Application for authorisation in the European Union of DAS-81419-2 × DAS-44406-6 soybean grain for all uses as for any other soybean, excluding cultivation, according to Articles 5 and 17 of Regulation (EC) No 1829/2003 on genetically modified food and feed

EFSA-GMO-NL-2016-132

Part VII

Summary

1. GENERAL INFORMATION

1.1 Details of application

a)	**Member State of application** The Netherlands.
b)	**Application number** EFSA-GMO-NL-2016-XX.
c)	**Name of the product (commercial and other names)** The development code for this genetically modified soybean is: DAS-81419-2 × DAS-44406-6. In countries where DAS-81419-2 × DAS-44406-6 will be cultivated, packages of this soybean will be marketed under the name of the variety, in association with the trademark (to be defined).
d)	**Date of acknowledgement of valid application** By EFSA: XXth XXXX 20XX.

1.2 Applicant

a)	**Name of applicant** Dow AgroSciences LLC represented by Dow AgroSciences Europe.
b)	**Address of applicant** Focal Point: Dow AgroSciences Europe European Development Centre 2nd Floor, 3B Park Square Milton Park, Abingdon Oxon OX14 4RN Dow AgroSciences LLC 9330 Zionsville Road Indianapolis, Indiana 46268-1054
c)	**Name and address of the representative of the applicant established in the Union (if the applicant is not established in the Union)** Dow AgroSciences Europe European Development Centre 2nd Floor, 3B Park Square Milton Park, Abingdon Oxon OX14 4RN

1.3 Scope of the application

a) GM food

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

b) GM feed

☒ Feed containing or consisting of GM plants
☒ Feed produced from GM plants or containing ingredients produced from GM plants

c) GM plants for food or feed use

☒ Products other than food and feed containing of consisting of GM plants with the exception of cultivation
☐ Seeds and plant propagating material for cultivation in the EU

1.4. Is the product or the uses of the associated plant protection product(s) already authorised or subject to another authorisation procedure within the Union?

Yes ☐	**No** ☒
If *yes*, specify	

1.5 Has the GM plant been notified under Part B of Directive 2001/18/EC?

Yes ☐	**No** ☒

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

The composition, expression, agronomic performance and environmental impact trials with DAS-81419-2 × DAS-44406-6 soybean have been performed at several locations in the US, in 2012, where soybean is commercially grown in order to gather data for the risk assessment.

A summary of the conclusions of the risk analysis that demonstrate the safety of DAS-81419-2 × DAS-44406-6 soybean to humans, animals and to the environment, has been presented in the respective sections throughout this summary.

1.6 Has the GM plant or derived products been previously notified for marketing in the Union under Part C of Directive 2001/18/EC?

Yes ☐	**No** ☒
If *yes*, specify	

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

Yes ☒	**No** ☐

If *yes*, specify the third country and provide a copy of the risk assessment conclusions, the date of the authorisation and the scope

Notification of intent to commercialize DAS-81419-2 × DAS-44406-6 has been sent to Canada, Japan, Australia and New Zealand, Argentina, Brazil, Paraguay and Uruguay. Additional applications for food and feed use are being prepared for Mexico, Columbia, South Africa, Japan, Korea, Taiwan, Philippines, and will be submitted throughout 2016.

1.8 General description of the product

a) Name of the recipient or parental plant and the intended function of the genetic modification

DAS-81419-2 × DAS-44406-6 was produced by crossing plants containing DAS-81419-2 and DAS-44406-6 using conventional breeding methods.

Expression of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins confer resistance against certain lepidopteran insect pests and tolerance to application of 2,4-D, glufosinate-ammonium and glyphosate herbicides, respectively.

b) Types of products planned to be placed on the market according to the authorisation applied for and any specific form in which the product must not be placed on the market (seeds, cut-flowers, vegetative parts, etc.) as a proposed condition of the authorisation applied for

The scope of this application according to Articles 5 and 17 of Regulation (EC) No 1829/2003 on genetically modified food and feed includes all uses of DAS-81419-2 × DAS-44406-6 soybean grain equivalent to the uses of any other soybean grain.

c) Intended use of the product and types of users

DAS-81419-2 × DAS-44406-6 soybean grain will be traded and used in the E.U. in the same manner as current commercial soybean varieties and by the same operators currently involved in the trade and use of conventional soybean.

d) Specific instructions and/or recommendations for use, storage and handling, including mandatory restrictions proposed as a condition of the authorisation applied for

No specific conditions or instructions are warranted or required for the placing on the market of DAS-81419-2 × DAS-44406-6 soybean grain, for all uses as any other soybean grain. DAS-81419-2 × DAS-44406-6 is substantially equivalent to other soybean varieties except for its resistance against certain lepidopteran insect pests and tolerance to application of 2,4-D, glufosinate,-ammonium and glyphosate herbicides, which are traits of agronomic interest. DAS-81419-2 × DAS-44406-6 was shown to be as safe and as nutritious as conventional soybean. Therefore DAS-81419-2 × DAS-44406-6 and derived products will be stored, packaged, transported, handled and used in the same manner as the commercial soybean products.

e) If applicable, geographical areas within the EU to which the product is intended to be confined under the terms of the authorisation applied for

DAS-81419-2 × DAS-44406-6 soybean grain, are suitable for import, processing

and food and feed uses throughout the E.U.

f) Any type of environment to which the product is unsuited

DAS-81419-2 × DAS-44406-6 soybean grain, are suitable for import, processing and food and feed uses throughout the E.U.

g) Any proposed packaging requirements

DAS-81419-2 × DAS-44406-6 is substantially equivalent to conventional soybean varieties (except for its resistance against certain lepidopteran insect pests and tolerance to application of 2,4-D, glufosinate-ammonium and glyphosate herbicides). Therefore, DAS-81419-2 × DAS-44406-6 and derived products will be used in the same manner as other soybean and no specific packaging is foreseen.

h) Any proposed labelling requirements in addition to those required by other applicable EU legislation than Regulation (EC) No 1829/2003 and when necessary a proposal for specific labelling in accordance with Articles 13(2) and (3), Article 25(2)(c) and (d) and Article 25(3) of Regulation (EC) No 1829/2003

In accordance with Regulations (EC) No 1829/2003 and 1830/2003, a labelling threshold of 0.9 % is applied for the placing on the market of DAS-81419-2 × DAS-44406-6 grain and derived products.

