. The shikimate pathway is found in all plants but is not present in mammals and other animal species.

More information on MON 89034, 1507 and NK603 can be obtained from the Center of Environmental Risk Assessment (CERA) and EuropaBio websites[3].

Worldwide plantings and regulatory status of MON 89034 x 1507 x NK603

Genetically modified crops protected against insect pests and/or tolerant to a specific herbicide are commercialized in the US by Monsanto and/or Dow since 1996. In 2012, 170.3 million hectares of GM crops were grown worldwide, from which 43.7 million hectares were expressing stacked traits (James, 2012). Of the 159 million hectares of global maize planted in 2012, more than one-third (35%) or 55.1 million hectares were biotech maize (James, 2012).

MON 89034 x 1507 x NK603 has already received regulatory approval for production in Argentina, Brazil, Canada, and the United States. Importation of MON 89034 x 1507 x NK603 and derived foods and feeds is approved in Japan, Korea, Mexico, Philippines, South Africa and Taiwan.

[1] USDA - http://usda01.library.cornell.edu/usda/fas/worldag-production//2010s/2013/worldag-production-07-11-2013.pdf - (Accessed on July 24, 2013)

[2] EC – http://ec.europa.eu/agriculture/cereals/trade/cereals/2010-2011_en.pdf (Accessed on July 24, 2013)

[3] CERA - http://www.cera-gmc.org/?action=gm_crop_database and http://www.europabio.org/information-operators-product-information (Accessed on July 24, 2013)

A strict regulatory system for genetically modified crops

In the EU, the regulatory system for GM crops comprises several regulations and directives, including Directive 2001/18/EC for deliberate release of GMOs in the environment (repealing Directive 90/220/EEC) and Regulation (EC) No 1829/2003 on GM Food and Feed (replacing Regulation (EC) No 258/97 on novel foods and novel food ingredients for GM products).

Regulation (EC) No 1829/2003 includes procedures for the authorisation of deliberate release (cultivation and/or import, and processing), in addition to food and feed use, according to the "one door, one key" principle.

A regulation on traceability and labeling of GMOs and products produced from GMOs (Regulation (EC) No 1830/2003) entered into force on 18 April 2004.

A regulation laying down the methods of sampling and analysis for the official control of feed as regards presence of genetically modified material for which an authorization procedure is pending or the authorisation of which has expired (Commission regulation (EU) No 619/2011) entered into force on 24 June 2011.

Regulatory status of MON 89034 x 1507 x NK603 in the EU

On 29 January 2009, Monsanto and Dow submitted an application for food and feed use of MON 89034 x 1507 x NK603 maize[4] as any other maize (excluding cultivation) under Regulation (EC) No 1829/2003 to the European Food Safety Authority (EFSA), via the Netherlands. This application was declared valid on 06 August 2009.

EFSA evaluated the application as well as additional information provided by Monsanto and Dow during the scientific review, scientific comments submitted by the Member States and relevant scientific publications. Furthermore, information from applications for placing on the market MON 89034, 1507 and NK603 were taken into account.

EFSA finalized the risk assessment and adopted a scientific opinion on 08 September 2010[5] for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 x 1507 x NK603 and all sub-combinations of the individual events as present in its segregating progeny. In its scientific opinion EFSA concludes that "*the maize* MON 89034 x 1507 x NK603 *is as safe as its conventional comparator and commercial maize varieties with respect to potential effects on human and animal health or the environment*"

The EFSA overall opinion, which fulfils the requirements of Articles 6 and 18 for the placing on the market of MON 89034 x 1507 x NK603, was published on 27 September 2010.

Following the Commission's mandate of 01 February 2011 to EFSA requesting to complement the overall EFSA opinion to cover all sub-combinations of their single events independently of their origin, in addition to the higher stack MON 89034 x 1507 x NK603, EFSA issued on 14 October 2011 a statement complementing the EFSA GMO Panel scientific opinion on maize MON 89034 x 1507 x NK603 to cover all sub-combinations independently of their origin[6].

The updated EFSA overall opinion was published on 10 November 2011.

On 10 June 2013, the European Commission presented the Draft Commission Implementing Decision authorizing the placing on the market of products containing, consisting of, or produced from genetically modified maize MON 89034 x 1507 x NK603 to the Standing Committee on the Food Chain and Animal Health (SCFCAH) for a vote. After this vote, the draft decision was passed to the Appeal Committee who met for a vote on 11 July 2013. The Appeal Committee forwarded the draft decision to the European Commission with a recommendation for an approval. The authorization was finally granted by the European Commission on November the 6th, 2013[7].

