Notification C/DE/98/6

Glufosinate tolerant Oilseed Rape Liberator pHoe6/Ac

In accordance with Directive 2001/18/EC

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*: Germany

b) *Notification number*: C/DE/98/6

c) *Name of the product (commercial and other names)*:

Progenies from transformation event Liberator pHoe6/Ac and commercial varieties derived from conventional breeding of Liberator pHoe6/Ac with non-transgenic lines will be placed on the market. These new lines will be marketed under their trade name followed by a logo such as Liberty Link®.

d) *Date of acknowledgement of notification*:

completed on 15 June 1998

2. Notifier

a) *Name of notifier*: Bayer CropScience GmbH

b) *Address of notifier*:

Industriepark Höchst, K607
D-65926 Frankfurt am Main

c) *Is the notifier: domestic manufacturer* Yes *importer* No

Progenies from Liberator pHoe6/Ac and commercial varieties derived from Liberator pHoe6/Ac will be manufactured and distributed by several companies under licence of Bayer CropScience.

d) *In the case of an import the name and address of the manufacturer shall be given:*

not relevant

3. General description of the product

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

The recipient is a commercial variety of winter oilseed rape named Liberator.

The genetic modification aims to develop oilseed rape tolerant for glufosinate-ammonium herbicides. They are replacing pre-emergence herbicides that have been the major tool used for weed control in conventional production. Post-emergence herbicides are fitter to Integrated Weed Control because they allow treatment according to the real weed infestation and adaptation of the rates and spraying time is possible.

Glufosinate-ammonium is a broad spectrum, non-systemic, non-selective herbicide with favourable environmental and safety characteristics. Glufosinate tolerant oilseed rape in concert with glufosinate-ammonium will be a valuable new weed management system that provides selective use of glufosinate-ammonium herbicides and can positively impact current agronomic practices by participating in the shift toward the use of post-emergence herbicides.

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

The GMHP *Brassica napus* will be used for exactly the same uses as are currently, and may in future be, allowed for conventionally bred varieties. The most significant use is cultivation for food, animal feed and industrial purposes. In this case the main users will be seed company/distributor, seed handling personnel, rape growers (farmers), processors and distributors for industrial, food and feed products.

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

The product will be used in exactly the same way as conventionally bred varieties. For field growing the product will be grown in areas of the EU where oilseed rape is normally grown. For import for crushing the grain will be handled and processed in exactly the same way as currently practised. The product, i.e. Liberator pHoe6/Ac and varieties of oilseed rape obtained by conventional plant breeding from this transformation event , carry the trait for tolerance to the herbicide glufosinate-ammonium. This enables the herbicide to be used selectively in the crop since the herbicide is detoxified by acetylation (via the enzyme phosphinothricin-N-acetyltransferase) to the inactive N-acetyl-phosphinothricin. The trait is specific to the detoxification of glufosinate (phosphinothricin) and has no influence on any other biological system. The use of glufosinate in weed control during the field growth of these varieties is the only difference in handling these modified lines.

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

The GMHP is intended for cropping on arable land in all EU countries where oilseed rape is normally grown.

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

None

g) Any proposed packaging requirements

For field growing seed bags of the type commonly used by distributors will be used.

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

All the varieties have to be registered in the EU by the official registration authorities and the trade label will be defined at this moment.

The varieties will get a special label which indicates that glufosinate can be used in these varieties as an additional option for weed control to other commercial herbicides *i) Estimated potential demand*

After launching in Europe, Liberator pHoe6/Ac and derived lines may cover 10 to 15% of the total oilseed market within the next ten years, depending on the agricultural performance of the new varieties.

Similar GM rape varieties containing the same new trait were first launched in Canada (in 1995) and have been approved for import into the community.

j) Unique identification code(s) of the GMO(s):

ACS-BN009-3

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

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

5. Is the product being simultaneously notified to another Member State ?

Yes ☐	No ☑
(i) If no, refer to risk analysis data on the basis of the elements of Part B of Directive 2001/18/EC. Several varieties derived from Liberator pHoe6/Ac have been extensively studied in field since 1995 under part B permits (see above). However the development activities have been temporarily suspended until granting the permit for placing on the market.	

Or

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

Yes ☐	No ☑
If yes, please specify:	

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

Yes ☐	No ☑
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

The transformation did not change the susceptibility of the GM rape to other herbicides which can be used to control undesired growth of oilseed rape.
Field studies have demonstrated that the GM rape can be controlled by traditional cultivation methods.

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: *Cruciferae*
b) Genus: *Brassica*
c) Species: *Napus*
d) Subspecies: *partim*
e) Cultivar/breeding line: Liberator
f) Common name: Oilseed rape

9. a) Information concerning reproduction

(i) Mode(s) of reproduction

Brassica napus is an annual species producing large numbers of self- compatible flowers. 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. Reproduction is sexual only via seeds.