Operators shall be required to label products containing or consisting of DAS-81419-2 × DAS-44406-6 soybean grain with the words “genetically modified soybean” or “contains genetically modified soybean”, and shall be required to declare the unique identifier in the list of GMOs that have been used to constitute the mixture that contains or consists of this GMO.

Operators shall be required to label foods and feeds derived from DAS-81419-2 × DAS-44406-6 soybean grain with the words “produced from genetically modified soybean”. In the case of products for which no list of ingredients exists, operators shall ensure that an indication that the food or feed product is produced from GMOs is transmitted in writing to the operator receiving the product.

Operators handling or using DAS-81419-2 × DAS-44406-6 soybean grain and derived foods and feeds in the E.U. are required to be aware of the legal obligations regarding traceability and labelling of these products. Given that explicit requirements for the traceability and labelling of GMOs and derived foods and feeds are laid down in Regulations (EC) No 1829/2003 and 1830/2003, and that authorised foods and feeds shall be entered in the Community Register, operators in the food/feed chain will be fully aware of the traceability and labelling requirements for DAS-81419-2 × DAS-44406-6 soybean grain. Therefore, no further specific measures are to be taken by the applicant for DAS-81419-2 × DAS-44406-6 soybean grain.

i) Estimated potential demand

(i) In the EU
Comparable to that of conventional soybean.

(ii) In EU export markets
Not applicable.

j) Unique identifier in accordance with Regulation (EC) No 65/2004

DAS-81419-2 × DAS-444Ø6-6.

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

Because this application is for consent to import and use DAS-81419-2 × DAS-44406-6 soybean grain, as any other soybean, not including the cultivation of DAS-81419-2 × DAS-44406-6 varieties, environmental release would be more likely to occur during import, storage and processing of DAS-81419-2 × DAS-44406-6 soybean grain. However, modern methods of grain handling minimise losses of grain, so there is little chance of germination of spilt grain resulting in the development of mature plants of DAS-81419-2 × DAS-44406-6 in the E.U. Moreover, in the event of incidental spillage, the establishment of volunteer plants would be unlikely, since soybean cannot survive without human assistance and is not capable of surviving as a weed. Although soybean seed can over-winter in mild conditions and can germinate the following year, the appearance of soybean in rotational fields is rare under European conditions. Soybean volunteers, if they occurred, would be killed by frost or could be easily controlled by the use of selective herbicides. Moreover, the information presented in this application established that DAS-81419-2 × DAS-44406-6 is unlikely to be different from other soybean and, therefore, is unlikely to pose any threat to the environment or to require special measures for its containment.

No specific conditions are warranted or required for the placing on the market of DAS-81419-2 × DAS-44406-6 soybean grain, for import, processing, or use for food and feed.

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

2.1. Complete name

a)	**Family name** Leguminosae.
b)	**Genus** *Glycine.*
c)	**Species** *Glycine max.*
d)	**Subspecies** N/A.
e)	**Cultivar/breeding line or strain** DAS-81419-2 × DAS-44406-6.
f)	**Common name** Soybean.

2.2 Geographical distribution and cultivation of the plant, including the distribution within the Union

Soybean can only cross with other members of *Glycine* subgenus *Soja*. The potential for such gene flow is limited by geographic isolation and by the fact that they are highly self-pollinating species. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR, and do not exist naturally in the EU.

2.3 Information concerning reproduction

a)	**Mode(s) of reproduction** Soybean is considered a self-pollinated species, propagated commercially by seed.
b)	**Specific factors affecting reproduction** The seed will germinate when the soil temperature reaches 10^{o}C and will emerge in a 5-7 day period under favourable conditions. In new areas of soybean production an inoculation with *Bradyrhizobium japonicum* is necessary, for optimum efficiency of the nodulated root system.
c)	**Generation time** From seeding to maturity, soybean passes through various growth stages (e.g., germination — seedling stage, third true-leaf stage); the entire growing period is 120–140 days.

2.4 Sexual compatibility with other cultivated or wild plant species

Gene transfer between cultivated soybean and wild species of subgenus *Soja* may occur, but not in Europe, where the wild relatives of subgenus *Soja* are not present.

2.5 Survivability (for environmental safety aspects)

a) Ability to form structures for survival or dormancy

Cultivated soybean seed rarely displays any dormancy characteristics and only under certain environmental conditions grows as a volunteer in the year following cultivation.

b) Specific factors affecting survivability

Soybean is a quantitative short day plant and hence flowers more quickly under short days. As a result, photoperiodism and temperature response are important in determining areas of cultivar adaptation.