Regulatory status of the parental lines

Approval of MON 89034 for import, food, feed and processing

On 14 December 2006, Monsanto submitted an application for food and feed uses, import and processing of MON 89034 under the Regulation (EC) No 1829/2003 on GM Food and Feed to the EFSA via the Netherlands. This application was declared valid by EFSA on 24 August 2007. EFSA evaluated the application as well as Monsanto's responses to comments and reasoned objections from certain Member States. EFSA finalized the risk assessment and adopted a scientific opinion on 03 December 2008, concluding that "*MON89034 is as safe as its non genetically modified counterpart with respect to potential effects on human and animal health or the environment*"[8]. The EFSA overall opinion, which fulfils the requirements of Articles 6 and 18 of Regulation (EC) No 1829/2003, was published on 18 December 2008. Subsequently, on 30 October 2009, MON 89034 maize was authorised by the European Commission for placing on the EU market

[4] Since maize grain is the product of genetic segregation and reunion of genetic components according to Mendelian law, F_2 grain produced from MON 89034 × 1507 × Nk603 hybrid (F_1) seeds, will include a mixture of MON 89034 × 1507 × NK603 and all combinations with fewer of these segregating events.

[5] EFSA – http://www.efsa.europa.eu/en/efsajournal/pub/1782.htm (Accessed on July 24, 2013)

[6] EFSA - http://www.efsa.europa.eu/fr/efsajournal/pub/2377.htm (Accessed on July 24, 2013)

[7] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:302:0038:0043:EN:PDF (Accessed on November the 29, 2013)

[8] EFSA - http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902216540.htm (Accessed on July 24, 2013)

for import, food and feed for 10 years (Commission Decision 2009/813/EC[9]).

Approval of 1507 maize import, processing and animal feed

Pioneer and Dow AgroSciences submitted the notification for 1507 maize import, processing and animal feed to the Dutch Competent Authority (CA) under Directive 90/220/EEC in November 2000 and updated it to meet the requirements of Directive 2001/18/EC on 6 November 2002. The Dutch CA issued a positive safety evaluation report on the notification in September 2003[10]. EFSA evaluated the notification and adopted a scientific opinion on 24 September 2004, concluding that '*1507 will not have an adverse effect on human and animal health or the environment in the context of its proposed use*'[11]. Subsequently, on 3 November 2005, 1507 maize was authorised by the European Commission for placing on the EU market for import, processing and animal feed use (Commission Decision 2005/772/EC). The final consent for placing on the market in accordance with Decision 2005/772/EC was granted by the Dutch CA on 16 March 2006 after approval of 1507 for food use[12].

Approval of1507 maize for food

Pioneer and Dow AgroSciences submitted the application for 1507 novel food use to the Dutch CA under Regulation (EC) NO 258/97 in February 2001. The application received a positive safety evaluation by the Dutch CA in November 2003, but had to be updated to meet the requirements under Regulation (EC) No 1829/2003 before being forwarded to EFSA by the Dutch CA. The GMO Panel of EFSA evaluated the application and adopted a scientific opinion on 19 January 2005, concluding once more '*that 1507 maize will not have an adverse effect on human and animal health in the context of its proposed use*'[13]. Subsequently, on 3 March 2006, 1507 maize was authorised by the European Commission for placing on the EU market for food use (Commission Decision 2006/197/EC)[14].

Approval of NK603 for import, processing and animal feed use of grain

In December 2000, Monsanto submitted an application for import and use of NK603 (excluding cultivation) under Directive 90/220/EEC to the Competent Authority of Spain, who evaluated the safety of NK603. The application was then amended to fulfill the new requirements of Directive 2001/18/EC. Spain issued a favourable Initial Assessment Report, which was forwarded to the EU Member States for further review in January 2003.

The European Food Safety Authority (EFSA) evaluated the notification as well as Monsanto's responses to comments and reasoned objections from certain Member States. EFSA issued a favourable scientific opinion on 25 November 2003 concluding that *"NK603 maize is as safe as conventional maize and therefore the placing on the market of NK603 maize for food or feed use of the grain or processing is unlikely to have an adverse effect on human or animal health or, in that context, on the environment."*[15].

After consideration by a Regulatory Committee, composed of Member State experts, and the Council of Environment Ministers, NK603 was approved for import, feed and processing by the European Commission on 19 July 2004 (Commission Decision 2004/643/EC)[16].

Approval of NK603 for novel foods and novel food ingredients

Monsanto submitted an application under the novel food and novel food ingredients Regulation (EC) No 258/97 to the Competent Authority of The Netherlands in April 2001. The Netherlands issued a favourable Initial Assessment Report, which was forwarded to the EU Member States for further review in January 2003 and, subsequently, to EFSA, which issued a favourable scientific opinion on 25 November 2003[17].

After consideration by a Regulatory Committee, composed of Member State experts, and the Council of Agriculture Ministers, NK603 was approved by the European Commission on 3 March 2005 (Commission Decision 2005/448/EC)[18].

