Application for import and use of Event MIR604 maize derived products

PART II: SUMMARY

A . GENERAL INFORMATION

1. Details of application

a) Member State of application UK
b) Application number Not available at the time of submission
c) Name of the product (commercial and other names) Maize Event MIR604
d) Date of acknowledgement of valid application Not available at the time of submission

2. Applicant

a) Name of applicant Syngenta Seeds S.A.S on behalf of Syngenta Crop Protection AG, Basel
b) Address of applicant Syngenta Seeds S.A.S. 12, chemin de l'Hobit BP 27 F-31790 Saint-Sauveur On behalf of Syngenta Crop Protection AG, Basel Switzerland and all affiliated companies Schwarzwaldallee 215 CH 4058 Basle Switzerland
c) Name and address of the person established in the Community who is responsible for the placing in the market, whether it be the manufacturer, the importer or the distributor, if different form the applicant (Comission Decision 2004/204/EC Art 3(a)(ii)) Not applicable

3. Scope of the application

☒ GM plants for food use

☒ Food containing or consisting of GM plants

☒Food produced from GM plants or containing ingredients produced from GM plants

☒GM plants for feed use

☒ Feed containing or consisting of GM plants

☒ Feed produced from GM plants

☒ Import and processing (Part C of Directive 2001/18/EC)

☐ Seeds and plant propagating material for cultivation in Europe (Part C of Directive 2001/18/EC)

4. Is the product being simultaneously notified within the framework of another regulation (e.g. Seed legislation?)?

Yes ☐	No ☒
If *yes*, specify	

5. Has the GM plant been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?

Yes ☒	No ☐
If *no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC	

6. Has the GM plant or derived products been previously notified for marketing in the Community under Part C of Directive 2001/18/EC or Regulation (EC) 258/97?

Yes ☐	No ☒
If *yes*, specify	

7. Has the product been notified in a third country either previously or simultaneously?

Yes ☒	No ☐
If *yes*, specify USA and RSA	

8. General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification Event MIR604 is a genetically modified (GM) maize developed to confer field protection against the Western Corn rootworm (WCRW) (*Diabrotica virgifera virgifera*, Le Conte), and the Northern Corn rootworm (NCRW) (*D. longicornis barberi* Smith and Lawrence) and other related coleopteran species. It also expresses a marker protein, PMI, that allows the plants to utilise mannose as a carbon source, acting as a selectable marker.
b) Types of products planned to be placed on the market according to the authorisation applied for The scope of the application includes all feed and food products containing, consisting or produced from the genetically modified maize Event MIR604 including products from inbreds and hybrids obtained by conventional breeding of Event MIR604. The application also covers the import and industrial processing of Event MIR604 for all potential uses, as any other maize.
c) Intended use of the product and types of users Use as any other conventional maize
d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for The characteristics of Event MIR604 maize and products derived from it are not different from those of its conventional counterpart. Event MIR604 has been shown to be as safe and as wholesome as existing varieties of maize. Therefore there are no specific instructions or recommendations for use, storage and handling of Event MIR604 maize.

e) Any proposed packaging requirements The characteristics of Event MIR604 maize and products derived from it are not different from those of its conventional counterpart. Event MIR604 has been shown to be as safe and as wholesome as existing varieties of maize. Therefore there are no specific instructions for packaging.
f) A proposal for labelling in accordance with Articles 13 and Articles 25 of Regulation ((EC) 1829/2003. In the case of GMOs, food and/or feed containing or consisting of GMOs, a proposal for labelling has to be included complying with the requirements of Article 4, B(6) of Regulation (EC) 1830/2003 and Annex IV of Directive 2001/18/EC A proposal for labelling has been included in the application following the guidance provided by EFSA. This includes the labelling requirements outlined by Regulation EC 1829/2003 and Annex IV of Directive 2001/18/EC. Event MIR604 maize grain will therefore be labelled as "genetically modified maize" and products derived from it will be labelled as "containing (or produced from) genetically modified maize". Since Event MIR604 maize and products derived from it are not different form those of its conventional counterpart, no additional labelling is required.
g) Unique identifier for the GM plant (Regulation (EC) 65/2004; does not apply to applications concerning only food and feed produced from GM plants, or containing ingredients produced from GM plants) SYN-IR6Ø4-5
h) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for. Any type of environment to which the product is unsuited Not applicable

9. Measures suggested by the applicant to take in case of unintended release or misuse as well as measures for disposal and treatment

The characteristics of Event MIR604 maize and products derived from it are not different from those of its conventional counterpart. Event MIR604 has been shown to be as safe and as wholesome as existing varieties of maize. Any unintended releases or misuse can be dealt with in the same way as any other conventional maize.

B. INFORMATION RELATING TO (A) THE RECIPIENT OR (B) (WHERE APPROPRIATE) PARENTAL PLANTS

1. Complete name

a) Family name Graminae
b) Genus *Zea*
c) Species *mays*
d) Subspecies
e) Cultivar/breeding line A Syngenta proprietary line of maize
f) Common name Maize

2 a. Information concerning reproduction

(i) Mode(s) of reproduction Sexual reproduction: *Zea mays* is an allogamous plant that propagates through seed produced predominantly by cross-pollination and depends mainly on wind borne cross-fertilisation. *Z. mays* is a plant with protandrous inflorescence; however, decades of conventional selection and improvement have produced varieties of maize with protogynous traits. *Z. mays* has staminate flowers in the tassels and pistillate flowers on the ear shoots. There is no asexually reproductive maize.
(ii) Specific factors affecting reproduction The key critical stages of maize reproduction are tasselling, silking, pollination and fertilization. Pollen dispersal is limited by several factors, including large size (0.1 mm diameter), rapid settling rate and short survivability. Most maize varieties are protoandrous so pollen shedding precedes silk emergence by up to five days. More than 98% of the pollen settles to the ground within a maximum distance of 25-50 meters of its source. Shed pollen typically remains viable for 10 to 30 minutes, but may remain viable longer under refrigerated and humid conditions.

