Notification C/BE/96/01

Oilseed Rape Ms8xRf3

Summary Information Format

January 2003

SUMMARY INFORMATION FORMAT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs)

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification : Belgium
b) Notification number: C/BE/96/01
c) Name of the product (commercial and other names): The product covered by notification C/BE/96/01 includes: • The female line containing event Ms8 and all progeny derived through traditional breeding crosses with non-genetically modified oilseed rape. • The male line (fertility restoration) containing event Rf3 and all progeny derived through traditional breeding crosses with non-genetically modified oilseed rape. • The hybrid seeds from traditional crossings between parental lines containing events Ms8 and Rf3. • Hybrid seeds will be commercialised under the trade name SeedLink® oilseed rape.
d) Date of acknowledgement of notification: 1st October 1996.

2. Notifier

a) Name of notifier: Bayer BioScience N.V.
b) Address of notifier: Jozef Plateaustraat, 22 B-9000 Gent.
c) Is the notifier : domestic manufacturer : X *importer ☐*
d) In the case of an import the name and address of the manufacturer shall be given. Not applicable.

3. General description of the product

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

The recipient organism is a commercial spring variety of oilseed rape commonly cultivated in Europe, which has been genetically modified to introduce a pollination control system (hybrid system), linked to a herbicide tolerant trait.

Pollination control:

Oilseed rape is a crop capable of undergoing both self-pollination (70%) as well as cross-pollination (30%). Therefore a system to ensure only cross-pollination is required for producing hybrids from two distinct parents. The SeedLink® hybridisation system allows that the female plants are pollinated by the desired male plants. It is based on:

- A female line obtained by the unique combination of a natural catabolic activity, a ribonuclease (Barnase protein produced by the *barnase* gene), and a DNA sequence limiting its expression to a specific stage and time during the development of the anthers. This female line does not produce pollen and thereby prevents self-pollination and enables the production of hybrids.
- A fertility restoration line (male line) harbouring a highly specific inhibitor (Barstar protein produced by the *barstar* gene) of the introduced ribonuclease (Barnase protein). Full fertility restoration is obtained after a cross between the female line and the fertility restoration line. Fertility restoration ensures that the hybrid seed is itself fully fertile in the farmer's field.

The resulting hybrid oilseed rape varieties have higher yields than conventional varieties, while their consistent growth and even ripening make harvesting easier.

Herbicide tolerance:

The SeedLink® hybridisation system is combined with the LibertyLink® trait of tolerance to Liberty® herbicide (active ingredient glufosinate-ammonium), through the PAT protein (produced by the *bar* gene) that degrades the herbicide. Liberty® enables farmers to use a broad spectrum herbicide with positive environmental characteristics and to avoid the precautionary pre-emergence herbicide treatments: it allows for postponing weed control operations and herbicide applications until really necessary, contributing an important tool in Integrated Crop Management. Farmers are thus given the flexibility to tolerate flora and fauna in their fields that do not pose a threat to either the quality or the yield of their crop.

b) 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

None

c) Intended use of the product and types of users

Cultivation and import in the EU for all uses as any other oilseed rape (food, feed and industrial uses).

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

No mandatory restrictions for use, storage and handling are proposed as a condition of the authorisation. All standard practices applicable to oilseed rape today remain adequate for the handling of hybrid varieties.

If genetically modified oilseed rape is co-mingled with non-genetically modified oilseed rape during use, storage and handling the corresponding batch will have to be labelled with the

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

Not applicable.

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

None

g) Any proposed packaging requirements

Like any other oilseed rape

h) Any proposed labelling requirements in addition to those required by law.

The label on the seed bag of SeedLink® oilseed rape will include the statement:

"This product contains genetically modified organisms"

together with the unique identification code ACS-BN005-8 x ACS-BN003-6.

This label will be adapted according to the provisions of the upcoming regulation on traceability and labelling of GMOs (2001/0180/COD)

h) Estimated potential demand

- in the Community: the level and amount of production in the Community will depend on the adaptation of the product to the growing areas in the specific Member States and the demand for the product by farmers. Total area cultivated with oilseed rape in the EU-15 is approx. 3 million hectares for a total production of approx. 9.4 million tonnes of seeds (3.5 million tonnes of oil).
- The Community also imports approx. 1 million tonnes of oilseed rape seeds and 500000 tonnes of oilseed rape cake.

In export markets for EC supplies: the European Community is not a major exporter of oilseed rape seeds (between 0.5 and 1 million tonnes of seeds exported depending on the EU

i) Unique identification code(s) of the GMO(s)

Female line Ms8 : ACS-BN005-8

Male line Rf3: ACS-BN003-6

Hybrid Ms8xRf3: ACS-BN005-8 x ACS-BN003-6

4. <u>Has the GMHP referred to in this product been notified under Part B of Directive 2001/18/EC and/or Directive 90/220/EEC?</u>

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

5. <u>Is the product being simultaneously notified to another Member State</u> ?