(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. Vegetative reproduction is not observed with *Brassica napus.*
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(iii) Generation time.

11 to 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*, *Raphanus raphanistrum* and *Sinapis arvensis*.

The frequency of gene flow from oilseed rape 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 a new trait in the weed species genome is confirmed to be extremely difficult.

10. Survivability

a) *Ability to form structures for survival or dormancy*

Oilseed rape is an annual plant. The only way of survival from one year to the next is by forming seeds. The seeds are all viable directly after harvest. But some may persist in soil for periods of more than five years under ideal conditions (Lutman 1993). Cultivation of the soil usually terminates this dormancy.

b) Specific factors affecting survivability, if any

Seeds are resistant to low temperatures during wintertime in moderate climates throughout Europe. Seed dormancy can be induced by ploughing volonteers into deeper fractions of the soil. The survival ability of the seed is affected by soil conditions such as temperature and moisture content.

11. Dissemination

a) Ways and extent of dissemination

Oilseed rape can only be disseminated by seed dispersal.

b) Specific factors affecting dissemination, if any

The primary agents in seed dispersal are: wind, water and animals. Since the seeds have no structural modifications to facilitate transfer by animals, their primary means of dispersal would be wind and, to a lesser extent, water. The distance of dispersal of the seed by wind would be dependent on the size of the seed. Grain-feeding birds do not contribute to the dissemination of *Brassica napus* seeds.

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

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 serves as an abundant supply of nectar for foraging insects such as honeybees, whilst a number of pathogens attack the crop (Heitefuss et al. 1984). The crop has no special survival characteristics that interact with different environmental conditions. Meal and oil are used for feed and food production.

15. Phenotypic and genetic traits

Liberator is a well-known commercial variety. It is an oilseed crop with high oil and protein content and low level of erucic acid and glucosinolates.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

The glufosinate tolerant transformant Liberator pHoe6/Ac was produced by the disabled *Agrobacterium tumefaciens* binary vector system (C 58 Rif pMP90RK and pHoe6/Ac) mediated transformation of hypocotyl fragments of the oilseed rape cultivar Liberator, line 8/92-01 (de Block et al., 1989).

5 mm pieces of the hypocotyl segment were cocultivated with Agrobacteria containing the binary transformation system. Selection was done in regeneration media containing 500 mg/l claforan and carbenicillin each for in vitro embryogenesis. Tolerant shoots were selelected in the presence of glufosinate. Glufosinate tolerant shoots were tested for the absense of Agrobacteria and further cultivated in antibiotic free media. At the size of 5 - 7 cm the plantlets were transferred to soil and brought to flowering in an S1 growth chamber. Selfing of these plants for at least 4 generations led to the transformed line which was homozygous for the introduced *pat* gene and used for field trials in Germany and UK. Since the native *pat* gene has a high G:C content, which is atypical for plants, a modified nucleotide sequence was synthesised using codons preferred by plants. The amino acid sequence of the enzyme remains unchanged. This synthetic version of the *pat* gene introduced into the disarmed vector was taken for the transformation process.

17. Nature and source of the vector used

The vector pHoe6/Ac is based on the original vector pPCV002 as described by Koncz and Schell, 1986, and PiAN7 (New England Biolabs Catalog 1986/87, p. 92).
Koncz and Schell describe the construction of a vector cassette for the transfer of foreign genes into plant genomes with the aid of the Ti plasmid system.
The cassette contains a replication and mobilisation unit, which allows the maintenance of the plasmid in *E. coli* and in *Agrobacterium tumefaciens.* The vector can be exchanged between both bacteria. But the RK2 type specific replication and mobilisation functions have to be provided in trans.

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

Table of the inserted genetic elements – origin and function		
Size	Source	Intended Function
25 bp	*Agrobacterium tumefaciens* T37 (Nopalin strain)	Right border sequence from Ti-plasmid pTiT37, necessary for DNA transfer.
184 bp	*Agrobacterium tumefaciens* T37 (Nopalin strain)	T-DNA partial sequence from Ti plasmid pTiT37, no function in plants.
51 bp	Synthetic	Polylinker sequence from pUC19.
530 bp	Cauliflower Mosaic Virus	Promoter from the 35S-RNA
552 bp	*Streptomyves viridochromogenes*	Synthetic nucleotide sequence of the *pat* gene
207 bp	Cauliflower Mosaic Virus	Terminator of the 35S-RNA
277 bp	*Agrobacterium tumefaciens* Ach5 (Octopin strain)	T-DNA partial sequence of the Ti plasmid pTiAch5, no function in plants.
24 bp*	*Agrobacterium tumefaciens* Ach5 (Octopin strain)	Left border sequence from Ti plasmid pTiAch5, necessary for DNA transfer.