(iii) Generation time

Maize is an annual crop. The generation time from sowing to harvesting varies according to the genetic background and the climate, it can range from as short as 60 to 70 days to as long as 43 to 48 weeks from seedling emergence to maturity.

2 b. Sexual compatibility with other cultivated or wild plant species

Other cultivated plant species: The sexual compatibility of maize with other cultivated plant species is limited to *Zea* species.

Wild plant species: No wild relatives of maize are present in Europe. Therefore, maize cannot exchange genes with any other wild species in the EU.

3. Survivability

a) Ability to form structures for survival or dormancy

Maize is an annual crop. Seeds are the only survival structures; they cannot be dispersed without mechanical disruption of the cobs and show little or no dormancy. Natural regeneration from vegetative tissue is not known to occur.

b) Specific factors affecting survivability

Survival of maize is dependent upon temperature, seed moisture, genotype, husk protection and stage of development. Maize cannot persist as a weed. Maize seed can only survive under a narrow range of climatic conditions. Volunteers are killed by frost or easily controlled by current agronomic practices including cultivation and the use of selective herbicides. Maize is incapable of sustained reproduction outside of domestic cultivation and is non-invasive of natural habitats.

4. Dissemination

a) Ways and extent of dissemination

Maize dissemination can only be accomplished through seed dispersal. Seed dispersal does not occur naturally due to the structure of the ear.

b) Specific factors affecting dissemination

Maize has a polystichous (arranged in many rows) female inflorescence (flower), called the ear, on a stiff central spike (cob) enclosed in husks (modified leaves). Because of the structure of the ears, seed dispersal of individual kernels does not occur naturally. Maize is non-invasive of natural habitats.

5. Geographical distribution and cultivation of the plant, including the distribution in Europe of the compatible species

Maize, which has very diverse morphological and physiological traits, is grown on approximately 140 million hectares worldwide. It is distributed over a wide range of conditions: from 56º N Lat to 40º S Lat, below sea level of the Caspian plains up to 3000 m in the Andes Mountains and from semi-arid regions to arid regions. The greatest maize production occurs where the warmest month isotherms range between 21º and 27º C and the freeze-free season lasts 120-180 days.

There are no wild relatives of maize in Europe.

6. In the case of plant species not normally grown in the Member State(s), description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts

Maize was introduced into Europe in the 15th century by Columbus and is widely grown in the European Union Member States.

7. Other potential interactions, relevant to the GM plant, of the plant with organisms in the ecosystem where it is usually grown, or used elsewhere, including information on toxic effects on humans, animals and other organisms

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and insect pests, as well as to competition from surrounding weeds. Maize is extensively cultivated and has a history of safe use for human food and animal feed. No significant native toxins are reported to be associated with the genus *Zea*.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Description of the methods used for the genetic modification

Transformation of Syngenta's maize Event MIR604, was conducted using immature maize embryos derived from a proprietary *Zea mays* line, *via Agrobacterium*-mediated transformation.

2. Nature and source of the vector used

Plasmid pZM26, a binary vector used for *Agrobacterium* mediated plant transformation, was used to generate event MIR604.

3. Size, source (name) of donor organism(s) and intended function of each constituent fragment of the region intended for insertion

Event MIR604 is a genetically modified (GM) maize expressing two transgenes:

- A modified *cry3A (mcry3A)* gene encoding the mCry3A protein that confers resistance to the Western Corn rootworm (WCRW) *(Diabrotica virgifera virgifera),* the Northern Corn rootworm (*Diabrotica longicornis barberi,* Smith and Lawrence) and other related coleopteran pests of maize. This gene is under the control of the promoter derived from maize metallothionein-like (MTL) gene that provides root preferential expression and the termination sequence of the nopaline synthase (NOS) gene, isolated from *Agrobacterium tumefaciens*.
- the *pmi* (*manA*) gene from *Escherichia coli*, which encodes the enzyme PMI as a selectable marker. PMI allows transformed corn cells to utilize mannose as a sole carbon source, while maize cells lacking the *pmi* gene fail to grow. The *pmi* gene is under the control of the ZmUbiIntron promoter derived from a maize ubiquitin gene together with the 1st intron of the gene and the termination sequence of the nopaline synthase (NOS) gene, isolated from *Agrobacterium tumefaciens.*

D. INFORMATION RELATING TO THE GM PLANT

1. Description of the trait(s) and characteristics which have been introduced or modified

Event MIR604 is a genetically modified (GM) maize that expresses a modified Cry3A protein (mCry3A) that confers resistance to the Western Corn rootworm (WCRW) *(Diabrotica virgifera virgifera),* the Northern Corn rootworm (NCRW) (*Diabrotica longicornis barberi,* Smith and Lawrence) and other related coleopteran pests of maize and the PMI protein that allows transformed corn cells to utilize mannose as a sole carbon source and acts as a selectable marker.