Yes : X for purposes other than placing on the market (part B releases)	*No ☐*
(i) If no*, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC*	

Or

<u>Has the product been notified in a third country either previously or simultaneously</u>?

Yes X	*No ☐*

If *yes*, please specify:

- Canada (commercial uses)
- USA (commercial uses)
- Australia
- Japan
- China
- Czech Republic
- Hungary

6. Has the same GMHP been previously notified for marketing in the Community?

Yes ☐	*No X*
If yes*, give notification number and Member State*	

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

In its opinion expressed on 19 May 1998, the European Scientific Committee on Plants (SCP) concluded that there is no evidence to indicate that the placing on the market of hybrid oilseed rape Ms8xRf3 with the purpose to be used as any other oilseed rape is likely to cause adverse effect on human health or the environment.

It is recommended to follow Good Agricultural Practices when placing the product on the market.

B. NATURE OF THE GMHP CONTAINED IN THE PRODUCT

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

8. Complete name

a) Family name : Brassicaceae
b) Genus : Brassica
c) Species : napus
d) Subspecies : oleifera
e) Cultivar/breeding line : various
f) Common name : oilseed rape

9. a) Information concerning reproduction

(i) Mode(s) of reproduction Autogamous and allogamous reproduction: oilseed rape is a crop capable of both self-pollination (approx. 70%) and cross-pollination (approx. 30%). The pollen, which is heavy and sticky, can be transferred from plant to plant through physical contact between neighbouring plants and by wind and insects.
(ii) Specific factors affecting reproduction, if any Temperature (insect visits), humidity (pollen viability) and wind. Pollinating insects, in particular honeybees (*Apis mellifera*) and bumblebees (*Bombus sp.)* play a major role in *B. napus* pollination.
(iii) Generation time Between 6 and 12 months.

9. b) Sexual compatibility with other cultivated or wild plant species

Successful hybrid formation depends not only on the sexual compatibility between the plants (whether the same or related species) but the two plants must flower simultaneously, share the same insect pollinator (if insect pollinated) and be sufficiently nearby for the transfer of viable pollen. The consequences of successful transfer will depend on the sexual fertility of the hybrid progeny, vigour and the fertility of subsequent generations or their ability to propagate vegetatively.

The possibility of gene flow from oilseed rape (*Brassica napus*) to wild relatives under natural conditions has been reported, mostly under optimal conditions, on five species: *Brassica rapa* (synonym *Brassica campestris*), *Brassica juncea*, *Hirschfeldia incana*,

10. Survivability

a) Ability to form structures for survival or dormancy

Oilseed rape is an annual plant. Seeds are formed as structures enhancing survival. They can persist in soil through dormancy during several years if they were ploughed in deeper soil. Cultivation of the soil usually terminates this dormancy.

b) Specific factors affecting survivability, if any

The survival ability of the seeds is affected by soil conditions such as temperature and moisture content.

11. Dissemination

Ways and extent of dissemination

Two development stages are relevant for dissemination: pollen and seeds.

- Pollen: oilseed rape pollen grains, which are heavy and sticky, can be transferred from plant to plant through physical contact between neighbouring plants and by wind and insects. Although pollen can be blown by wind or carried away by insect pollinators over large distances, the bulk of cross-pollination has been observed to occur over very short distances. Successful pollination declines exponentially with increasing distance between the pollen source and the nearest recipient plant.
- Seeds: oilseed rape seeds are small and may be left in and near the field (essentially seeds that shattered or leaked from the transport equipment) or may be carried away (essentially seeds that leaked from the transport carriers).

b) Specific factors affecting dissemination, if any

Pollen dissemination is mainly affected by wind and insects. Pollinating insects, in particular honeybees (*Apis mellifera*) and bumblebees (*Bombus sp.)* play a major role in *B. napus* pollination. The dynamics of bee-mediated pollen movement depend on the quantity of pollen available (size and density of donor population) and the size and location of the receiving populations, as well as environmental conditions and insect activity.

There is no specific factor affecting seed dissemination (oilseed rape seeds have no special adaptations to encourage transport), which is mainly due to human activity.

12. Geographical distribution of the plant

Since the second world war, rapeseed production in Europe and Canada has increased dramatically as a result of improved oil and meal quality. China, India, Europe and Canada are now the top producers. Today two species of *Brassica (B. napus and B. rapa*) have commercialised varieties with double low characteristics, low erucic acid content in the oil and very low glucosinolate content in the meal, characteristics desirable for high-quality vegetable oil and high quality animal feed.

B. napus is grown as a winter annual crop in regions where winter conditions do not result in very low temperatures. In North America and Northern Europe, a spring biotype of *B. napus* that requires no vernalisation prior to flowering is grown.

Oilseed rape is now one of the major global sources of vegetable oil and the major crop grown in Europe for the production of vegetable oil.

The total cultivation area of rapeseed in the world is approx. 24 million hectares, with approx. 14 million hectares in Asia (particularly India and China), 10 million hectares in North America (mainly Canada), 3 million hectares in the EU and 0.7 million hectares in Australia.