Approval of NK603 for feed materials, feed additives and food additives

In April 2005, existing feed materials, feed additives and food additives produced from NK603 were listed in the community register, according to Regulation (EC) No 1829/2003 concerning GM food and feed. Processed feeds such as corn gluten feed and meal are therefore approved in the EU[19].

In August 2005, Monsanto applied for the renewal of the existing feed materials, feed additives, and food additives produced from NK603 listed in the Community Register, pursuant to Regulation (EC)

[9] EUR-LEX -http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:289:0021:0024:EN:PDF (Accessed on July 24, 2013)

[10] JRC - http://gmoinfo.jrc.ec.europa.eu/csnifs/C-NL-00-10_AssessmentReport.pdf (Accessed on July 24, 2013)

[11] EFSA - http://www.efsa.europa.eu/en/scdocs/doc/124.pdf

[12] EUR-LEX - http://eur-lex.europa.eu/LexUriServ/site/en/oj/2005/l_291/l_29120051105en00420044.pdf (Accessed on July 24, 2013)

[13] EFSA - http://www.efsa.europa.eu/en/scdocs/scdoc/182.htm (Accessed on July 24, 2013)

[14] EUR-LEX - http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:070:0082:0086:EN:PDF (Accessed on July 24, 2013)

[15] EFSA - http://www.efsa.europa.eu/en/efsajournal/doc/10.pdf (Accessed on July 24, 2013)

[16] EUR - LEX http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32004D0643:EN:HTML (Accessed on July 24, 2013)

[17] EFSA - http://www.efsa.europa.eu/en/scdocs/doc/opinion_gmo_02_final_en1,3.pdf . (Accessed on July 24, 2013)

[18] EUR – LEX - http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:158:0020:0022:EN:PDF (Accessed on July 24, 2013)

[19] EC - http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=16 (Accessed on July 24, 2013)

No 1829/2003. A positive EFSA overall opinion, fulfilling the requirements of Articles 6 and 18 of Regulation (EC) No 1829/2003 and confirming the conclusions of the original safety assessment, was adopted on 27 May 2009 (and published on 11 June 2009)[20]. According to the legal framework these authorised products remain lawfully on the market until a decision on re-authorization is taken by the European Commission.

Traceability, labelling, unique identifier

Once authorised for commercialisation, operators importing, handling or using MON 89034 x 1507 x NK603 grain and derived foods and feeds in the EU are required to be aware of the legal obligations regarding traceability and labelling of these products, laid down in Regulation (EC) No 1830/2003 and in the conditions of placing on the market of the consent.

The unique identifier for MON 89034 × 1507 × NK603 is MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6.

In August 2008, MON 89034 × 1507 × NK603 samples of food and feed and control samples were provided to the Joint Research Centre (JRC), acting as the European Union Reference Laboratory (EURL). The EURL considers that the detection methods validated on the parental lines, MON 89034, 1507 and NK603, show a comparable performance when applied to MON 89034 x 1507 x NK603. The detection methods for MON 89034, 1507 and NK603 had been previously validated by the EURL and were published at the EURL website[21]. The validation report for MON 89034 x 1507 x NK603, prepared by the EURL in collaboration with the ENGL, was published on 04 August 2010 on the same website.

Food, feed and environmental safety of MON 89034 x 1507 x NK603

Food and feed safety

MON 89034 x 1507 x NK603 is a traditionally bred maize, produced by the crossing of three GM maize lines: MON 89034, 1507 and NK603. The safety assessment was essentially carried out in two steps:

- Demonstration that the characteristics of the parental single-trait lines are maintained in MON 89034 x 1507 x NK603.
- Assessment based on that of the parental single-trait lines.

Molecular analysis of the DNA inserts present in MON 89034 x 1507 x NK603 confirmed that the insert structures of the parental single-traits were retained. Also, Cry1A.105, Cry2Ab2, Cry1F, PAT, and CP4 EPSPS protein levels in forage and grain of MON 89034 x 1507 x NK603 were comparable to the levels in the corresponding single-trait maize (MON 89034, 1507 and NK603).

The conclusions of safety for the Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins, as already demonstrated in the context of the MON 89034, 1507 and NK603 maize lines, remain applicable when these proteins are produced in combination in MON 89034 x 1507 x NK603. It is unlikely that interactions between these proteins would occur that would raise any safety concerns[22].

Compositional and phenotypic/agronomic analyses showed that there are no biologically relevant differences in the characteristics of MON 89034 x 1507 x NK603 as compared with its conventional counterpart and that the composition fell within the range of non-GM maize varieties, except that MON 89034 x 1507 x NK603 expressed the Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins.