Event MIR604 is an alternative tool for growers in the protection of their fields from WRCW and NCRW infestations. The genetic modification is not intended to change any of the typical crop characteristics of maize (except for the resistance against WCRW and NCRW) and the handling and use of Event MIR604 can be the same as non-genetically modified maize.

2. Information on the sequences actually inserted or deleted

a) The copy number of all detectable inserts, both complete and partial

Data from Southern analysis demonstrates that single copies of the *mcry3A* gene, *pmi* gene, MTL promoter and ZmUbiInt promoter are present in Syngenta's maize Event MIR604. Event MIR604 does not contain any of the backbone sequences from the transformation plasmid pZM26.

b) In case of deletion(s), size and function of the deleted region(s)

Not applicable

c) Chromosomal location(s) of insert(s) (nucleus, chloroplasts, mitochondria, or maintained in a non-integrated form), and methods for its determination

The inheritance pattern of the insert derived from pZM26 in Event MIR604 was investigated. The results showed that insertion had taken place in the nucleus. Statistical analysis confirmed the expected Mendelian inheritance ratio for both *mcry3A* and *pmi.*

d) The organisation of the inserted genetic material at the insertion site

The entire insert and the 5' and 3' flanking regions were sequenced. Sequence analysis confirmed that the overall integrity of the insert and the contiguousness of the functional elements as intended in pZM26 have been maintained.

3. Information on the expression of the insert

a) Information on developmental expression of the insert during the life cycle of the plant

To characterize the range of expression of mCry3A and PMI proteins in maize plants derived from Event MIR604, the concentrations of these proteins were determined by ELISA at four growth stages (whorl, anthesis, seed maturity and senescence). Quantifiable levels of mCry3A protein were detected in all Event MIR604-derived plant tissues analysed except pollen across all plant stages.

PMI protein was detected in most of the Event MIR604-derived plant tissues analysed, albeit at low levels. Across all plant stages, mean PMI levels measured in leaves, roots and whole plants ranged from not detectable to a maximum of ca 0.4µg/g fresh wt.

b) Parts of the plant where the insert is expressed

To characterize the range of expression of mCry3A and PMI proteins in maize plants derived from Event MIR604, the concentrations of these proteins were determined by ELISA in several plant tissues (leaves, roots, kernels, silk tissues, pollen and whole plants). Quantifiable levels of mCry3A protein were detected in all Event MIR604-derived plant tissues analysed except pollen. PMI protein was detected in most of the Event MIR604-derived plant tissues analysed, albeit at low levels.

4. Information on how the GM plant differs from the recipient plant in

a) Reproduction

No changes in the reproduction, dissemination or survivability compared to non-GM maize have been observed in field trials conducted with Event MIR604.

b) Dissemination

No changes in the reproduction, dissemination or survivability compared to non-GM maize have been observed in field trials conducted with Event MIR604.

c) Survivability

No changes in the reproduction, dissemination or survivability compared to non-GM maize have been observed in field trials conducted with Event MIR604.

d) Other differences

No changes in the reproduction, dissemination or survivability compared to non-GM maize have been observed in field trials conducted with Event MIR604.

5. Genetic stability of the insert and phenotypic stability of the GM plant

Genetic stability:

Southern analysis was conducted to confirm the presence of a single copy of the *mcry3A* gene over several generations of Event MIR604 plants. The hybridization data demonstrate that the insert from pZM26 incorporated into Event MIR604 is stable over several generations

Phenotypic stability:

The stability of mCry3A and PMI protein expression over multiple generations was evaluated. Seed from four successive backcross generations (representing genotypes that were hemizygous for the Event MIR604 transgenes) was grown under greenhouse conditions and leaf material was collected at anthesis for analysis of mCry3A and PMI protein levels.

Overall, levels of mCry3A and PMI were similar across the four generations analysed. There was no evidence of any significant trend either up or down, indicating that the expression of mCry3A protein and of the PMI protein is stable.

6. Any change to the ability of the GM plant to transfer genetic material to other organisms

a) Plant to bacteria gene transfer

The horizontal gene transfer from GM plants to bacteria with subsequent expression of the transgene is regarded as a highly unlikely event under natural conditions and especially in the absence of selective pressure. The inserted DNA has been evaluated for possible enhancement of gene transfer potential. Southern analysis showed that the origin of replication has not been transferred to Event MIR604 and can therefore be excluded from the analysis. The *pmi* gene is from bacterial origin but adapted for optimal expression in plants and does not contain sequences that might enhance recombination. Similarly the *mcry3A* gene synthetically created by Syngenta scientists was optimised for maize expression and does not contain sequences that could enhance recombination.

b) Plant to plant gene transfer

The scope of this application does not include authorization for the cultivation of Event MIR604 maize.

The genetic modification was not intended to change any of the typical crop characteristics of maize (except for the resistance against Western Corn rootworm). Observations from field trials have confirmed that the agronomic characteristics of Event MIR604 have not changed in comparison with controls, and therefore, that there is no increase or decrease in the potential for plant-to-plant gene transfer.

In the unlikely event that small amounts of grain of maize Event MIR604 accidentally found their way into the environment in the EU, their survival would be very unlikely as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. Gene transfer from Event MIR604 maize to other sexually compatible plant species is not possible since maize has no wild relatives in the EU. Dissemination of pollen to other cultivated maize plants is not likely to occur since these plants are not intended for cultivation in Europe. Any spilled grain could be easily controlled by standard agronomic practices. In the highly unlikely event that cross-pollination did occur this would not lead to establishment of the transgene in the maize genetic pool as commercially grown hybrids are used only for grain production and volunteers can easily be controlled using standard agronomic practices.

7. Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed

7.1 Comparative assessment

Choice of the comparator

Event MIR604 was compared with relevant control maize lines that had not been genetically modified. Commercial varieties were also included in the comparison whenever possible.

7.2 production of material for comparative assessment

a) number of locations, growing seasons, geographical spreading and replicates

To confirm that Event MIR604-derived maize plants are substantially equivalent to the non-transgenic isolines, replicate trials of transgenic and corresponding isogenic controls were planted in 12 locations over two growing seasons. The locations of the trial sites were selected to be representative of the range of environmental conditions under which the hybrid varieties are expected to be grown. At each location, three replicate plots of each genotype were planted.

b) the baseline used for consideration of natural variations

The levels of multiple nutritive components were compared in maize kernels (grain) or whole plants (forage) produced from Event MIR604-derived maize plants and simultaneously grown isogenic control plants. The mean values were also compared with the range of data published in the literature, where data was available.

7.3 Selection of compounds for analysis

As recommended by OECD, grain from transgenic MIR604-derived maize plants and isogenic non-transgenic control plants were analysed for proximates (including starch), minerals, amino acids and selected fatty acids, vitamins, anti-nutrients and secondary metabolites. Forage (whole plants) from transgenic MIR604-derived maize plants and isogenic non transgenic control plants were analysed for proximates and minerals.

As it is often the case with this type of analysis, occasional statistically significant differences between Event MIR604 transgenic plants and isogenic controls were observed for some parameters, no consistent patterns emerged to suggest that biologically significant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of the transformation process or expression of the transgene. All the components recommended by OECD evaluated were within the range of reported literature values for maize.

7.4 Agronomic traits

Event MIR604-derived hybrids were grown in 22 locations in 8 states during 2002 and 2003. Up to 18 separate agronomic traits were assessed. Most of the agronomic traits evaluated showed no statistically significant differences between Event MIR604-derived hybrids and the non-transformed isogenic counterparts. While some differences between transgenic and control were found to be significant, as it is often the case in such type of analysis, there were no consistent trends in the data across locations or across years that would indicate that any of these differences were due to the presence of the transgene. These differences were within the normal range of variation experienced in agronomic field trials conducted with transgenic events by Syngenta over the past decade.

Field trials performed in the US require USDA permits and notifications for which one of the requirement is to record any abnormal agronomic occurences observed during the course of the trial. For the 2002-2003 growing seasons, no adverse effects relating to non-target and pest insects were recorded. Similarly, there were no reports of enhanced susceptibility to insect pests.

7.5 Product specification

Maize as a product has a history of safe use for human food and animal feed. No significant native toxins are reported to be associated with the genus *Zea*. The information presented in this application confirms that Event MIR604 maize and products derived from it are not different from those of its conventional counterpart.

7.6 Effect of processing

Maize from Event MIR604 will be produced and processed in the same way as any non-GM maize and there is no evidence to suggest that the expression of the mCry3A and PMI proteins will influence this processing in any way.

Presence of modified Cry3A protein was quantitatively analysed in standard wet-milled and dry-milled processing fractions, corn oil and corn chips from the maize grain derived from Event MIR604. Control grain was obtained from non-transgenic hybrid field corn plants that were derived from similar germplasm as the MIR604-derived plants. The analysis of different samples derived from Event MIR604 plants showed mCry3A protein could be found in some of the fractions in both wet and dry-milling processes, but no quantifiable levels were found in corn chips or corn oil.

7.7 Anticipated intake/extent of use

It is expected that the introduction of Event MIR604 will replace some of the maize in existing food and feed products. However, the genetic modification was not intended to change any of the compositional parameters in food and feed (and it did not so, as shown in Section 7.3). Therefore no nutritional changes are expected from this replacement. No impact on the extend of use of maize can be expected.

7.8 Toxicology

7.8.1 Safety assessment of newly expressed proteins

Maize derived from Syngenta's transformation Event MIR604 expresses two proteins: the mCry3A and the PMI protein. To demonstrate the safety of these protein a series of studies were conducted. The data obtained showed that both newly expressed proteins, mCry3A and PMI, are not structurally and functionally related to proteins which have the potential to adversely affect human or animal health. They are sensitive to processing and rapidly degraded in simulated gastric fluid. Neither of the proteins showed any sequence homology with known toxins. In addition, acute oral toxicity studies of mCry3A and PMI in the mouse confirmed that these proteins are not acutely toxic to mice at the highest dose tested.
Supplemental information was also provided by a 90-day whole food safety study in rodents and a poultry feeding study showing no adverse effects on rats and chickens.

7.8.2 Testing of new constituents other than proteins

Maize is a common source of food and feed and has a long history of safe use. Event MIR604 has been modified to express two proteins: the mCry3A and the PMI protein. No other new constituents apart from these two proteins are expected to be produced in Event MIR604 and compositional analyses have confirmed the substantial equivalence of Event MIR604 and conventional maize (see Section 7.8.3 below) therefore no testing of any other constituent is considered necessary.

7.8.3 Information on natural food and feed constituents

The presence and levels of natural food and feed constituents such as macro- and micronutrients, secondary plant metabolites as well as natural toxins and antinutritional factors have been analysed in Event MIR604 and compared with non-genetically modified isolines and data from the literature.

These analyses showed that the levels of the components measured had not changed beyond the natural variation in maize. As it is often the case with this type of analysis, occasional statistically significant differences between Event MIR604 transgenic plants and isogenic controls were observed for some parameters. No consistent patterns emerged to suggest that biologically significant changes in composition or nutritive value of the grain or forage had occurred as an unintended result of the transformation process or expression of the transgene.