The total cultivation area of rapeseed in the EU in 2000 was 3.035 million hectares with 1.2 million hectares in France, 1.1 million hectares in Germany and 0.4 million hectares in the UK.

13. 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

Not applicable, as the crop is grown normally in the Member States.

14. Potentially significant interactions of the plant with other organisms in the ecosystem where it is usually grown, including information on toxic effects on humans, animals and other organisms

Oilseed rape is not pathogenic or harmful. There are no major interactions with the ecosystem except for being a crop.

Oilseed rape serves as an abundant supply of nectar for foraging insects such as honeybees.

Oilseed rape plants or seeds may be occasionally consumed by flea beetles, animal browsers (e.g. rabbits) and birds.

A number of diseases (e.g. *Sclerotinia sclerotiorum*) may infest the crop.

Concerns about the nutritional safety of erucic acid in oilseed rape oil and of glucosinolates in oilseed rape meal led to the development of varieties of oilseed rape which have combined low levels of both glucosinolates and erucic acid (also known as "double zero" varieties), characteristics desirable for high-quality vegetable oil and high quality animal feed.

15. Phenotypic and genetic traits

The recipient organism is a commercial spring variety of oilseed rape commonly cultivated in Europe (Drakkar variety) with no specific phenotypic or genetic trait.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

Insertion of genetic material by *Agrobacterium tumefaciens* mediated transformation

17. Nature and source of the vector used

Line Ms8 was produced with plasmid **pTHW107** and line Rf3 with plasmid **pTHW118**, both derived from *Escherichia coli.*

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

Female line Ms8 - plasmid **pTHW107** contains between the left and right borders:

1. PTA29-*barnase*-3'nos:

- the tapetum cell-specific promoter PTA29 from *Nicotiana tabacum*
- the *barnase* gene from *Bacillus amyloliquefaciens*
- part of the 3' non-coding region (3' nos) of the nopaline synthase gene of *Agrobacterium tumefaciens*

2. PssuAra-*bar*-3'g7:

- the PssuAra promoter from *Arabidopsis thaliana*
- the *bar* gene isolated from *Streptomyces hygroscopicus*
- the 3' untranslated sequence of the TL gene 7 of *Agrobacterium tumefaciens*

Male line Rf3- plasmid **pTHW118**contained between the left and right borders:

1. PTA29-*barstar*-3'nos:

- the tapetum cell-specific promoter PTA29 from *Nicotiana tabacum*
- the *barstar* gene from *Bacillus amyloliquefaciens*
- part of the 3' non-coding region (3' nos) of the nopaline synthase gene of *Agrobacterium tumefaciens*

2. PssuAra-*bar*-3'g7:

- the PssuAra promoter from *Arabidopsis thaliana*
- the *bar* gene isolated from *Streptomyces hygroscopicus*
- the 3' untranslated sequence of the TL gene 7 of *Agrobacterium tumefaciens*

Tables 1 to 2 show the size of each constituent DNA fragment and their source and intended function

Table 1 Genetic Elements of T-DNA Component of pTHW107

Abbreviation	**Definition**	**Source**	**Size (bp)**	**Function**
RB	Right border sequence	*A. tumefaciens*	25	T-DNA integration
PLS	Polylinker sequence	Synthetic	72	Plasmid cloning
3'g7	Terminating signal from TL-DNA gene 7	*A. tumefaciens*	212	Stop signal
PLS	Polylinker sequence	Synthetic	21	Plasmid cloning
Bar	Glufosinate tolerance gene	*S. hygroscopicus*	552	Selectable marker and herbicide tolerance
PSsuAra	Promoter	*A. thaliana*	1726	Constitutive promoter targeting expression mainly to green tissue
PLS	Polylinker sequence	Synthetic	50	Plasmid cloning
3'nos	Polyadenylation region of nopaline synthase gene	*A. tumefaciens*	261	Stop signal
3'UTR	Terminating signal of barnase gene	*B. amyloliquefaciens*	112	Stop signal
barnase	Ribonuclease gene	*B. amyloliquefaciens*	336	Male sterility
PTA29	Promoter	*N. tabacum*	1510	Expression only in anthers
PLS	Polylinker sequence	Synthetic	44	Plasmid cloning
LB	Left border sequence	*A. tumefaciens*	25	T-DNA integration