2.6 Dissemination (for environmental safety aspects)

a) Ways and extent of dissemination

Soybean is considered a self-pollinated species, propagated commercially by seed.

b) Specific factors affecting dissemination

The soybean flower stigma is receptive to pollen approximately 24 hours before anthesis and remains receptive 48 hours after anthesis. The anthers mature in the bud and directly pollinate the stigma of the same flower. As a result, soybeans exhibit a high percentage of self-fertilisation, and cross pollination is usually less than one percent.

2.7 Geographical distribution within the Union of the sexually compatible species (for environmental safety aspects)

Soybean can only cross with other members of *Glycine* subgenus *Soja*. The potential for such gene flow is limited by geographic isolation and by the fact that they are highly self-pollinating species. Wild soybean species are endemic in China, Korea, Japan, Taiwan and the former USSR, and do not exist naturally in the EU.

In addition small amounts of soybean are commercially produced within Europe with Italy producing the highest amount at approximately 160,000 hectares in 2010, followed by France producing 50,000 hectares and Hungary producing 40,000 hectares.

2.8 In the case of plant species not normally grown in the Union description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts (for environmental safety aspects)

Soybeans are grown in the EU commercially.

2.9 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 (for environmental safety aspects)

Soybean it has a history of safe use for human food and animal feed. However, soybean 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 nematode, insect and mite pests.

3. MOLECULAR CHARACTERISATION

3.1 Information relating to the genetic modification

a) Description of the methods used for the genetic modification

DAS-81419-2 × DAS-44406-6 was produced by crossing plants containing DAS-81419-2 and DAS-44406-6 using conventional breeding methods.

Both DAS-81419-2 and DAS-44406-6 were developed through *Agrobacterium*-mediated transformation of soybean.

b) Nature and source of the vector used

DAS-81419-2 × DAS-44406-6 has been obtained by conventional breeding of DAS-81419-2 and DAS-44406-6 and no vector has been used to produce this soybean stack.

c) Source of donor DNA used for transformation, size and intended function of each constituent fragment of the region intended for insertion

By crossing DAS-81419-2 and DAS-44406-6, by conventional breeding, DAS-81419-2 × DAS-44406-6 inherits the inserted DNA from both parental soybean lines.

The individual components and the function of these inherited DNA sequences are given in Tables 1 and 2.

Table 1. Components of the inserted DNA inherited from DAS-81419-2

Feature Name	Feature Start	Feature Stop	Feature Length	Description
5' Flanking border	1	1297	1297	Soybean genomic DNA flanking the 5' end of the insert in DAS-81419-2 soybean
Re-arranged sequence	1298	1321	24	Re-arranged DNA fragment at the 5' end of the insert
cry1Ac(synpro) partial fragment	1322	1419	98	Complementary *cry1Ac(synpro*) partial fragment at the 5' end of the insert that is 99% identical to 1990 - 2087 bp of the full-length *cry1Ac(synpro)* gene
Re-arranged sequence	1420	1432	13	Re-arranged DNA fragment at the 5' end of the insert
Partial T-DNA Border B	1433	1433	1	Last nucleotide from T-DNA Border B which is required for transfer of DNA from *Agrobacterium tumefaciens* into plant cells
Intervening sequence	1434	1704	271	Non-specific DNA sequences necessary for cloning
AtUbi10 promoter	1705	3026	1322	AtUbi10 promoter along with the 5' untranslated region and intron from *Arabidopsis thaliana* polyubiquitin 10 (UBQ10) gene
Intervening sequence	3027	3034	8	Non-specific DNA sequences necessary for cloning
cry1F v3	3035	6481	3447	*cry1F v3* (synthetic version of the *cry1F* gene from *Bacillus thuringiensis* subsp. *aizawai* strain PS811)
Intervening sequence	6482	6583	102	Non-specific DNA sequences necessary for cloning
AtuORF23 3' UTR	6584	7040	457	AtuORF23 3' UTR (3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of *Agrobacterium tumefaciens* pTi15955
Intervening sequence	7041	7103	63	Non-specific DNA sequences necessary for cloning
CsVMV promoter	7104	7620	517	CsVMV promoter along with the 5' untranslated region derived from Cassava Vein Mosaic virus
Intervening sequence	7621	7629	9	Non-specific DNA sequences necessary for cloning
cry1Ac(synpro)	7630	11100	3471	*cry1Ac (synpro)* (synthetic version of the *cry1Ac* gene from *Bacillus thuringiensis* subsp. *kurstaki* strain HD73)

Intervening sequence	11101	11133	33	Non-specific DNA sequences necessary for cloning
AtuORF23 3' UTR	11134	11590	457	AtuORF23 3' UTR (3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of *Agrobacterium tumefaciens* pTi15955)
Intervening sequence	11591	11704	114	Non-specific DNA sequences necessary for cloning
CsVMV promoter	11705	12221	517	CsVMV promoter along with the 5' untranslated region derived from Cassava Vein Mosaic virus
Intervening sequence	12222	12228	7	Non-specific DNA sequences necessary for cloning
pat	12229	12780	552	*pat* (synthetic version of phosphinothricin acetyl transferase gene from *Streptomyces viridochromogenes*)
Intervening sequence	12781	12882	102	Non-specific DNA sequences necessary for cloning
AtuORF1 3' UTR	12883	13586	704	AtuORF1 3' UTR (3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 1 (ORF1) of *Agrobacterium tumefaciens* pTi15955)
Intervening sequence	13587	13784	198	Non-specific DNA sequences necessary for cloning
Re-arranged sequence	13785	13793	9	Re-arranged DNA fragment at the 3' end of the insert
3' Flanking border	13794	15172	1379	Soybean genomic DNA flanking the 3' end of the insert in DAS-81419-2 soybean