7.8.4 Testing of the whole GM food/feed

In addition to the compositional analysis, the wholesomeness and safety of maize Event MIR604 was confirmed in a 90-day whole food safety study in rats and a 49-day poultry feeding study.

The 90-day whole food safety study was conducted at two dose levels representing the maximum human chronic dietary intake level maize and the maximum dose level achievable without causing nutritional imbalance. No treatment related effects were observed on any of the parameters tested: bodyweight, food consumption, clinical condition (including ophtalmoscopy and functional observation), clinical pathology, organ weights or hystopathology. It can therefore be concluded that when fed to rats for 90 consecutive days, Event MIR604 maize grain did not cause any treatment related effects and there were no differences with the non-GM controls.

A 49-day poultry feeding study was also conducted to evaluate whether standard poultry diets prepared with Event MIR604 grain had any adverse effect on male or female broiler chickens as compared to diets prepared with non-transgenic isoline control grain and a commercial source of corn. The study showed that the transgenic corn had no deleterious effects on broiler chickens.

7.9 Allergenicity

7.9.1 Assessment of allergenicity of the newly expressed protein

According to recommendations of the Codex *ad hoc* Intergovernmental Task Force on Foods derived from Biotechnology, an integrated stepwise approach has been used for the assessment of potential allergenicity of the newly expressed proteins, mCry3A and PMI of Event MIR604.

The source of the transgenes has been considered. The source of native Cry3A protein is *Bacillus thuringiensis*. Bacteria have no history of allergenicity. Additionally, despite decades of widespread use of Bt insecticides on food crops, there have been no reports of oral allergies to these preparations. The source of the *pmi* gene is *E. coli*. Bacteria have no history of allergenicity. Neither of these donor organisms is known to be allergenic.

An extensive bioinformatics search for sequence homologies and structural similarities between the expressed proteins and known allergens was performed. The results demonstrated that the mCry3A protein as expressed in maize Event MIR604 shows no homology to any known or putative allergenic proteins. The PMI protein was also assessed for its allergenic potential, homology searches and sera analysis confirmed that PMI is unlikely to be allergenic.

The susceptibility of mCry3A and PMI proteins to proteolytic degradation was evaluated in simulated mammalian gastric fluid (SGF) containing pepsin. Both proteins were readily degraded in SGF. No intact mCry3A, PMI or immunoreactive fragments were detected following digestion in SGF for 2 minutes. These data support the conclusion that mCry3A and PMI expressed in transgenic plants will be readily digested as conventional dietary protein under typical mammalian gastric conditions.

Many food allergens are known to be stable to heat. A study of the heat stability of the PMI and mCry3A proteins determined that incubation for 30 minutes at 65ºC inactivated PMI and reduced the activity of mCry3A against WCRW. At 95ºC the mCry3A protein was completely inactivated.

In summary, it can be concluded that the mCry3A and PMI proteins expressed in Event MIR604 maize plants are unlikely to be allergenic.

7.9.2 Assessment of allergenicity of the whole GM plant or crop

Maize has been extensively cultivated and has a history of safe use for human food and animal feed. Maize is not considered to be an allergenic food crop and Event MIR604 does not express any new proteins with allergenic characteristics.

7.10 Nutritional assessment of GM food/feed

7.10.1 Nutritional assessment of GM food

Genetically modified Event MIR604 is not intended to improve the nutritional status of individuals of populations or to be processed in products with enhanced functionality. Compositional analysis and whole food safety tests have demonstrated that no unexpected alterations in nutrients and other food components have occurred and that no nutritional imbalances were introduced.

7.10.2 Nutritional assessment of GM feed

Genetically modified Event MIR604 is not intended to improve the nutritional status of livestock animals. Compositional analysis and whole food safety tests have demonstrated that no unexpected alterations in nutrients and other food components have occurred and that no nutritional imbalances were introduced.

7.11 Post-market monitoring of GM food/feed

As described in sections 7.1 to 7.10 above, the presence of Event MIR604 in food and feed will not result in any nutritional changes, therefore post-market monitoring is not considered appropriate.

8. Mechanism of interaction between the GM plant and target organisms (if applicable)

Maize derived from Syngenta's transformation Event MIR604 expresses a mCry3A protein that confers resistance to the Western Corn rootworm *(Diabrotica virgifera virgifera),* the Northern Corn rootworm (*Diabrotica longicornis barberi*, Smith and Lawrence) and other related coleopteran pests of maize.

The scope of this application does not include cultivation of maize in the EU, therefore interaction with target organisms is highly unlikely.

9. Potential changes in the interactions of the GM plant with the biotic environment resulting from the genetic modification

9.1 Persistence and invasiveness

Cultivation of maize derived from Event MIR604 in the EU is not within the scope of this application. In the unlikely event that small amounts of maize kernels of Event MIR604 could accidentally find their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention, especially under normal European climatic conditions. The expression of the mCryA and PMI proteins does not affect the agronomic characteristics or weediness potential of Event MIR604, as demonstrated in field trials conducted to evaluate the agronomic performance of this event in comparison with the isogenic control. In the unlikely event that these maize plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, the probability of the genetically modified plants becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats as a result of importing maize kernels of this event into the EU can be considered negligible.

9.2 Selective advantage or disadvantage

Event MIR604 plants have been modified to express a mCry3A protein conferring tolerance to Western Corn rootworm, along with a marker protein, pmi, that allows the plants to utilise mannose as a carbon source.