Additionally, the food and feed safety of MON 89034 x 1507 x NK603 was established through:

- The long history of safe use of Cry proteins (Betz, et al., 2000), CP4 EPSPS and PAT proteins in general;
- The evaluation of CP4 EPSPS activity and its homology to EPSPS proteins present in a diversity of plants, including those used for foods;
- The rapid digestibility of the introduced proteins in *in vitro* digestive models;
- The lack of toxicity or allergenicity of the introduced proteins, as demonstrated with bioinformatics as well as *in vitro* and *in vivo* safety studies of the proteins;
- A large margin of safety resulting from the low dietary exposure to the introduced proteins.

In conclusion, MON 89034 x 1507 x NK603 was shown to be as safe and nutritious as the parental lines and the conventional maize control.

Environmental safety

The environmental safety of MON 89034 x 1507 x NK603 was established through extensive laboratory and field testing of plant tissue or purified Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins, and with a wide range of non-target species. No adverse effects have been observed in non-target species exposed to Cry1A.105, Cry2Ab2, Cry1F, PAT and CP4 EPSPS proteins. In addition, these proteins are expected to degrade rapidly in the environment. Furthermore, agronomic, morphological and pest susceptibility data have been recorded in multiple field trials conducted in major maize growing regions. Results from these trials confirm that MON 89034 x 1507 x NK603 is phenotypically equivalent to conventional maize except for its protection against target lepidopterans and its

[20] EFSA - http://www.efsa.europa.eu/fr/efsajournal/doc/1137.pdf (Accessed on July 24, 2013)

[21] JRC - http://gmo-crl.jrc.ec.europa.eu/StatusOfDossiers.aspx (Accessed on July 24, 2013)

[22] EFSA – http://www.efsa.europa.eu/en/efsajournal/pub/1782.htm (Accessed on July 24, 2013)

tolerance to glufosinate-ammonium and glyphosate.

MON 89034 x 1507 x NK603, the benefits

MON 89034 x 1507 x NK603 will benefit both farmers and the environment.

Its potential benefits combine the benefits provided by parental lines: MON 89034 and 1507, which confer protection against certain lepidopteran insect pests; NK603, which additionally confers tolerance to glyphosate and 1507, which additionally confers tolerance to glufosinate-ammonium.

The benefits of MON 89034 x 1507 x NK603 include:

- Multiple modes-of-action to help protect plants above ground: Protected shoots to enhance photosynthesis and grain production. In addition, insect resistance has a much lower likelihood when plants present dual and triple modes of protection. The use of unique multiple modes-of-action provides the enhanced insect protection—while maintaining long-term durability of the trait technology. Overall, the product provides substantial economic benefits to growers by limiting yield losses from lepidopteran insect pests as well as from weed pressure.
- A method to control corn borers, other lepidopteran pests of maize compatible with integrated pest management (IPM) approaches, that offers improved pest control and higher yields, while at the same time being safe for humans and the environment. This is combined with a successful broad-spectrum weed control option that allows over-the-top applications of glufosinate-ammonium and/or glyphosate in maize on an "as needed basis"
- An effective insect resistance management tool for lepidopteran insect pests due to the presence of three insecticidal proteins, Cry1A.105, Cry2Ab2 and Cry1F;
- Decreased occurrence of fungal mycotoxins associated with adverse health effects, as a result of lower damage to maize plants by lepidopteran pests (Bakan, et al., 2002; Brookes, 2008; de la Campa, et al., 2005; Munkvold, 2003; Wu, 2006);
- Negligible to no risks for adverse effects on beneficial, non-target organisms when compared to fields treated with conventional pesticides or with untreated controls, attributed to the reduction in insecticide use, low toxicity of glyphosate and compatibility with conservation tillage practices (Ammann, 2003; Fawcett and Towery, 2002; Giesy, et al., 2000; Lozzia, 1999; Orr and Landis, 1997; Pilcher, et al., 1997; Reyes, 2005).
- Control of a wide spectrum of weeds using a smaller number of herbicides. This is particularly important since a number of active ingredients are being re-assessed for toxicological and environmental safety under Directive 91/414/EEC. Glyphosate has already been approved under this directive and can provide an environmentally sustainable, flexible, and profitable alternative to existing weed control programs (Dewar, 2009); [
- Increased benefits for farmers linked to the reduced exposure to insecticides, ease of use and handling, time and labor savings, as well as better pest control (Brookes and Barfoot, 2008; Marra, et al., 2002)
- Resource conservation linked to reduced insecticide and herbicide use, *e.g.* less diesel fuel consumed in the manufacture and delivery of insecticides, less water used for insecticide application, conservation of aviation fuel and reduced use of insecticide containers (Carpenter, et al., 2002; Phipps and Park, 2002)[23];
- An excellent fit with reduced tillage systems, which are linked to many environmental advantages including improved soil and water quality, reduced soil erosion and runoff, improved wildlife habitat and reduced fuel use and C_{O2} emissions (Fawcett and Towery, 2002; Phipps and Park, 2002);

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