Table 2 Genetic Elements of T-DNA Component of pTHW118

Abbreviation	Definition	Source	Size (bp)	Function
RB	Right border sequence	*A. tumefaciens*	25	T-DNA integration
PLS	Polylinker sequence	Synthetic	28	Plasmid cloning
	TL-DNA sequence	*A. tumefaciens*	37	None
PLS	Polylinker sequence	Synthetic	7	Plasmid cloning
3'g7	Terminating signal from TL-DNA gene 7	*A. tumefaciens*	212	Stop signal
PLS	Polylinker sequence	Synthetic	21	Plasmid cloning
Bar	Glufosinate tolerance gene	*S. hygroscopicus*	552	Selectable marker and herbicide tolerance
PSsuAra	Promoter	*A. thaliana*	1726	Constitutive promoter targeting expression mainly to green tissue
PLS	Polylinker sequence	Synthetic	50	Plasmid cloning
3'nos	Polyadenylation region of nopaline synthase gene	*A. tumefaciens*	261	Stop signal
PLS	Polylinker sequence	Synthetic	21	Plasmid cloning
3'UTR	Terminating signal of barstar gene	*B. amyloliquefaciens*	40	Stop signal
barstar	Ribonuclease inhibitor gene	*B. amyloliquefaciens*	273	Fertility Restoration
PTA29	Promoter	*N. tabacum*	1510	Expression only in anthers
PLS	Polylinker sequence	Synthetic	45	Plasmid cloning
LB	Left border sequence	*A. tumefaciens*	25	T-DNA integration

INFORMATION RELATING TO THE GMHP

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

Pollination control system (female line Ms8 and male (fertility restoration) line Rf3)

The female line Ms8 contains a *barnase* gene (origin *Bacillus amyloliquefaciens*) coding for a ribonuclease expressed only in the tapetum cells during anther development that leads to lack of viable pollen.

The male (fertility restoration) line Rf3 contains a *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for an inhibitor of *barnase* expressed only in the tapetum cells during anther development that leads to restoration of fertility in the hybrid plant.

Herbicide tolerance

Both the female and male lines (and the resulting hybrid) contain a *bar* gene (bialaphos resistance, origin *Streptomyces hygroscopicus*) coding for phosphinotricin acetyl transferase conferring tolerance to herbicides based on glufosinate ammonium. The *bar* gene is driven by a plant promoter that is active in all green tissues of the plant.

20. Information on the sequences actually inserted/deleted/modified

a) Size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced in the GMHP or any carrier or foreign DNA remaining in the GMHP

The size and structure of the inserts have been characterised in detail using Southern Blot analysis. PCR analysis has shown that the integrated DNA is restricted to the DNA between the T-DNA border repeats. The insert has been completely sequenced.

In line Ms8 the inserted DNA has been shown to consist of a single copy of T-DNA insert.

In line Rf3 there is a T-DNA copy arranged in an inverted repeat structure with a second, incomplete T-DNA copy. The second copy includes a functional part of promoter PTA29, the coding region of *barstar*, the 3' nos and a non-functional part of promoter PssuAra.

Sequences outside the T-DNA borders of the vector are not present. Based on Southern blots as well as detailed PCR analyses it is confirmed that no sequences of the backbone of plasmids pTHW107 or pTHW118 are present in the plant. No *Agrobacterium* DNA containing the Ti-plasmid remains present in the genetically modified plant.

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

Not applicable

c) Location of the insert in the plant cells (integrated in the chromosome, chloroplast, mitochondrion, or maintained in a non-integrated form), and methods for its determination.

In both Ms8 and Rf3, based on Southern and segregation analyses, it was demonstrated that the DNA has integrated in a single genetic locus in the oilseed rape nuclear genome (chromosomes).

Following analyses of the regions flanking the insert there is no indication of insertion of T-DNA in a functional gene.

d) Copy number and genetic stability of the insert

Based on Southern blot and PCR analyses,

In line Ms8 the inserted DNA has been shown to consist of a single copy of T-DNA insert.

In line Rf3 there is a T-DNA copy arranged in an inverted repeat structure with a second, incomplete T-DNA copy. The second copy includes a functional part of promoter PTA29, the coding region of *barstar*, the 3' nos and a non-functional part of promoter PssuAra.

Based on phenotypic and molecular techniques it was shown that the genes are stable and follow standard mendelian inheritance.

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

21. Information on the expression of the insert

a) Information on the expression of the insert and methods used for its characterisation

Linked to the plant promoter PssuAra, the expression of the *bar* gene is mainly targeted to green tissue of the plant.

The plant promoter PTA29 allows the activity of the *barnase* and the *barstar* genes to be limited in time (only when flowering, during anther development) as well as place (tapetum cells of the pollen sac).

Expression level was measured by Northern blot analysis and PAT protein specific ELISA. The PAT protein activity was assessed by enzymatic assays.

b) Parts of the plant where the insert is expressed (e.g. roots, stem, pollen, etc.)

Linked to the plant promoter PssuAra, the expression of the *bar* gene is mainly targeted to green tissue of the plant (e.g. leaves). The PAT protein can also be detected in very low amounts in dry seed (approx. 0.1 µg/g seed).

The plant promoter PTA29 allows the activity of the *barnase* and the *barstar* genes to be limited in time (only when flowering, during anther development) as well as place (tapetum cells of the pollen sac). The barnase and the barstar proteins are not detected in seeds.

New proteins are not expected to be present in pollen because the genes coding for these proteins (*bar, barnase* and/or *barstar* genes) were linked to plant tissue specific promoters that are not known to lead to significant gene expression in pollen (Pssu Ara, TA29). This was confirmed through Northern Blot analyses.