The expression of resistance to Western Corn rootworm in certain areas of Europe where this pest is found could be considered a selective advantage for maize that grows outside the managed agricultural environment. However, as described above, maize is incapable of surviving under these conditions and is incapable of survival without human intervention.

Cultivation of maize in the EU is not within the scope of this application.

In summary, it is not anticipated that the expression of these genes will confer any environmental advantage or disadvantage to Event MIR604 maize plants over the unmodified plants.

9.3 Potential for gene transfer

Gene transfer from Event MIR604 maize to other sexually compatible plant species is not possible since there are not any maize wild relatives in the EU (EEA, 2002).

Gene transfer from Event MIR604 maize to other maize could occur through pollen dispersal during the cultivation of the crop. Maize is predominantly wind pollinated. Plants produce pollen for 10-13 days and shed pollen typically remains viable only a short time. 98% of pollen settles to the ground within 25-50 meters of its source (EEA, 2002). Cultivation of maize Event MIR604 is not within the scope of this application. It is therefore highly unlikely that this maize could grow in any significant quantity in the EU. In the unlikely event that small amounts of grain from maize Event MIR604 could accidentally find their way into the environment this would represent extremely low levels of exposure and the survival of these grain would be very unlikely for the reasons stated above. In addition, they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize. Therefore maize derived from Event MIR604 is extremely unlikely to flower and fertilise other varieties of maize in Europe.

In summary, the potential for gene transfer between Event MIR604 maize plants and other maize plants or sexually compatible wild relatives in the EU can be considered negligible under the scope of this application.

9.4 Interactions between the GM plant and target organisms

Since the scope of this application does not include cultivation of maize plants of Event MIR604, there will not be significant direct or indirect interactions with target organisms.

In the unlikely event that small amounts of maize kernels of Event MIR604 could accidentally find their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention, especially under normal European climatic conditions. Moreover, if these plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, interactions between Event MIR604 and target organisms are highly unlikely under the scope of this application.

9.5 Interactions of the GM plant with non-target organisms

The scope of this application does not include cultivation of maize plants of Event MIR604, therefore interactions with non-target organisms are highly unlikely.

9.6 Effects on human health

Event MIR604 will be imported in the EU and could enter the food chain. Human exposure to this maize could therefore occur though food consumption. However, the recipient organism, maize, has a history of safe use throughout the world. Event MIR604 express a mCry3A protein conferring tolerance to Western Corn rootworm and Northern Corn rootworm, along with a marker protein, PMI, that allows the plants to utilise mannose as a carbon source. Studies comparing the composition of Event MIR604 maize plants and non-modified maize have been performed. These studies lead to the conclusion that this maize is substantially equivalent to conventional maize. Both proteins, mCry3A and PMI, are expressed at extremely low levels in the plant, are sensitive to processing and heat and rapidly degraded in simulated gastric fluids. None of the proteins show any sequence homology with known toxins.
In addition, the acute oral toxicity studies conducted with mCry3A and PMI in the mouse, confirmed that these proteins are not acutely toxic to mice at the highest dose tested. No test substance related mortalities occurred during the study and no clinical signs attributable to the test substance were observed. There were no treatment related effects on body weight, food consumption or organ weights, nor were any treatment related effects observed following macroscopic or microscopic examination of tissues.

An allergenicity assessment has been conducted showing that these proteins are not likely to be allergenic.

The inserted DNA has been sequenced. A detailed open reading frame analysis could find no evidence for sequences of any other proteins.

The wholesomeness and safety of maize Event MIR604 was confirmed in a 90-day toxicity study in rats and a poultry feeding study, where no treatment related effects were observed and no differences with the non-GM controls were detected.

In summary, it can be concluded that grain from maize Event MIR604 is safe for food consumption and no differences in wholesomeness are expected with comparable non-GM maize varieties.

9.7 Effects on animal health

Event MIR604 will be imported in the EU and could enter the feed chain. Animal exposure to this maize could therefore occur though feed consumption. However, the recipient organism, maize, has a history of safe use throughout the world. Event MIR604 express a mCry3A protein conferring tolerance to Western Corn rootworm and Northern Corn rootworm, along with a marker protein, PMI, that allows the plants to utilise mannose as a carbon source. Studies comparing the composition of Event MIR604 maize plants and non-modified maize have been performed. These studies lead to the conclusion that this maize is substantially equivalent to conventional maize. Both proteins, mCry3A and PMI, are expressed at extremely low levels in the plant, are sensitive to processing and heat and rapidly degraded in simulated gastric fluids. None of the proteins show any sequence homology with known toxins.

In addition, the acute oral toxicity studies conducted with mCry3A and PMI in the mouse, confirmed that these proteins are not acutely toxic to mice at the highest dose tested. No test substance related mortalities occurred during the study and no clinical signs attributable to the test substance were observed. There were no treatment related effects on body weight, food consumption or organ weights, nor were any treatment related effects observed following macroscopic or microscopic examination of tissues.

The inserted DNA has been sequenced. A detailed open reading frame analysis could find no evidence for sequences of any other proteins.

The wholesomeness and safety of maize Event MIR604 was confirmed in a 90-day toxicity study in rats and a poultry feeding study, where no treatment related effects were observed and no differences with the non-GM controls were detected.

In summary, it can be concluded that grain from maize Event MIR604 is safe for feed consumption and no differences in wholesomeness are expected with comparable non-GM maize varieties.