Table 2. Components of the inserted DNA inherited from DAS-44406-6

Feature Name	Feature Start	Feature Stop	Feature Length	Description
T-DNA Border B	1	24	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells
Intervening sequence	25	160	136	Non-specific DNA sequences necessary for cloning
RB7 MAR	161	1326	1166	Matrix attachment region from the *Nicotiana tabacum* rb-7-5A gene
Intervening sequence	1327	1365	39	Non-specific DNA sequences necessary for cloning
Histone H4A748 3' UTR	1366	2026	661	3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of the histone H4A748 gene from *Arabidopsis thaliana*
Intervening sequence	2027	2049	23	Non-specific DNA sequences necessary for cloning
2mepsps	2050	3387	1338	Native 5-enolpyruvylshikimate-3-phosphate synthase gene from *Zea mays* with two mutations providing glyphosate tolerance
TPotp C	3388	3759	372	Optimized chloroplast transit peptide derived from maize and sunflower RuBisCO
Intervening sequence	3760	3763	4	Non-specific DNA sequences necessary for cloning

Histone H4A748 promoter	3764	5193	1430	Promoter along with the 5' untranslated region of the Histone H4A748 gene from *Arabidopsis thaliana* including an intron from the Histone 3 gene from *Arabidopsis thaliana*
Intervening sequence	5194	5285	92	Non-specific DNA sequences necessary for cloning
AtUbi10 promoter	5286	6607	1322	Promoter along with the 5' untranslated region and intron from the *Arabidopsis thaliana* polyubiquitin 10 (UBQ10) gene
Intervening sequence	6608	6615	8	Non-specific DNA sequences necessary for cloning
aad-12	6616	7497	882	Plant-optimized aryloxyalkanoate dioxygenase gene from *Delftia acidovorans* encoding an enzyme with an alpha ketoglutarate-dependent dioxygenase activity which results in metabolic inactivation of the herbicide(s)
Intervening sequence	7498	7599	102	Non-specific DNA sequences necessary for cloning
AtuORF23 3' UTR	7600	8056	457	3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 23 (ORF23) of plasmid pTi15955 from *Agrobacterium tumefaciens*
Intervening sequence	8057	8170	114	Non-specific DNA sequences necessary for cloning
CsVMV promoter	8171	8687	517	Promoter along with the 5' untranslated region derived from the Cassava Vein Mosaic virus
Intervening sequence	8688	8694	7	Non-specific DNA sequences necessary for cloning
pat	8695	9246	552	Plant-optimized version of phosphinothricin acetyl transferase (PAT) gene, isolated *from Streptomyces viridochromogenes*, encoding a protein that confers tolerance to glufosinate-ammonium
Intervening sequence	9247	9348	102	Non-specific DNA sequences necessary for cloning
AtuORF1 3' UTR	9349	10052	704	3' untranslated region (UTR) comprising the transcriptional terminator and polyadenylation site of open reading frame 1 (ORF1) of plasmid pTi15955 from *Agrobacterium tumefaciens*
Intervening sequence	10053	10280	228	Sequence from Ti plasmid C58
T-DNA Border A	10281	10304	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells
Intervening sequence	10305	10323	19	Sequence from Ti plasmid C58
T-DNA Border A	10324	10347	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells, aiming to prevent vector DNA being transferred into plant genome
Intervening sequence	10348	10634	287	Sequence from Ti plasmid pTi15955
T-DNA Border A	10635	10658	24	Required for transfer of T-DNA insert from *Agrobacterium tumefaciens* into plant cells, aiming to prevent vector DNA being transferred into plant genome

Plasmid backbone sequences	10659	11037	379	Plasmid backbone sequences from RK2 plasmid
Ori Rep	11038	12057	1020	Replication origin sequences from RK2 plasmid
Plasmid backbone sequences	12058	12602	545	Plasmid backbone sequences from RK2 plasmid
Trf A	12603	13751	1149	Plasmid replication sequences for Trf A protein from RK2 plasmid
Plasmid backbone sequences	13752	14955	1204	Plasmid backbone sequences from RK2 plasmid
Spec R	14956	15744	789	Sequences for Spectinomycin resistance gene
Plasmid backbone sequences	15745	16018	274	Plasmid backbone sequences for cloning

3.2 Information relating to the GM plant

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

DAS-81419-2 × DAS-44406-6 soybean expresses the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins, derived from *Bacillus thuringiensis, Delftia acidovorans, Streptomyces viridochromogenes*and *Zea mays*, providing tolerance to application of 2,4-D, glufosinate-ammonium and glyphosate herbicides, respectively.

Commercialisation of DAS-81419-2 × DAS-44406-6 will therefore provide substantial benefits to growers by limiting yield losses from insect pests and weed pressure.

3.2.2 . Information on the nucleic acid(s) sequences actually inserted or deleted

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

DAS-81419-2 and DAS-44406-6 each contain a single DNA insert with a single functional copy of the introduced DNA fragment.

The genome of DAS-81419-2 × DAS-44406-6 contains two different inserts, one derived from DAS-81419-2 and one derived from DAS-44406-6. The presence of these inserts in the hybrid was confirmed through Southern blot analysis.

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

Not applicable.

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

The conventionally bred DAS-81419-2 × DAS-44406-6 contains the single product inserts in the nuclear genome, as they were present in the single products DAS-81419-2 and DAS-44406-6, respectively.

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

Since the inserts present in DAS-81419-2 × DAS-44406-6 correspond to those of the parental lines, the characteristics of the insertions and the 5’ and 3’ flanking sequences are likely to have been conserved in this combined trait product.