22. Information on how the GMHP differs from the recipient plant in

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

Reproduction occurs through seed production.

In the female line Ms8 there is no pollen production.

In the male line Rf3 and the hybrid line Ms8xRf3 the mode and rate of reproduction are similar to the recipient plant.

b) Dissemination

Dissemination of the plants happens through the seed stage. The trait may also be conveyed via the pollen stage. No differences in dissemination capacity have been observed between genetically modified and non-genetically modified plants, with the exception of the female line being incapable of releasing pollen.

Studies show that the genetic modification did not modify the characteristics of the plants that could have an impact on seed dispersal :

- no differences in seed shattering ability have been observed between the genetically modified oilseed rape plants and non-genetically modified oilseed rape.
- the shape and size of the seeds is identical to that of the original non-genetically modified variety; there is no development of structures facilitating transport (such as hairs or needles);
- the germination ability (a key-parameter to test seed dormancy) and evolution of germination ability of the seeds of the genetically modified oilseed rape did not differ from their non-genetically modified counterpart (cf. field trials and germination tests);
- no differences in regrowth ability have been observed between the genetically modified and non-genetically modified oilseed rape under greenhouse and field conditions.

c) Survivability

Survival is essentially determined at the seed stage. There is no indication on any change in seed characteristics as a result of the genetic modification. No difference in survival was recorded at the vegetative stage.

Although non-genetically modified oilseed rape as well as genetically modified oilseed rape can be volunteers and following crops, current agricultural practices (including cultivation, rotation, selective herbicides) are able to control both modified and unmodified volunteer rape plants.

d) Other differences

The female, male and hybrid lines have been made tolerant to the Liberty® herbicide (active ingredient glufosinate ammonium) and can therefore survive treatment with glufosinate ammonium.

23. Potential for transfer of genetic material from the GMHP to other organisms

a) Transfer of genetic material to other higher plants.

Comparative data on germination, establishment, plant phenotype and parameters of normal agronomic performance suggest that genetically modified oilseed rape will not behave differently from non-genetically modified oilseed rape regarding its ability for gene transfer or dispersal. Available evidence shows no differences in their ability to out-pollinate between transformed and untransformed oilseed rape plants.

Data generated from pollen dispersal studies show that the rate of cross-pollination between two adjacent fields of oilseed rape is measurable and decreases over the distance. The level of hybridisation will depend on several factors, such as the weather, the wind direction, field topography, presence of pollinators, synchrony of flowering.

There is no evidence of genetic transfer and exchange under natural conditions with organisms other than those with which oilseed rape is able to produce fertile crosses through sexual reproduction. There are no indications that the potential for successful exchange has changed due to the genetic modification. The possibility of gene flow to wild relatives under natural conditions has been reported, mostly under optimal conditions, on five species *Brassica rapa, Brassica juncea, Hirschfeldia incana, Raphanus raphanistrum,* and *Sinapis arvensis*.

The frequency of gene flow to wild relatives under natural conditions is considered very low, the fitness of the interspecific hybrids is generally reduced compared to the parents and the stable introgression of the herbicide tolerance trait in the weed species genome is confirmed to be extremely difficult.

When gene flow occurs, the consequences first depend on the sexual fertility of the hybrid progeny and the vigour and fertility of subsequent generations. Successful hybrid formation depends not only on the sexual compatibility of the recipient species (whether the same or related species) but the two species must flower simultaneously, share the same insect pollinator (if insect pollinated) and be sufficiently nearby for the transfer of viable pollen. The consequences of successful transfer will depend on the sexual fertility of the hybrid progeny, vigour and the fertility of subsequent generations or their ability to propagate vegetatively.

Environmental studies have demonstrated that the genetic stability, fertility and vigour of the offspring from inter-specific crosses are generally reduced. Potential transgenic exchange is therefore unlikely to lead to establishment as a result of reduced viability of any hybrid plants and competition.

In addition, specific studies conducted during the environmental risk assessment of oilseed rape Ms8 Rf3 have shown that:

- The number of fertile hybrids resulting from pollination between oilseed rape and wild *Brassicaceae* relatives will be very limited.
- Any viable progeny of a hybrid oilseed rape-wild relative carrying the herbicide tolerance gene will have

no competitive advantage in the absence of selective pressure by herbicide containing glufosinate ammonium.

- Any viable progeny of an hybrid oilseed rape-wild relative carrying the herbicide tolerance gene can be controlled by current agronomic practices, either mechanically by cultivation in the rotation cycle, or chemically by many other active ingredients than glufosinate ammonium.

b) *Transfer of genetic material to bacteria.*

In order for any horizontal gene transfer to lead to a new type of microorganism and therefore to introduce a significant impact, some of the following conditions will have to be fulfilled:

- the uptake should result in the incorporation of complete undegraded DNA;
- the plant targeted genes should result in significant expression in a prokaryotic background;
- the expression should represent a significant increase over the background level;
- the traits should convey a competitive advantage to the strain in which they are incorporated.