9.8 Effects on biogeochemical processes

Since the scope of this application does not include cultivation of maize plants of Event MIR604, effects on biogeochemical processes are highly unlikely. In the unlikely event that small amounts of maize kernels of Event MIR604 could accidentally find their way into the environment their survival would be very unlikely as maize is highly domesticated and cannot survive without human intervention, especially under normal European climatic conditions. Moreover, if these plants were to survive they could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

In summary, effects on biogeochemical processes resulting from the use of Event MIR604 under the scope of this application are highly unlikely.

9.9 Impacts of the specific cultivation, management and harvesting techniques

The scope of this application does not include cultivation of maize plants of Event MIR604 in the EU.

10. Potential interactions with the abiotic environment

The scope of this application does not include cultivation of Event MIR604 maize in the EU, therefore interactions of maize Event MIR604 with the abiotic environment are highly unlikely. In the unlikely event that small amounts of maize kernels of event MIR604 could accidentally find their way into the environment these plants will not have different interactions with the abiotic environment to similar non-GM plants. They will not be able to survive under European climatic conditions, and if they would, they could easily be controlled using conventional agronomic measures normally used on commercial maize plants.

11. **Environmental monitoring plan** (not if application concerns only food and feed produced from GM plants, or containing ingredients produced from GM plants)

11.1 General (risk assessment, background information)

The scope of this application does not include cultivation of Event MIR604 maize in the EU. Environmental exposure to Event MIR604 could only occur in the unlikely event that small amounts of grain of maize Event MIR604 accidentally found their way into the environment in the EU. However, the survival of this grain would be very unlikely as maize is a highly domesticated plant and cannot survive without human intervention, especially under normal European climatic conditions. This grain, if germinated could be easily controlled using any of the current agronomic measures taken to control other commercially available maize.

An environmental risk assessment (e.r.a) has been conducted as recommended by the Guidance document of the Scientific Panel of Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA Journal, 2004). According to this guidance the e.r.a should follow the principles outlined in Directive 2001/18/EC (Annex II). The risk assessment is described as *"a process of evaluation, including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s)/event(s) occuring to man or the environment following exposure under defined conditions to a risk source(s)"*. This risk assessment has been conducted taking into account the scope of this application, which covers the import of grain for use as any other conventional maize grain, but does not include the cultivation of Event MIR604. A sequential approach to the e.r.a, as recommended by Comission Decission 2002/623/EC has been followed, so the characteristics of Event MIR604 that may cause an adverse effect have been identified, their potential consequences evaluated, the likelihood of their occurrence assessed and the overall risk posed by each characteristic identified. Comparison of Event MIR604 maize with conventional maize has been used as a baseline.

The conclusions of this e.r.a confirm that the effects to the environment arising from the use of Event MIR604 can be considered as negligible as those from any other commenrcial maize, under the scope of this application.

11.2 Interplay between environmental risk assessment and monitoring

In general two types of environmental monitoring can be described:

a. case-specific monitoring, designed to evaluate potential adverse effects linked to the genetic modification, identified in the ERA.
b. general surveillance, which is aimed to identify adverse unforeseen effects that were not anticipated in the environmental risk assessment.

An environmental risk assessment (e.r.a) has been conducted in accordance with Annex II of Directive 2001/18/EC to evaluate potential adverse effects of Event MIR604 on human and animal health and the environment. The conclusions of this e.r.a confirm that the potential risks to human and animal health or the environment arising from the placing on the market of Event MIR604 maize can be considered negligible, under the scope of this application. Therefore, a case-specific monitoring plan is not considered necessary under the scope of this application. However, a general surveillance plan based on Annex II of the Directive 2001/18/EC is outlined below (Section 11.4).

11.3 Case-specific GM plant monitoring (approach, strategy, method and analysis)

An environmental risk assessment (e.r.a) has been conducted in accordance with Annex II of Directive 2001/18/EC to evaluate potential adverse effects of Event MIR604 on human and animal health and the environment. The conclusions of this e.r.a confirm that the potential risks to human and animal health or the environment arising from the placing on the market of Event MIR604 maize can be considered negligible, under the scope of this application. Therefore, a case-specific monitoring plan is not considered necessary under the scope of this application. However, a general surveillance plan based on Annex II of the Directive 2001/18/EC is outlined below (Section 11.4).

11.4 General surveillance of the impact of the GM plant (approach, strategy, method and analysis)

The objective of general surveillance is to identify unforeseen adverse effects of the GM plant or its use, on human health and the environment, which were not predicted in the risk assessment. The scope of this application is limited to import of Event MIR604 and excludes cultivation practices. Cultivation outside the EU is accompanied by stewardship and surveillance programmes which include the provision of information to traders and processors of bulk mixtures of grain.

The provisions concerning traceability and labelling for placing on the market of Event MIR604 maize will allow the prompt identification of products containing or consisting of this maize, and thus enable any unanticipated adverse effects to be effectively traced.

The majority of imported Event MIR604 material will be used for feed purposes. Therefore, traders and processors as well as the European feed industry serve as a good focal point to address questions related to any unanticipated effects that might be associated with the use of Event MIR604.

Syngenta is committed to inform traders, processors and the European feed and food industry with details on the safety of Event MIR604 and to establish a communication network where unforeseen effects can be reported. If unusual observations are reported, more focussed in-depth studies can be carried out in order to determine cause and relationship with the specific event. Final decisions on whether any identified effects are significant can only be made if causality is clear and endpoints are determined.