(e) In case of modifications other than insertion or deletion, describe function of the modified genetic material before and after the modification as well as direct changes in expression of genes as a result of the modification

Not Applicable.

3.2.3 Information on the expression of the insert

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

The levels of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins in various tissues of DAS-81419-2 × DAS-44406-6 were assessed by validated enzyme-linked immunosorbent assays (ELISA).

Tissue samples for analysis were collected from nine test sites during field trials conducted in the U.S.A. in 2012. The locations of these trials represent the major soybean producing regions of the U.S.A. and provide a variety of environmental conditions.

The data show that the levels of Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins in grain of DAS-81419-2 × DAS-44406-6 are comparable to protein levels in the positive controls substances, DAS-81419-2 and DAS-44406-6, as appropriate.

b) Parts of the plant where the insert is expressed

Results of the analyses confirm expression of Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins throughout key development stages of DAS-81419-2 × DAS-44406-6. Expression levels in grain are most relevant for food and feed safety evaluation.

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

Based on the molecular characterisation of DAS-81419-2 × DAS-44406-6, it is highly likely that the insert sequences of DAS-81419-2 × DAS-44406-6 are conserved with their inherent properties.

3.2.5 Information on how the GM plant differs from the recipient plant in

a) Mode(s) and/or rate of reproduction

Agronomic data collected from trials performed with DAS-81419-2 × DAS-44406-6 have demonstrated that DAS-81419-2 × DAS-44406-6 has not been altered in survival, multiplication or dissemination characteristics when compared to conventional soybean varieties. The trait for herbicide tolerance has no influence on soybean reproductive morphology and hence no changes in seed dissemination would be expected.

b) Dissemination

The inherited traits have no influence on soybean reproductive morphology and hence no changes in seed dissemination are to be expected.

c) Survivability

Soybean is known to be a weak competitor in the wild, which cannot survive outside cultivation without human intervention. Field observations have demonstrated that DAS-81419-2 × DAS-44406-6 has not been altered in its survivability when compared to conventional soybean.

d) Other differences

Comparative assessments in the field did not reveal any biologically significant differences between DAS-81419-2 × DAS-44406-6 and conventional soybean varieties, except for the introduced trait that is of agronomic interest.

3.2.6 Any change to the ability of the GM plant to transfer genetic material to other organisms (for environmental safety aspects)

a) Plant to bacteria gene transfer

None of the genetic elements inserted in DAS-81419-2 × DAS-44406-6 have a genetic transfer function. Therefore, no changes are expected in the ability of these soybean lines to transfer genetic material to bacteria.

b) Plant to plant gene transfer

There is a negligible probability for plant to plant gene transfer as the scope of the current application does not include the cultivation of DAS-81419-2 × DAS-44406-

6 varieties in the E.U.

4 COMPARATIVE ANALYSIS

4.1 Choice of the conventional counterpart and additional comparators

DAS-81419-2 × DAS-44406-6 was compared with a conventional control soybean with similar genetic background, as well as with other commercially available soybean varieties.

4.2 Experimental design and statistical analysis of data from field trials for comparative analysis

DAS-81419-2 × DAS-44406-6 and the conventional control soybean were grown at nine field sites in major soybean-growing areas of the U.S.A. during the 2012 field season.

The compositional study compared DAS-81419-2 × DAS-44406-6 to the non-transgenic near-isogenic control soybean Maverick. Reference lines were grown in the same field locations and under the same conditions as the test and control. Where statistical differences occurred, the measured analyte was compared to reference ranges.

4.3 Selection of material and compounds for analysis

The numerous compounds that were selected for analysis in the compositional study were chosen on the basis of internationally accepted guidance provided by the OECD (*See* consensus document for compositional analysis of soybean), in addition to other selected compounds.

Based on the positive results of these extensive, compositional analyses conducted for DAS-81419-2 × DAS-44406-6 compared to conventional soybean varieties, there is no indication to further analyse other selected compounds in this soybean.

4.4 Comparative analysis of agronomic and phenotypic characteristics

Field trials with DAS-81419-2 × DAS-44406-6 were performed and the set of agronomic observations supports a conclusion that from an agronomic and phenotypic (morphological) point of view, DAS-81419-2 × DAS-44406-6 is equivalent to conventional soybean, except for the resistance against certain lepidopteran insect pests and tolerance to application of 2,4-D, glufosinate,-ammonium and glyphosate herbicides.

4.5 Effect of processing

Soybean is converted into a diverse range of food and feed products and derivatives used as food and feed ingredients or additives. As DAS-81419-2 × DAS-44406-6 is substantially equivalent and as safe and as nutritious as conventional soybean, the use of DAS-81419-2 × DAS-44406-6 soybean grain for the production of foods and feeds is no different from that of conventional soybean. Consequently, any effects of the production and processing of DAS-81419-2 × DAS-44406-6 soybean grain are not expected to be any different from the production and processing of the equivalent foods and feeds, originating from conventional soybean.

5 TOXICOLOGY

a) Toxicological testing of newly expressed proteins

DAS-81419-2 × DAS-44406-6 expresses the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins. The conclusion of safety to humans of these proteins was based upon the following considerations:

- The proteins have a history of safe use;
- They have no structural similarity to known toxins or other biologically active proteins that could cause adverse effects in humans or animals;
- They do not exert any acute toxicity to mammals.

In addition, their low concentration in tissues that are consumed and their rapid digestibility in simulated digestive fluids provide additional assurance for their safety.