In the very unlikely case where both horizontal gene transfer from genetically modified plants to bacteria would occur and where due to genetic recombination the genes would be expressed in micro-organisms (the *bar, barnase* and *barstar* genes are under the control of plant promoters which are not functional in bacteria), this would have no impact since the transgenes involved would not provide a selective advantage (the only known substrate of the PAT protein is the herbicide glufosinate ammonium and ribonucleases and inhibitors are ubiquitous among bacteria).

24. Information on any harmful effects on human health and the environment, arising from the genetic modification

No harmful effects on human health arising from the genetic modification are expected since:

- There are no indications that the PAT, barnase and barstar proteins would cause allergenic reactions.
- There are no indications that growing the genetically modified oilseed rape would induce or change the intensity of an allergic reaction towards oilseed rape pollen in occupationally or daily exposed personnel.
- The only known substrate of the PAT protein is the herbicide glufosinate ammonium and there is evidence available concerning the safety of PAT.
- The ribonuclease and its inhibitor (barnase and barstar proteins) represent activities widely occurring among common bacteria and with no foreseeable risks associated with them.
- The PAT protein is detected in very low amounts in seeds and the barnase and barstar proteins are not detected. In addition virtually no protein is present in the oil extracted from the plants.

- The nutritional quality of food products derived from the genetically modified oilseed rape is not different from the nutritional quality of food product derived from non-genetically modified oilseed rape. Compositional analyses carried out on oil content, glucosinolate levels, fatty acid profiles including erucic acid content, vitamin E and mineral content showed that the ranges for genetically modified oilseed rape fell within the range for non-modified oilseed rape.

25. Information on the safety of the GMHP to animal health, where the GMHP is intended to be used in animal feedstuffs, if different from that of the recipient/parental organism(s)

The safety of the GMHP to animal health is not different from that of non-genetically modified oilseed rape. No adverse effects on animal health are expected since:

- There are no indications that the PAT, Barnase and Barstar proteins would alter feed safety of the genetically modified oilseed rape.
- The amounts of PAT present in seed-meal fed to animals would be too low to cause even theoretical concern.
- The low levels of PAT protein and the weight of evidence available concerning the PAT protein lead to the conclusion that there is no risk to livestock following ingestion of the gene product.
- The nutritional quality of feed products derived from the genetically modified oilseed rape is not different from the nutritional quality of feed products derived from non-genetically modified oilseed rape.
- Feeding studies already submitted show no differences between animals fed with genetically modified and non-genetically modified oilseed rape.

26. Mechanism of interaction between the GMHP and target organisms (if applicable) , if different from that of the recipient/parental organism(s)

Not applicable since there are no target organisms.

27. Potentially significant interactions with non-target organisms, if different from the recipient or parental organism(s)

There are no non-target organisms specific to the GMHP compared to non-genetically modified oilseed rape. There are no observed effects of the herbicide tolerant (hybrid) oilseed rape on non-target organisms:

- In the very unlikely case where both horizontal gene transfer from genetically modified plants to bacteria would occur and where due to genetic recombination the genes would be expressed in micro-organisms (the *bar, barnase* and *barstar* genes are under the control of plant promoters which are not functional in bacteria), this would have no impact since the transgenes involved would not provide a selective advantage (the only known substrate of the PAT protein is the herbicide glufosinate ammonium and ribonucleases and inhibitors are ubiquitous among bacteria).
- The different studies conducted confirm that the GMHP has no effect on honeybees (attractiveness, behaviour, pollination activity, mortality, etc.) and predatory arthropods of oilseed rape.
- No effect could be observed on birds and small mammals.

28. Description of detection and identification techniques for the GMHP, to distinguish it from the recipient or parental organism(s)

- DNA-based methods:

DNA-based methods available include PCR and Southern-Blot methodology. They allow detection and identification of the events Ms8 and Rf3 through detection of nucleotide sequences that are specific to these events.

- Protein-based methods:

Protein-based methods available include quantitative methods (e.g. specific PAT protein ELISA test) or qualitative methods (e.g. Trait LL Leaf Test kit) based on the specific interaction between antibodies and the PAT protein produced by the introduced gene. They allow detection and identification of glufosinate-ammonium herbicide tolerance trait through detection of the PAT protein in the product.

Protein-based methodology offers an easier-to-use alternative to DNA-based methodology.

In addition to these methods, control samples of the product genetic material are available.

INFORMATION ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OF THE GMHP

29. Potential environmental impact from the release or the placing on the market of GMOs (Annex II, D2 of Directive 2001/18/EC), if different from a similar release or placing on the market of the recipient or parental organism(s)

The following conclusions were drawn (see items listed Annex II D2 of Directive 2001/18/EC):

1) The herbicide-tolerant hybrid oilseed rape neither becomes more persistent than the recipient plant in agricultural habitats, nor shows any changed behaviour with respect to invasiveness in natural habitats.
2) A selective advantage to the herbicide-tolerant hybrid oilseed rape could only be identified upon treatment with glufosinate ammonium.
3) Potential for gene transfer to oilseed rape and/or wild relatives is the same as with non-genetically modified oilseed rape: gene transfer to other oilseed rape can occur while gene transfer to wild relatives is very difficult. The same selective advantage, tolerance to treatment with glufosinate ammonium, would be conferred to those plants.
4) There are no target organisms.
5) No impact could be identified on non-target organisms such as honeybees, birds and small mammals.
6) No adverse effects on human health from contact or handling have been identified.
7) No adverse effect on animal health or the feed/food chain following animal feed use have been identified.
8) No effect or alteration on biogeochemical processes was observed.
9) Adaptations of cultivation and management techniques for the genetically modified oilseed rape are limited to changes in herbicide use, without any adverse environmental impact.