Although not a formal part of the surveillance plan, it is appropriate to note that there is an extensive information network, with global reach, which will provide additional information on possible adverse effects arising from the use of GM crops. These include new and rapid means of access to information from across the globe through telecommunications, the media and Internet access. Through these means, many groups, including agronomists, ecologists, health professionals, and the general public now have unprecedented access to reports on the use world-wide of GM crops. In addition, electronic discussion sites, for example those of WHO, OECD, FAO, and consumer organisations, are valuable sources of information and communication for professionals and, in many cases, the general public.

11.5 Reporting the results of monitoring

The notifier/consent holder is responsible, under Regulation (EC) No 1829/2003, to inform the Rapporteur and the Commission of the results of the surveillance. Consistent with the EFSA guidance, the notifier will submit a General Surveillance Report containing information related to the monitoring on an annual basis.

12. Detection and event-specific identification techniques for the GM plant

A method for detection of Event MIR604 has been developed. The proposed method is based on specific detection of Event MIR604 genomic DNA a real-time quantitative TaqMan® PCR.

E. INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT AND/OR DERIVED PRODUCTS

1. History of previous releases of the GM plant notified under Part B of the Directive 2001/18/EC and under Part B of Directive 90/220/EEC by the same notifier

a) Notification number B/ES/03/14
b) Conclusions of post-release monitoring No agronomic differences between Event MIR604 and the isogenic controls were observed, nor in occurrence of volunteers, persistence or weediness.
c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC) No evidence for adverse effects on human health or the environment was found.

a) Notification number B/FR/03/03/03
b) Conclusions of post-release monitoring No differences between Event MIR604 and the isogenic controls in occurrence of volunteers, persistence or weediness were observed.
c) Results of the release in respect to any risk to human health and the environment (submitted to the Competent Authority according to Article 10 of Directive 2001/18/EC) No evidence for adverse effects on human health or the environment was found.

2. History of previous releases of the GM plant carried out outside the Community by the same notifier

a) Release country Argentina
b) Authority overseeing the release Secretaria de Agricultura, Ganaderia, Pesca y Alimentación -SAGPyA Authorization number: S01: 206838/2003
c) Release site Santa Isabel, Provincia de Santa FE
d) Aim of the release Efficacy Trial
e) Duration of the release Five months
f) Aim of post-releases monitoring Control of volunteer plant carrying the event to avoid any accidental gene release.
g) Duration of post-releases monitoring One year after harvest
h) Conclusions of post-release monitoring No differences between Event MIR604 and the isogenic controls in occurrence of volunteers, persistence or weediness were observed.
i) Results of the release in respect to any risk to human health and the environment No evidence for adverse effects on human health or the environment was found.

a) Release country US
b) Authority overseeing the release Event MIR604 hybrids have been planted in several states under USDA-APHIS comprehensive permit and notification since 2001.

c) Release site

Year	USDA Notification or Permit No.	Approved Trial Sites by State
2001	01-018-01n	IL, MN, HI
	01-022-07 r/m	IL, PR
2002	02-022-01 r/m	FL, IL, MN, NE
	02-022-02 r/m	HI
2003	03-021-01 r/m	AL,AR,AZ,CA,CO,FL,GA,IA,ID,IL,IN,KS,KY,LA,ME, MN,MO,MS,NC,NE,NM,NY,OH,PR,SD,TN,TX,WI
	03-021-02 r/m	HI
	03-038-07n	IL, TX
	03-287-07n	PR
	03-287-06n	IN
	03-287-05n	FL, HI, IL
	03-287-02n	HI, IL, PR
	03-287-01n	HI
	03-287-10n	HI, IA
	03-287-11n	HI, NE
	03-287-03n	WI
	03-287-12n	HI, IN
	03-287-04n	IL
	03-287-13n	IL
	03-300-04n	HI, IA, PR
2004	04-072-06n	AR,CA,CO,FL,HI,IA,ID,IL,IN,KS,KY,LA,MD,MN, MO,MS,NC,NE,NM,NY,OH,PA,PR,SD,TX,VA,WI
	04-076-04n	FL,HI,IA,ID,IL,IN,KY,MN,MS,NC,NE,NY,PA,PR,SD, WI
	04-085-08n	IA, IL, MN, NC
	04-086-03n	IL, MN, NC
	04-096-02n	HI, IL, NE, PR
	04-140-01n	IA, IL, NC, MN
	04-203-09n	IL, MN, NC, PR

d) Aim of the release

Efficacy, Agronomy and compositional analysis trials

e) Duration of the release

Various depending on location.

f) Aim of post-releases monitoring

Control of volunteers.

g) Duration of post-releases monitoring Various, typically one year
h) Conclusions of post-release monitoring No differences between Event MIR604 and the isogenic controls in occurrence of volunteers, persistence or weediness were observed.
i) Results of the release in respect to any risk to human health and the environment No evidence for adverse effects on human health or the environment was found.

3. Links (some of these links may be accessible only to the competent authorities of the Member States, to the Commission and to EFSA):

a) Status/process of approval To be provided
b) Assessment Report of the Competent Authority (Directive 2001/18/EC) To be provided
c) EFSA opinion To be provided
d) Commission Register (Commission Decision 2004/204/EC[1]) To be provided
e) Molecular Register of the Community Reference Laboratory/Joint Research Centre To be provided
f) Biosafety Clearing-House (Council Decision 2002/628/EC[2]) To be provided
g) Summary Notification Information Format (SNIF) (Council Decision 2002/812/EC) To be provided