*) The molecular analysis of the transformant showed, that only one copy of the T-DNA has entered the Liberator genome with at least 34 subterminal bp of the T-DNA missing in the transformant .

INFORMATION RELATING TO THE GMHP

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

The objective of the modification is to insert the trait for tolerance to the herbicide glufosinate-ammonium (GA). The transformed plant has the same phenotypical characteristics as the recipient Liberator. The only difference is the expression of an additional protein, the phosphinothricin N-acetyl-transferase that inactivates GA via N-acetylation. No other sequences have been inserted during the transformation process.

GA is a non-selective herbicide active ingredient, which destroys all the treated green parts of the plants. Oilseed rape is normally very susceptible to GA. It can be irreversibly damaged by sprays of no more than 300 g/ha of GA. The transformed plants can support more than 1500 g/ha of GA without expressing significant symptoms.

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 inserted fragments consist of the right and left border of *Agrobacterium tumefaciens* with short stretches of adjacent Agrobacteria DNA and the *pat* expression cassette including the cauliflower mosaic virus 35S promoter/terminator system and the synthetic version of the *Steptomyces viridochromogenes* pat gene.

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*

Restriction enzyme digestion, Southern analysis and probing with PAT indicate one copy of the DNA is present in the nuclear genome of the transformed plant.

d) *Copy number and genetic stability of the insert*

One single copy of the T-DNA fragment of the disarmed vector was inserted into the Genome of the recipient plant Liberator during the transformation process.

Both the nature and the stability of transformed line Liberator pHoe6/Ac were assessed by observing the expression of the *pat* gene over multiple generations. A single dominant characteristic such as glufosinate tolerance will segregate according to a defined pattern as described by Mendel. Segregation analysis was used to assess the stability of transformed materials.

Different combinations are possible in the F2-generation. When start with one copy of the single dominant Glufosinate tolerance gene, a 3:1 phenotypic and a 1:2:1 genetic segregation is expected. A distinction between homo- and hemizygous plants after Glufosinate spraying is not possible, because the plants are after Glufosinate treatment either not damaged, or completely damaged.

The theoretically expected segregation was examined in several F2-generations. The results of this experiment are presented in the table 1 .

Table 1 : Segregation pattern of F2-generations

breeding lines	number of plants			% sensible
	total	sensible	tolerant	
846/6Ac	98	20	78	20,41
846/6Ac	138	30	108	21,74
846/6Ac	88	28	60	31,82
849/6AC	83	23	60	27,71
849/6AC	91	22	69	24,18
849/6AC	129	26	103	20,16
854/6Ac	124	25	99	20,16
854/6Ac	129	34	95	26,36
854/6Ac	136	31	105	22,79
854/6Ac	111	36	75	32,43
856/6Ac	129	31	98	24,03
856/6Ac	91	22	69	24,18
856/6Ac	92	19	73	20,65
857/6Ac	108	21	87	19,44
857/6Ac	136	38	98	27,94
857/6Ac	98	23	75	23,47
866/6Ac	106	34	72	32,08
866/6Ac	103	30	73	29,13
866/6Ac	209	64	145	30,62
866/6Ac	100	33	67	33,00
866/6Ac	101	30	71	29,70
total:	2400	620	1780	25,83

The routinely checked F1-generations showed homogenous Glufosinate tolerance.
The segregation expected in the F2-generation in relation to the pat gene gives the following combinations :

- PP homozygous plants with Glufosinate tolerance
- P- hemizygous plants with Glufosinate tolerance
- -- homozygous plants without Glufosinate tolerance

in a proportion of 1:2:1.

This means that 25% of the plants in the F2- generation are Glufosinate-susceptible. These 25% is found back as an average of all tested F2-plants which are Glufosinate susceptible (see table). This proves that a single dominant Pat gene is stably inherited to the following generations. The heredity of the Pat gene followed the Mendel`s law.

The segregation data for Liberator pHoe6/Ac clearly show that the gene is expressed stably over subsequent generations. Furthermore the data show that the original transformant that was obtained from Agrobacterium mediated gene transfer into diploid cells of the hypocotyl was hemizygous for PAT.

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*

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

The concentrations of the PAT protein was assayed in different tissues of the transformant. PAT protein was detectable in all parts ofthe transgenic crop Liberator pHoe6/Ac. The concentration ranged from 0.05 % PAT of the total soluble protein in the stem to 0.004 % in pollen. No PAT protein could be detected in tissue samples of non transgenic control plants.