It is therefore highly unlikely that the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins would cause any toxic effects on human or animal health.

b) Testing of new constituents other than proteins

Since soybean is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world and as DAS-81419-2 × DAS-44406-6 was shown to be substantially equivalent to conventional soybean, no testing of any constituent other than the inherited proteins are indicated.

c) Information on natural food and feed constituents

Soybean is known as a common source of food and feed with a centuries-long history of safe use and consumption around the world. No particular natural constituents of soybean are considered to be of significant concern to require additional information or further risk assessment.

d) Testing of the whole GM food/feed

Evaluation of the nutrient composition of DAS-81419-2 × DAS-44406-6 soybean proved its equivalency to non-GM control soybean with comparable genetic background and to representative commercial lines. In addition it's been shown that the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins expressed in DAS-81419-2 × DAS-44406-6 soybean are safe for humans, animal health and the environment. On that basis, no additional studies are required.

6. ALLERGENICITY

a) Assessment of allergenicity of the newly expressed protein

The Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins have been assessed for their potential allergenicity according to the recommendations of Codex Alimentarius Commission. The proteins are from non-allergenic sources, lack structural similarity to known allergens, are rapidly digested in simulated gastric fluid, constitute a very small portion of the total protein present in the grain of DAS-81419-2 × DAS-44406-6. Taken together, these data lead to the conclusion that the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins are unlikely to have any allergenic potential; hence, DAS-81419-2 × DAS-44406-6 is as safe as conventional soybean regarding the risk for allergenicity.

b) Assessment of allergenicity of the whole GM plant

Compositional analyses, comparative phenotypic assessments and animal feeding studies have demonstrated that DAS-81419-2 × DAS-44406-6 is substantially equivalent to traditional soybean, with the exception of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins (which are unlikely to have any allergenic potential). Moreover, the whole food derived from DAS-81419-2 × DAS-44406-6 shows no potential change in the overall allergenicity when compared to a non transgenic control.

7. NUTRITIONAL ASSESSMENT

a) Nutritional assessment of GM food

The introduced traits in DAS-81419-2 × DAS-44406-6 are of agronomic interest, and are not intended to change any nutritional aspects of this soybean. Hence this soybean is not expected to be more or less attractive for use as food (or feed), for processing, or as a food (or feed) ingredient. Therefore, anticipated dietary intake of soybean-derived foods and feeds is not expected to be altered upon commercialisation of DAS-81419-2 × DAS-44406-6 soybean grain, and no nutritional imbalances are expected as a result of the use of DAS-81419-2 × DAS-44406-6 soybean grain.

b) Nutritional assessment of GM feed

As discussed throughout this application, animal feed products from DAS-81419-2 × DAS-44406-6 soybean are substantially equivalent to, nutritionally equivalent to, and as safe as feed derived from commercial soybean.

8. EXPOSURE ASSESSMENT – ANTICIPATED INTAKE/EXTENT OF USE

There are no anticipated changes in the intake and/or extent of use of soybean or derived products for use as such or in food or feed as a result of the addition of DAS-81419-2 × DAS-44406-6 soybean grain to the conventional soybean supply. DAS-81419-2 × DAS-44406-6 soybean grain is expected to replace a portion of current soybean varieties such that their intake or use will represent some fraction of the total products derived from soybean.

9. RISK CHARACTERISATION

Assessments show that DAS-81419-2 × DAS-44406-6 demonstrates agronomic, phenotypic and compositional equivalence to non-transgenic soybean. It has also been established that it is highly unlikely that Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins will be toxic or allergenic making it negligible that DAS-81419-2 × DAS-44406-6 will cause adverse effects in humans or animals.

10. POST-MARKET MONITORING ON GM FOOD OR FEED

The assessment of the human and animal safety of DAS-81419-2 × DAS-44406-6 was conducted on the basis of its substantial equivalence to conventional soybean (except for the introduced traits) and by extensive characterisation of the introduced traits, which are of agronomic interest, resulting in the expression of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins.

The pre-market risk characterisation for food and feed use of DAS-81419-2 × DAS-44406-6 demonstrates that the risks of consumption of DAS-81419-2 × DAS-44406-6 or its derived products are consistently negligible and no different from the risks associated with the consumption of conventional soybean and soybean-derived products.

As a consequence, specific risk management measures are not indicated, and post-market monitoring of the use of this soybean for food, feed or processing is neither warranted, nor appropriate.

11. ENVIRONMENTAL ASSESSMENT

11.1 Mechanism of interaction between the GM plant and target organisms

The Cry1F and Cry1Ac proteins produced in DAS-81419-2 × DAS-44406-6 provide protection against lepidopteran pests. Those lepidopteran insects may be considered the target organisms which interact with DAS-81419-2 × DAS-44406-6. The AAD-12, PAT and 2mEPSPS proteins (conferring tolerance to application of 2,4-D, glufosinate-ammonium and glyphosate herbicides), also present in DAS-81419-2 × DAS-44406-6, do not have target organisms.

A generalized mode of action of Cry proteins includes the following steps: ingestion of the protoxin by the insect, solubilization of the crystal in the insect midgut, proteolytic processing of the released Cry protein by digestive enzymes to produce an active toxin termed delta-endotoxin, binding of the endotoxin to receptors on the surface of midgut epithelial cells of target organisms, formation of membrane ion channels or pores, and consequent disruption of cellular homeostasis. Electrolyte imbalance and pH changes render the gut paralyzed, which causes the insect to stop eating and die.

Any significant interactions of DAS-81419-2 × DAS-44406-6 with its target pest organisms are, however, limited to those countries where the cultivation of this soybean will be authorised. The cultivation of DAS-81419-2 × DAS-44406-6 in the E.U. are not within the scope of this application. The likelihood that the import and use of DAS-81419-2 × DAS-44406-6 grain for food, feed or processing will result in plants of these soybean lines being present in the environment is negligible.