The overall conclusion is that:

- The potential adverse effect identified is establishment of transgenic herbicide tolerance, be it through herbicide-tolerant oilseed rape volunteers or through transfer of the herbicide tolerance gene to wild *Brassica* relatives.
- Standard Good Agricultural Practices allow adequate management of both herbicide-tolerant oilseed rape volunteers and herbicide-tolerant wild *Brassica* relatives i.e. allow adequate risk management of the identified adverse effects.
- The overall risk of herbicide-tolerant hybrid oilseed rape Ms8/Rf3, taking into account the risk management strategies available, is therefore nil.

30. <u>Potential environmental impact of the interaction between the GMHP and target organisms (if applicable), if different from that of the recipient or parental organism(s)</u>

Not applicable : there are no target organisms.

31. <u>Possible environmental impact resulting from potential interactions with non-target organisms, if different from that of the recipient or parental organism(s)</u>

a) Effects on biodiversity in the area of cultivation

No adverse effects on biodiversity are expected in the area of cultivation (no adverse effects on non-target organisms).

The herbicide tolerance gene provides a selective advantage in cultivated habitats (fields) if the glufosinate ammonium is used as a herbicide for weed control.

The incorporation and expression of the genes *barnase* (in female plants) and *barstar* (in male plants restoring fertility) will not give the recipient plants any selective advantage over non-genetically modified plants.

In agricultural habitats (fields and field borders, with potential selective pressure through herbicide treatment), the herbicide tolerant (hybrid) oilseed rape is not likely to become more persistent:

- The herbicide tolerant (hybrid) oilseed rape will not create additional volunteer problems compared to non-genetically modified oilseed rape
- Standard Good Agricultural Practices provide adequate control of genetically modified oilseed rape volunteers

b) Effects on biodiversity in other habitats

No adverse effects on biodiversity in other habitats are expected (no adverse effects on non-target organisms).

No selective advantage has been conferred to the GMHP.

In natural habitats (absence of selective pressure), the herbicide tolerant hybrid oilseed rape is not likely to become more invasive since there is no selective advantage conferred to the herbicide tolerant hybrid oilseed rape in the absence of selective pressure through herbicide treatment.

In absence of treatment with glufosinate ammonium the presence of the *bar* gene does not confer any selective advantage to herbicide tolerant hybrid oilseed rape. As a consequence, the herbicide tolerance gene is not providing a selective advantage (or disadvantage) in natural habitats or habitats that are not exposed to glufosinate ammonium treatment.

The incorporation and expression of the genes *barnase* (in female plants) and *barstar* (in male plants restoring fertility) will not give the recipient plants any selective advantage over non-genetically modified plants. The male sterility trait in the female line will rather confer a disadvantage to the multiplication of the plant.

c) Effects on pollinators

In oilseed rape, honeybees (*Apis mellifera*) are considered to be the principal pollinators searching for nectar and pollen, though other insects such as several bumble-bees, some solitary honeybees and some dipteran, lepidopteran, hemipteran and coleopteran insects may have a pollinator role as well.

Experimentation was performed on the plants themselves, floral morphology, pollen characteristics and morphology, nectar production and total sugar and glucose content. These data show that there are no pleiotropic effects of the genetic modification on the attractiveness of the herbicide tolerant (hybrid) oilseed rape for the pollinators.

Furthermore, a large number of studies have been conducted over the last years to study the impact of the genetic modification and herbicide spray on bee behaviour: foraging activities, hive activities, life cycle, and development of the populations: no difference could ever be detected in the bee behaviour that could be due to an effect of the genetic modification. No adverse effect on honeybee colonies and their brood has ever been observed.

d) Effects on endangered species

No adverse effects are expected on endangered species since no adverse effects have been observed on non-target organisms including birds and small mammals.