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

Comparison of the recipient Liberator and the transformed organism Liberator pHœ6/Ac raised in a growth chamber showed no major differences in important characteristics which might lead to a higher probability of survival, multiplication or dissemination in the field. Extensive greenhouse and field studies with similar transformants of oilseed rape (Falcon GS 40/90pHoe6/Ac) in Germany and canola (HCN 92) in Canada came to the same conclusion: the newly introduced trait did not change the overall characteristics of *Brassica napus.*

Other differences

The GMO Liberator pHoe6/Ac has been extensively tested in Europe since 1995 and no differences could be seen in comparison to conventional oilseed rape or to the non transgenic counterpart. No differences could be detected by comparing the response to different agronomical treatments including chemical treatments and bebaviour to plant diseases. The only difference is that the transgene Liberator pHoe6/Ac has been made tolerant to the Liberty® herbicide (active ingredient glufosinate ammonium) and can therefore survive a treatment with glufosinate ammonium.Growth parameters, germination rate, flowering time and pollen production were similar to non transgenic oilseed rape.

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 Liberator pHoe6/Ac 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 *pat* gene 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).

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

No human health effects are predicted for the product, i.e. the live GMO (seed, plant). The possibility of allergenicity is ruled out by studies on protein homology and by experience of exposure of humans to the crop and seed over more than 5 years in Europe. In addition the food health and safety of the processed oil has been confirmed by the BGVV (Bundesinstitut für gesundheitlichen Verbrauscherschutz). Compositional studies have shown that the plant is substantially equivalent to non-transgenic counterparts and that the *pat* gene and protein are denatured in gastric juices and that humans will therefore not be exposed to it via the animal food chain. The oil from oilseed rape is used for human consumption. Due to the oil production process it is very unlikely that the PAT protein will be carried over to the oil fraction. The compositional analysis of the oil from the transgenic plant and the non-transgenic counterpart did not show any difference.

No changes in the interactions of oilseed rape with the environment have been observed using transgenic glufosinate tolerant Liberator in field trials in Europe.

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)

Should there be any PAT enzyme remaining after food and feed processing of oilseed rape, the only route of exposure for livestock to PAT would be oral ingestion. Bayer CropScience has confirmed experimentally that PAT protein and *pat* DNA in glufosinate tolerant oilseed rape is degraded in vitro by the gastric juices from swine, chicken, and cattle (bovine rennet-bag fluid and paunch). In these experiments the natural fluids were adjusted to different pH's (1.5, 2.5, 3.5, 4.5, 5.5, 7.7) with base or acid, in the case of paunch. The physiological pH of swine, chicken and rennet fluid is pH 1.5. The physiological pH of paunch fluid is pH 7.1. Degradation of total transgenic oilseed rape DNA at pH 1.5 was complete after one hour incubation at 37°C. Degradation was less efficient at the higher pH's. PCR products specific to the *pat* gene could be recovered from all pH treatments after the 60-minute incubation with the exception of pH 1.5. Inactivation of the PAT protein present in a crude protein extract from transgenic canola by swine and cattle stomach fluids is very rapid. At physiological pH the inactivation of PAT (inability to acetylate PPT) was 100% by 1 minute in swine stomach fluid; 100% by 2.5 minutes in rennet-bag fluid; and 90% by 30 minutes in bovine paunch fluid. Inactivation was less rapid with increasing pH in swine and rennet fluids (paunch and chicken fluids were not tested), but was greater than 50% in 15 minutes at pH 5.5, the most alkaline pH tested. Swine, chicken and cattle represent the three primary types of gastric systems among livestock.

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 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 *pat* gene is 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).
- 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 event Liberator pHoe6/Ac 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 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 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 Liberator pHoe6/Ac, taking into account the risk management strategies available, is therefore nil.

30. 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)

Not applicable because there are no target organisms

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

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.
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 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 oilseed rape is not likely to become more invasive since there is no selective advantage conferred to the herbicide tolerant oilseed rape in the absence of selective pressure through herbicide treatment.

In absence of treatment with glufosinate ammonium the presence of the *pat* 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.

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 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

- Germany : B/D/95/35, B/D/95/36, BD/95/37, B/D/96/52, B/D/96/53
- Great Britain : 96/R27/1

b) *Conclusions of post-release monitoring*

In all trials the crop following on the site was a cereal and the antidicot herbicides used on it killed all 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 risks observed

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

a) Inside the Community: **See above**
b) Outside the Community: **None**
c) Release site: Not relevant
d) Aim of the release: Not relevant
e) Duration of the release: Not relevant
f) Aim of post-releases monitoring: Not relevant
g) Duration of post-releases monitoring: Not relevant
h) Conclusions of post-release monitoring: Not relevant

Results of the release in respect to any risk to human health and the environment:

Not relevant

D. 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-Commercialisation Monitoring Plan for genetically modified oilseed rape Liberator GS40/90 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.