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

a) Persistence and invasiveness

Like for conventional soybean, the likelihood of DAS-81419-2 × DAS-44406-6 spreading in the environment is negligible, as soybean is neither persistent nor invasive and these parameters are unaltered in DAS-81419-2 × DAS-44406-6 when compared to conventional soybean. In the unlikely event of the establishment of DAS-81419-2 × DAS-44406-6 plants in the environment, the introduced traits would confer only a limited selective advantage of: resistance against certain lepidopteran insect pests and tolerance to application of 2,4-D, glufosinate-ammonium and glyphosate herbicides of short duration, narrow spatial context and with negligible consequences for the environment. Hence, the risk of establishment and spreading of DAS-81419-2 × DAS-44406-6 soybean grain into the environment is negligible.

b) Selective advantage or disadvantage

Compared with conventional soybean, the presence of the introduced traits in DAS-81419-2 × DAS-44406-6 would only confer a meaningful advantage under specific conditions, i.e. high lepidopteran insect pressure or where plants would be treated with 2,4-D, glufosinate-ammonium and glyphosate herbicides; if no other more important factors limiting its survival in the environment were present. This introduced “advantage” is only relevant in agricultural habitats (i.e. in soybean fields) and is short in duration. The risk of 2,4-D, glufosinate-ammonium and glyphosate tolerance traits in DAS-81419-2 × DAS-44406-6 to be the cause of any adverse effects resulting from a competitive advantage or disadvantage is negligible, as soybean is unlikely to establish outside cultivation under European conditions (see Section E.3.1). When viewed in the context of today’s baseline

agronomic practices for the production of soybean, these advantages present negligible risk to the agricultural environment.

c) Potential for gene transfer

DAS-81419-2 × DAS-44406-6 is unchanged in its potential for gene transfer compared to conventional soybean. There is no potential for gene transfer from DAS-81419-2 × DAS-44406-6 to wild plant species in the E.U. and negligible likelihood for gene transfer to other soybean crops, as this application is not for consent to cultivate DAS-81419-2 × DAS-44406-6 varieties in the E.U. The environmental risk of potential gene transfer is negligible. Further to this searches against microbial databases indicate it is highly unlikely a Homologous Recombination event can happen between DAS-81419-2 and DAS-44406-6 sequences, and any sequence present in the bacteria, plasmid and virus sequence databases, meaning the potential for horizontal gene transfer is negligible.

d) Interactions between the GM plant and target organisms

Since the likelihood is negligible that the import, processing and food and feed use of DAS-81419-2 × DAS-44406-6 will result in plants of this soybean being present in the environment at meaningful levels, it is not expected that organisms will be exposed to the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins.

e) Interactions of the GM plant with non-target organisms

Given the scope of the current application, which does not include the cultivation of DAS-81419-2 × DAS-44406-6 varieties in the E.U., the likelihood for direct or indirect interactions of these soybean lines with non-target organisms is considered to be negligible. In addition, the newly expressed proteins present a negligible hazard to non-target organisms, even if incidental spillage of DAS-81419-2 × DAS-44406-6 grain during import, storage, transport or use would lead to the short survival of DAS-81419-2 × DAS-44406-6 plants in the environment. As a consequence, there is negligible risk for harmful effects of DAS-81419-2 × DAS-44406-6 on non-target organisms, either through direct or indirect interactions with this soybean or through contact with the newly expressed protein.

Furthermore, no adverse effects were brought forward by the people handling these products during the field trials conducted in the U.S.A.

f) Effects on human health

The likelihood for any adverse effects occurring in humans as a result of their contact with this soybean is no different from conventional soybean. DAS-81419-2 × DAS-44406-6 expresses the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins, which have negligible potential to cause any toxic or allergenic effects in humans. Therefore, the risk of changes in the occupational health aspects of this soybean is negligible.

g) Effects on animal health

The likelihood of potential adverse effects in animals fed on DAS-81419-2 × DAS-44406-6 and in humans, consuming those animals, is negligible. Therefore, the risk of DAS-81419-2 × DAS-44406-6 for the feed/food chain is also negligible.

h) Effects on biogeochemical processes

There is no evidence that DAS-81419-2 × DAS-44406-6 plants would be any different from conventional soybean regarding their direct influence on biogeochemical processes or nutrient levels in the soil, as DAS-81419-2 × DAS-44406-6 is compositionally equivalent and has equivalent growth and development as conventional soybean.

i) Impacts of the specific cultivation, management and harvesting techniques

Not applicable. This application is for consent to import DAS-81419-2 × DAS-44406-6 soybean grain in the E.U. and for the use of these soybean lines as any other soybean, excluding the cultivation of varieties in the E.U.

11.3 Potential interactions with the abiotic environment

No adverse impact of DAS-81419-2 × DAS-44406-6 on the abiotic environment is expected to result from the import, processing or use of this product for food and feed in the E.U. Although the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins are introduced proteins in soybean, they already have a safe history and have no known negative interactions with the abiotic environment. The *Bacillus huringiensis*, *Delftia acidovorans*, *Streptomyces viridochromogenes* and *Zea mais*. 2mfrom which the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins are derived are widespread in nature and found all over the world. The Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins are innocuous and are ubiquitous in nature. The families of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins have no known negative interactions with the abiotic environment.