C. INFORMATION RELATING TO PREVIOUS RELEASES

32. History of previous releases 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

Belgium: B/B/94/W2, B/B/94/VSP1, B/B/95VSP6, B/B/95/VSP6-96, B/B/95/VSP6-97, B/B/95/VSP6-98, B/B/95/VSP6-99, B/BE/00/VWSP9, B/BE/00/VWSP9-2001, B/BE/00/VWSP9-2002, B/BE/00/VWSP10

Denmark: B/DK/98/04

France: B/FR/95.05.10, , , B/FR/96.01.14, B/FR/96.07.04, B/FR/97.01.10, B/FR/97.08.06, B/FR97.08.07, B/FR/99-01-15, B/FR/99-06-04, B/FR/99-06-05, B/FR/00-07-03

Germany: 6786-01-090 (SNIF: B/DE/98/90)

Spain: B/ES/97/41

Sweden: 22-120/95, 22-726/96, 22-58/97, 22- 4291/97, 22-1825/99, 22-1911/99, 22-2769/99

United-Kingdom: 95/R15/7, 95/R15/8, 96/R15/16, 96/R15/17, 97/R15/18, 97/R15/19, 97/R15/20, , 97/R15/21, 97/R15/22, , 98/R19/18, 99/R19/20, 00/R33/1, 00/R33/5, 00/R33/5S, 00/R33/6, 00/R33/6S, 00/R33/7, 00/R33/7S, 00/R33/9, 00/R33/9, 00/R33/10, 00/R33/10S, 00/R33/11, 00/R33/11S

b) Conclusions of post-release monitoring

Standard Good Agricultural Practices provide adequate control of genetically modified oilseed rape volunteers.

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 adverse effects on human health and the environment were observed.

33. History of previous releases carried out inside or outside the Community by the same notifier

a) Inside the Community:

Belgium: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : Belgian Biosafety Council

Information on the releases at www.biosafety.be or at http://gmoinfo.jrc.it

Aim of the releases: research and development, safety data, breeding, seed production.

Denmark: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : Ministry of Environment

Information on the releases at www.sns.dk or at http://gmoinfo.jrc.it

Aim of the releases: herbicide trial and variety registration

France: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : Ministry of Agriculture and Fisheries

Information on the releases at www.agriculture.gouv.fr Or at http://gmoinfo.jrc.it

Aim of the releases:

Germany: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : Robert Koch Institute

Information on the releases at www.rki.de Or at http://gmoinfo.jrc.it

Aim of the releases: breeding, seed production, herbicide trial and variety registration

Spain: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : Ministry of Environment

Information on the releases at www.mma.es Or at http://gmoinfo.jrc.it

Aim of the releases:

Sweden: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : Swedish Board of Agriculture

Information on the releases at www.sjv.se or at http://gmoinfo.jrc.it

Aim of the releases: winterhardiness nursery

United Kingdom: Previous releases : see notification numbers in section C.32

Authority overseeing the releases : DEFRA (England) and SEERAD (Scotland)

Information on the releases at www.defra.gov.uk www.scotland.gov.uk or at http://gmoinfo.jrc.it

Aim of the releases: Farm Scale Evaluation, Research and Development, variety registration

Czech Republic:

Notification: 8/1999;1/200; 2/2000; 8/2000; 9/2000; 11/2000; 12/2000; 117/OER/GMO/01; 1060/OER/GMO/1; 1059/OER/GMO/01

Authority overseeing the releases : Ministry of Environment/Agriculture/Health

Information on the releases at www.env.cz

Hungary:

Notification 52.058/1999/3

Authority overseeing the releases : Ministry of Agriculture and Rural Development

Information on the releases at www.biosafety.abc.hu

Canada (commercial uses since 1999)

Authority overseeing the releases : Food Inspection Agency

Information on the releases at www.inspection.gc.ca

USA (commercial uses since 1999)

Authority overseeing the releases : Department of Agriculture

Information on the releases at www.aphis.usda.gov

Australia:

Authority overseeing the releases : Department of agriculture, fisheries and forestry

Information on the releases at www.affa.gov.au

China:

Authority overseeing the releases : Ministry of Agriculture

Japan:

Authority overseeing the releases : Ministry of agriculture, fisheries and forestry

Information on the releases at www.maff.go.jp

E INFORMATION RELATING TO THE MONITORING PLAN - IDENTIFIED TRAITS, CHARACTERISTICS AND UNCERTAINTIES RELATED TO THE GMO OR ITS INTERACTION WITH THE ENVIRONMENT THAT SHOULD BE ADDRESSED IN THE POST COMMERCIALISATION MONITORING PLAN

The Post-Commercilisation Monitoring Plan for genetically modified oilseed rape Ms8xRf3 will be divided in two parts:

1. Case-specific monitoring that focuses on the two case-specific potential adverse effects that were identified in the environmental risk assessment

- Volunteers: establishment of genetically modified oilseed rape volunteers
- Outcrossing: gene transfer to wild *Brassicaceae* relatives and establishment of herbicide tolerant weeds

It will confirm that any assumptions regarding the occurrence and impact of the potential adverse effects identified in the environmental risk assessment are correct. The case-specific monitoring plan will also confirm the effectiveness of the risk management strategies that were developed following the environmental risk assessment and will demonstrate that the potential adverse effects identified in small-scale trials (volunteers and outcrossing) are fully manageable in a practical way in farmers' fields.

2. General surveillance monitoring for the occurrence of adverse effects of the GMHP or its use on human health or the environment which were not anticipated in the environmental risk assessment, including potential long-term environmental impacts.