11.4 Risk characterisation for the environmental risk assessment

Considering the scope of this application is for import for food and feed uses of DAS-81419-2 × DAS-44406-6 and that cultivation of DAS-81419-2 × DAS-44406-6 soybean varieties in the EU is not planned; any exposure to the environment from the import of DAS-81419-2 × DAS-44406-6 soybean will be limited to unintended release via spillage during transportation of the grain. There are no target organisms for the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins expressed in DAS-81419-2 × DAS-44406-6, which confers tolerance to certain herbicides.

Therefore, the likelihood that the import and use of DAS-81419-2 × DAS-44406-6 for food, feed or processing will result in plants of this soybean being present in the environment is negligible.

12. ENVIRONMENTAL MONITORING PLAN

a) General (risk assessment, background information)

As required by Article 5(5)(b) and 17(5)(b) of Regulation (EC) No 1829/2003 the proposed monitoring plan for DAS-81419-2 × DAS-44406-6 has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC. The structure of the monitoring plan also takes into account the guidance on presentation of applications provided in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed.

b) Interplay between environmental risk assessment and monitoring

An environmental risk assessment (e.r.a.) was carried out for DAS-81419-2 × DAS-44406-6 according to the principles laid down in Annex II to Directive 2001/18/EC and Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC. The scientific evaluation of the characteristics of DAS-81419-2 × DAS-44406-6 in the e.r.a. (Section E.3) has

shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses of DAS-81419-2 × DAS-44406-6 soybean grain.

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

The scientific evaluation of the characteristics of DAS-81419-2 × DAS-44406-6 in the e.r.a. has shown that the risk for potential adverse effects on human and animal health or the environment is negligible in the context of the intended uses. It is therefore considered that there is no need for case-specific monitoring.

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

In accordance with Council Decision 2002/811/EC, general surveillance is not based on a particular hypothesis and it should be used to identify the occurrence of unanticipated adverse effects of the viable GMO or its use for human and animal health or the environment that were not predicted in the e.r.a.

The authorisation holders are not involved in commodity trade with DAS-81419-2 × DAS-44406-6 soybean grain. The monitoring methodology hence needs to be predominantly based on collaboration with third parties, such as operators involved in the import, handling and processing of viable DAS-81419-2 × DAS-44406-6 soybean grain. They are exposed to the imported viable DAS-81419-2 × DAS-44406-6 soybean grain and therefore are the best placed to observe and report any unanticipated adverse effects in the framework of their routine surveillance of the commodities they handle and use.

The general surveillance information reported to and collected by the authorisation holders from the European trade associations or other sources will be analysed for its relevance. Where information indicates the possibility of an unanticipated adverse effect, the authorisation holder will immediately investigate to determine and confirm whether a significant correlation between the effect and DAS-81419-2 × DAS-44406-6 soybean grain can be established. If the investigation establishes that DAS-81419-2 × DAS-44406-6 soybean grain was present when the adverse effect was identified, and confirms that DAS-81419-2 × DAS-44406-6 soybean grain is the cause of the adverse effect, the authorisation holders will immediately inform the European Commission, as described in Section E.4.3.4.

e) Reporting the results of the monitoring

The authorisation holders will submit an annual monitoring report containing information obtained from participating networks, and/or in case of an effect that was confirmed. If information that confirms an adverse effect which alters the existing risk assessment becomes available, Dow AgroSciences LLC will submit a report, consisting of a scientific evaluation of the potential adverse effect and a conclusion on the safety of the product. The report will also include, where appropriate, the measures that were taken to ensure the safety of human or livestock health and/or the environment.

13. DETECTION AND IDENTIFICATION TECHNIQUES FOR THE GM PLANT

The PCR detection methods to confirm the molecular identity of DAS-81419-2 × DAS-44406-6 soybean along with complementary information and samples of DAS-81419-2 × DAS-44406-6 soybean and non-GM soybean that have been provided to the JRC-IHCP (Joint Research Centre-Institute of Health and Consumer Protection).

The Institute for Reference Materials and Measurements (IRMM) has collaborated with Dow AgroSciences, to develop certified reference materials for DAS-81419-2 and DAS-44406-6 soybeans. The sales conditions of certified reference materials for DAS-68416-4 are available from the IRMM website (http://irmm.jrc.ec.europa.eu/html/homepage.htm). Detailed information on these materials is given in the IRMM reports and sample certificates, posted on the corresponding websites.

14 INFORMATION RELATING TO PREVIOUS RELEASES OF THE GM PLANT (FOR ERA ASPECTS)

14.1 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

None.

b) Conclusions of post-release monitoring

N/A.

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)

N/A.

14.2 History of previous releases of the GM plant carried out outside the Union by the same notifier

a) Release country

DAS-81419-2 × DAS-44406-6 has been field tested in the U.S.A. in 2012.

b) Authority overseeing the release

U.S.A: United States Department of Agriculture (USDA).
Argentina: CONABIA.

c) Release site

U.S.A.: Multiple sites in soybean producing states of the U.S.A.
Argentina: multiple growing regions.

d) Aim of the release

	U.S.A.: assess performance, efficacy, variety evaluation, seed production, yield, and collection of regulatory data. Argentina: Assess performance, efficacy, yield, and collection of regulatory data.
e)	**Duration of the release** 12 months per release.
f)	**Aim of post-releases monitoring** Assessment/removal of volunteers.
g)	**Duration of post-releases monitoring** 12 months per release.
h)	**Conclusions of post-release monitoring** Volunteers have been eliminated to prevent potential persistence in the environment.
i)	**Results of the release in respect to any risk to human health and the environment** No evidence that DAS-81419-2 × DAS-44406-6 is likely to cause any adverse effects to human or animal health or the environment.